United States Environmental Protection Agency
Office of Water
Washington, DC 20460
EPA-822-R-02-022
March 2002

Methods for evaluating wetland condition

#12 Using Amphibians in Bioassessments of Wetlands

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METHODS FOR EVALUATING WETLAND CONDITION

#12 Using Amphibians in Bioassessments of Wetlands

Major Contributors

USGS Patuxent Wildlife Research Center
Donald W. Sparling

King County Department of Natural Resources, Seattle, WA
Klaus O. Richter

Department of Applied Biology, University of Maine
Aram Calhoun

Ohio EPA, Division of Surface Water
Mick Micacchion

Prepared jointly by:

The U.S. Environmental Protection Agency
Health and Ecological Criteria Division (Office of Science and Technology)

and

Wetlands Division (Office of Wetlands, Oceans, and Watersheds)

Notice

Appropriate Citation

U.S. EPA. 2002. *Methods for Evaluating Wetland Condition: Using Amphibians in Bioassessments of Wetlands.* Office of Water, U.S. Environmental Protection Agency, Washington, DC. EPA-822-R-02-022.

Acknowledgments

EPA acknowledges the contributions of the following people in the writing of this module:
Aram Calhoun (Department of Applied Biology, University of Maine), Douglas G. Hoskins (U.S. Environmental Protection Agency), Mick Micacchion (Ohio Environmental Protection Agency), Klaus O. Richter (King County Department of Natural Resources, Seattle, WA), Don W. Sparling (United States Geological Survey), and the King County Department of Natural Resources, Washington State, for supporting this work.

This entire document can be downloaded from the following U.S. EPA websites:

http://www.epa.gov/ost/standards

http://www.epa.gov/owow/wetlands/bawwg

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Foreword

In 1999, the U.S. Environmental Protection Agency (EPA) began work on this series of reports entitled *Methods for Evaluating Wetland Condition*. The purpose of these reports is to help States and Tribes develop methods to evaluate (1) the overall ecological condition of wetlands using biological assessments and (2) nutrient enrichment of wetlands, which is one of the primary stressors damaging wetlands in many parts of the country. This information is intended to serve as a starting point for States and Tribes to eventually establish biological and nutrient water quality criteria specifically refined for wetland waterbodies.

This purpose was to be accomplished by providing a series of "state of the science" modules concerning wetland bioassessment as well as the nutrient enrichment of wetlands. The individual module format was used instead of one large publication to facilitate the addition of other reports as wetland science progresses and wetlands are further incorporated into water quality programs. Also, this modular approach allows EPA to revise reports without having to reprint them all. A list of the inaugural set of 20 modules can be found at the end of this section.

This series of reports is the product of a collaborative effort between EPA's Health and Ecological Criteria Division of the Office of Science and Technology (OST) and the Wetlands Division of the Office of Wetlands, Oceans and Watersheds (OWOW). The reports were initiated with the support and oversight of Thomas J. Danielson (OWOW), Amanda K. Parker and Susan K. Jackson (OST), and seen to completion by Douglas G. Hoskins (OWOW) and Ifeyinwa F. Davis (OST). EPA relied heavily on the input, recommendations, and energy of three panels of experts, which unfortunately have too many members to list individually:

- Biological Assessment of Wetlands Workgroup
- New England Biological Assessment of Wetlands Workgroup
- Wetlands Nutrient Criteria Workgroup

More information about biological and nutrient criteria is available at the following EPA website:

http://www.epa.gov/ost/standards

More information about wetland biological assessments is available at the following EPA website:

http://www.epa.gov/owow/wetlands/bawwg

List of "Methods for Evaluating Wetland Condition" Modules

Module #	Module Title
1	Introduction to Wetland Biological Assessment
2	Introduction to Wetland Nutrient Assessment
3	The State of Wetland Science
4	Study Design for Monitoring Wetlands
5	Administrative Framework for the Implementation of a Wetland Bioassessment Program
6	Developing Metrics and Indexes of Biological Integrity
7	Wetlands Classification
8	Volunteers and Wetland Biomonitoring
9	Developing an Invertebrate Index of Biological Integrity for Wetlands
10	Using Vegetation to Assess Environmental Conditions in Wetlands
11	Using Algae to Assess Environmental Conditions in Wetlands
12	Using amphibians in Bioassessments of Wetlands
13	Biological Assessment Methods for Birds
14	Wetland Bioassessment Case Studies
15	Bioassessment Methods for Fish
16	Vegetation-Based Indicators of Wetland Nutrient Enrichment
17	Land-Use Characterization for Nutrient and Sediment Risk Assessment
18	Biogeochemical Indicators
19	Nutrient Load Estimation
20	Sustainable Nutrient Loading

Summary

Amphibians are important ecological components of both wetlands and dry land. Among vertebrates they are distinctive in many ways. For biological assessments, they are especially promising because of their capability of linking wetlands with surrounding landscapes. Surveying amphibians and obtaining reliable data on community characteristics may require multiple samplings of each wetland within a year because of differences in breeding and developmental phenology among species and the complex life histories of amphibians. Thus, more work may be necessary to conduct an adequate monitoring program for this assemblage than for macrophytes, fish, algae, or aquatic invertebrates. Published studies using amphibians in developing indices of biological integrity currently do not exist. However, such studies are in progress with several attributes proposed, and more can be developed from a thorough search of literature dealing with limiting factors in amphibians. Most of the specific metrics will have to be developed on a regional basis owing to differences in continent-wide distributions of amphibian species. We encourage investigators to examine amphibian communities as a possible source of metrics in the development of their indices of biological integrity.

Purpose

This module is intended to encourage the use of amphibian metrics in wetland bioassessment. To facilitate use of this valuable information, we describe briefly the basic ecology of amphibians and their assemblages, and the best available science on their responses to physical, chemical, and ecological stress. Monitoring protocols for the assessment of amphibian responses along a gradient of human disturbance are described at various levels of sampling effort.

Introduction

Carl Linnaeus, arguably among the most famous biologists of the 18th century, characterized reptiles and amphibians as "foul and loathsome animals, abhorrent because of their cold body, pale color, cartilaginous skeleton, filthy skin, fierce aspect, calculating eye, offensive voice, squalid habitation, and terrible venom" (quoted in Hunter et al. 1992). Three centuries later, amphibians (and their squalid habitation) have gained stature in human eyes, as their ecological importance becomes clearer. For example, amphibians may constitute the highest biomass among vertebrates in some ecosystems and, depending on scale, may be keystone species (Burton and Likens 1975, Windmiller 1996, Wyman 1998, Fauth 1999, Petranka and Murray 2001). Within the last decade, amphibians have the dubious distinction of being in the global spotlight owing to worldwide declines (Barinaga 1990, Wyman 1990, Wake 1991, Griffiths and Beebee 1992). Suggested causes of declines include various human-induced processes, including habitat loss or degradation (Reh and Seitz 1990, Griffiths and Beebee 1992, deMaynadier and Hunter 2000, Turtle 2000), acid deposition (Freda 1986, Horne and Dunson 1994), climate warming (Wyman 1990, Pounds et al. 1999), increases in UV radiation (Blaustein et al. 1994), spread of toxic substances (Sparling et al. 2000, 2001), introduction of predators (Funk and Dunlap 1999, Lawler et al. 1999), and pathogens (Carey and Bryant 1995, Jancovich et al. 1997, Morrell 1999, Daszak et al. 2001).

These declines raise the global eyebrow because amphibians are indicators of ecosystem health (Wake 1991). A thin, moist, highly permeable skin; jellied, unshelled eggs; possession of aquatic and terrestrial life histories; restricted home range; and limited dispersal abilities of many species make amphibians effective biomonitors. Dramatic changes in their populations and increased incidences of diseases and malformations, particularly in seemingly pristine areas, highlight concerns about general environmental deterioration.

Scientists studying amphibians have had difficulties sorting out local versus global and single versus multiple (e.g., synergistic) causes behind population declines and malformations. The formulation of a useful Index of Biological Integrity (IBI) depends on identifying biological attributes (or metrics) that provide reliable signals about the condition of the resource (Karr and Chu 1999). For amphibians, the poor resolution in identifying relationships between potential causative factors and effects complicates metric development.

Amphibians as a group are much more closely associated with water and wetlands than are most reptiles, birds, or mammals. Most anurans (i.e., frogs and toads) and many caudates (i.e., salamanders and newts) lay their eggs in water, have aquatic larvae, and inhabit forests or other upland habitats as adults. Treefrogs (*Hyla* spp.), toads (*Bufo* spp.), and salamanders belonging to the mole salamander group *Ambystoma*, for example, exhibit such characteristics. Numerous North American frogs, especially those in the common "true frog" genera *Rana* and the chorusfrog genera *Pseudacris*, live their entire lives in wetlands. Still others, such as some mole salamanders including the common northwestern salamander (*Ambystoma gracile*) and the widely distributed tiger salamander (*A. tigrinum*), may turn into neotenes (i.e., gilled adults), a life form that is entirely restricted to water (Plate 1). Species in the genera *Plethodon* and *Ensatina* lay eggs in damp locations on land and spend their entire life in upland habitats (Plate 2).

Among amphibians breeding in aquatic habitats, different genera, and even species within genera, select specific aquatic environments on the basis of hydroperiod, current velocity, and other wetland characteristics. Some (e.g., wood frog, *R. sylvatica*; long-toed salamander, *A. macrodactylum*) prefer to breed in standing water of vernal wetlands or temporary pools. Others (e.g., green frog, *R. clamitans*; bullfrogs, *R. catesbeiana*) select quiescent water of permanent ponds (Plate 3).

Plate 1. Among other species, the northwestern salamander *(Ambystoma gracile)* commonly occurs as a neotene (e.g. gilled adult) and consequently can only successfully breed in permanently flooded wetlands.
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Plate 2. The Ensatina *(Ensatina eschscholtzii)* is a species of amphibian that lives almost entirely on land.
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Plate 3. The bullfrog *(Rana catesbeiana)* breeds mostly in permanent ponds.

Interestingly, others still (e.g., tailed frog; *Ascaphus truei*; torrent salamander larvae, *Rhyacotriton* spp.) are restricted to fast-moving water of small mountain streams, spring heads, and seepages (Plates 4 and 5).

Those that use both uplands and wetlands for different phases of their life cycles often have unique physiological and behavioral requirements for both habitat types. Consequently, some amphibian species may be very useful in biological assessments of wetland health. However, the constellation of species that inhabits a particular region may be better in assessing the health of both uplands and wetlands. In this way, amphibians can provide useful metrics to assess the health or condition of entire landscapes.

Plate 4. Tailed frogs (*Ascaphus truei*) breed in fast-moving streams and live and feed along their shorelines.

Plate 5. Tailed frog tadpoles possess ventral sucotorial discs that allow attachment to the substrate in swift moving currents.

Amphibian community composition may vary dramatically across the country, and even across comparatively narrow regions. It is therefore important for those involved in assessments to be aware of regional and local amphibian fauna and to avoid broad generalizations. Readers are referred to the following atlases, natural histories, and descriptions of species distributions for their areas: (a) overall North America (Behler and King 1979, Stebbins and Cohen 1995, Wright and Wright 1995, Lannoo 1998, Petranka 1998); (b) Northeastern North America (DeGraaf and Rudis 1983, Hunter et al. 1999, Hulse et al. 2001); (c) Southeastern North America (Dundee and Rossman 1996, Bartlett and Bartlett 1999); (d) Eastern and Central North America (Pfingsten and Downs 1989, Conant and

Collins 1998, Mitchell and Reay 1999); (f) Western North America (Stebbins 1985); (g) Northwestern North America (Nussbaum et al. 1983, Corkran and Thoms 1996, Leonard et al. 1993); and (h) Southwestern North America (Stebbins 1972).

On a continental scale, McDiarmid and Mitchell (2000) recognized six natural regions for herpetofauna (i.e., amphibians and reptiles) (Figure 1). Species richness follows somewhat different patterns for caudates than for anurans. In North America there are more species of salamanders and newts (155) than frogs and toads (91), and salamanders tend to be more terrestrial than frogs. The highest species richness for salamanders is in the Appalachian Highlands, Gulf and Atlantic Coastal Plains, and Pacific Northwest. Several species in the Appalachian Highlands have very restricted ranges. Other areas with high species richness of salamanders include the western Sierra Nevadas, the Edwards Plateau, and the Interior Highlands of the Central Plain. Frogs also reach their highest species richness in the southeastern United States but are most diverse in the lowlands of the Coastal Plains. South-central Texas is another area of high salamander species richness. Within each of these

Figure 1: Natural regions of continental herpetofauna (after McDiarmid and Mitchell 2000).

major natural regions there is considerable local variation in taxa and abundance of which biologists must be aware.

What Is the Value of Using Amphibians in Bioassessments?

Contrary to Linnaeus' quote above, amphibians are of particular importance in wetland ecosystems and can perform a significant function in landscape assessments. To a large extent, they have been historically ignored in favor of other vertebrates, but amphibians serve as vital links in food webs and between wetland and upland habitats. In addition, recent media attention on declines and malformations in amphibians has made them a popular and conspicuous element in nature. This attention is useful in creating incentives for recruiting agency and volunteer support in developing monitoring programs.

Specific advantages of using amphibians in bioassessments include:

1. *Sensitivity.* Because of their unique physiology and habitat requirements, amphibians are often regarded as more exposed and potentially more vulnerable to changes in their environment than many other vertebrates (see Sparling et al. 2000). Amphibians may serve as the proverbial "canaries in the coal mine" in their response to factors such as habitat fragmentation, hydrologic modifications, alterations in water chemistry, water and airborne contamination, and large-scale climatic variation. This is exacerbated by production of embryos in clear, unprotected jelly egg-masses; thin, highly permeable skin exposed to water and the atmosphere; and their limited dispersal and home ranges (but utilization of a wide spectrum of habitats across the aquatic/terrestrial continuum) (Plate 6).

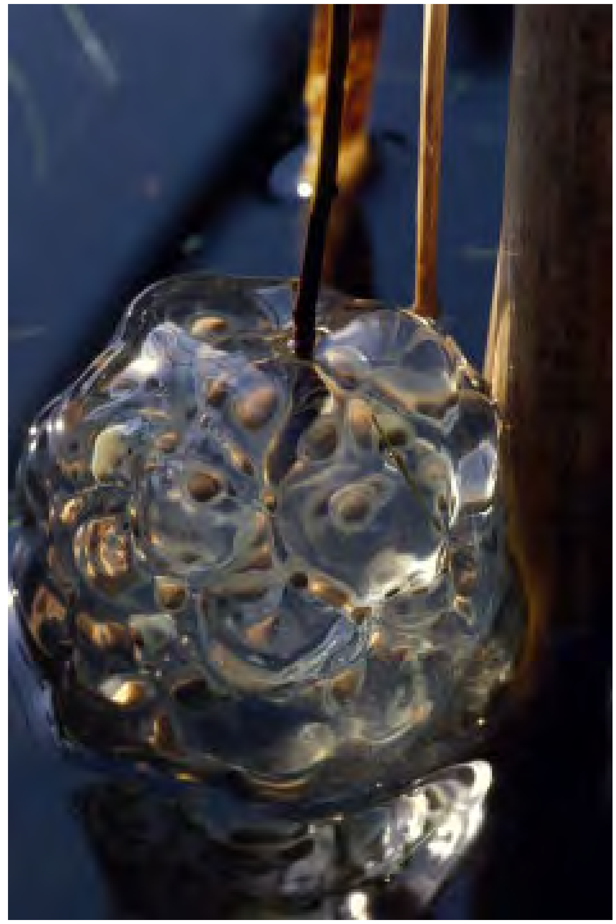

Plate 6. Embryos of wetland-breeding amphibians are "shell-less" and therefore sensitive to water pollutants and other environmental influences. Consequently, development, abnormalities, and mortality can readily be determined and studied.

2. *Complex life history.* Wetland-breeding amphibians exhibit complex life histories, often undergoing dramatic and irreversible morphological and physiological change from sedentary eggs, to free-swimming aquatic larvae, to semiaquatic or terrestrial adults. Especially because of their utilization of wetland/upland transitional areas, members of this class are appropriate for assessing impacts on these habitats. For instance, the first effects of hydrologic modifications become apparent to eggs that are exposed to freezing and to tadpoles and newly metamorphosed individuals that may face desiccation (Plate 7). Whereas some amphibians move into the adjacent uplands to live the majority of their life cycle, others spend most of their time on this

Plate 7. The health of amphibian eggs can provide valuable information on wetland hydrologic conditions. This totally frozen northwestern salamander egg mass was attributable to dropping water levels between spawning and survey date.

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fringe between wetland and upland, and both could serve as "early warning systems" for pending impacts to their aquatic environments.

3. *Easily studied.* Anurans are very visible, tangible, and popularly enjoyed animals of wetlands and nearby environments. Adult males tend to be vocal during the breeding season and tadpoles generally are easily caught, particularly when they reside in small, isolated, seasonally flooded wetlands and vernal pools. The attractiveness of amphibians stimulates volunteer recruitment, as exemplified by programs such as the North American Amphibian Monitoring Project (NAAMP) and North American Reporting Center for Amphibian Malformations (NARCAM) (see also Module #8: Volunteers and Wetland Biomonitoring).

4. *Selecting amphibians for specific purposes.* Researchers can utilize amphibians' multiple life stages, varied species-specific habitat preferences, and broad and diverse landscape utilization to assess a variety of wetland classes and habitats over a relatively long annual cycle. There are regional differences in habitat preferences, such as for permanent wetlands, seasonally flooded wetlands, and vernal pools; but similarities can be drawn upon as well to facilitate comparisons among amphibians (Plates 8–10). For example, Plethodons are terrestrial, ambystomids use water and land, and ranids

Plate 8. Permanent wetlands are the preferred breeding habitats for bullfrogs, green frogs, northwestern salamanders, and a select few other species of amphibians.

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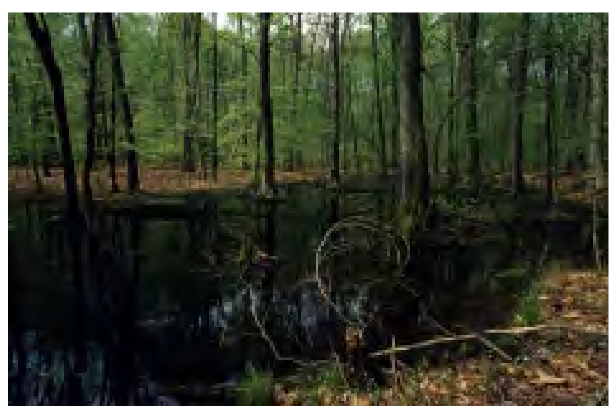

Plate 9. Vernal pools are the preferred breeding habitats for many species of ranids, chorus frogs, and treefrogs.

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Plate 10. After rains, the Great Basin spadefoot toads *(Spea intermontana)* often breed and lay their eggs in puddles, roadside ditches, and other shallow ephemeral waters. Their eggs hatch in a few days and they metamorph within a few weeks.

tend to prefer stable bodies of water to breed and live near aquatic environments for more of their life cycle than do bufonids (toads).

5. *Existing information.* Although there are obvious gaps in our knowledge about amphibian ecology, there is also enough scientific literature on the effects of environmental stressors on amphibians to assist in understanding their needs and limitations (see below).

6. *Further study will lead to better understanding.* The additional information that would be gathered during amphibian biological assessments should fill in some of these data gaps and contribute to a better understanding of amphibian relationships to gradients of human disturbance and the reasons for their population declines.

Necessary Refinements To The Basic Study Plan

Modules #4: Study Design for Monitoring Wetlands and 6: Developing Metrics and Indexes of Biological Integrity provide the basics for bioassessment of wetlands and construction of an index of biological integrity, and we will not repeat that information here in detail. Each group of organisms—macrophytes, algae, invertebrates, fish, and amphibians—requires special procedures or modifications of that basic protocol. In this section we discuss some of the considerations necessary in using amphibians in bioassessments.

1. *Uplands and wetlands are important*. Compared with other assemblages such as macrophytes or aquatic invertebrates, one difference in using amphibians in assessing wetlands is that many species are restricted to water for only part of their life cycle whereas others are totally aquatic. Most amphibians breed and lay eggs in water (or moist environments); their larval stages depend on standing water but the metamorphosed juveniles and adults outside of the breeding season are terrestrial or arboreal. For example, many adult toads, treefrogs, and metamorphosed ambystomid salamanders spend more of their time on land than in water. Therefore, inclusion of upland areas adjacent to wetlands may be essential in developing a disturbance gradient and understanding local amphibian distributions. This is especially true for forested wetlands and other sites where uplands are integral components of a wetland/upland complex.

2. *Amphibian populations fluctuate widely*. Reliance on the presence or absence of amphibian species from limited studies may not be a good indicator of overall wetland health. Short-term studies especially may be misleading because of substantial annual variation in numbers at a given body of water (Pechmann et al. 1989, Pechmann 1991) and because many amphibian species exist as metapopulations, using several nearby wetlands interchangeably (Gill 1978, Dodd and Cade 1998, Semlitsch 2000). Numbers of breeding amphibians at any wetland can vary widely for several reasons. Specifically, in some species only a small percentage of the adult population breeds in any given year, and the reasons for not breeding are unknown in most instances. In others, annual variation in weather can have a large effect on the num-

bers of species and individuals migrating or breeding (Packer 1960, 1963, Hurlbert 1969, Semlitsch 1985).

3. *Repeated visits may be necessary.* In most regions it is impractical or even a mistake to monitor amphibian species by making only one visit to a wetland. The sampling windows for some amphibians, especially adults, are short in duration, noncontinuous among species, and vary from year to year within a species. Following breeding there is typically a larval period lasting from a few weeks to several months and then a comparatively short period of metamorphosis. Individual species may have discrete breeding seasons, although larval periods may overlap. Investigators need to know the life histories, approximate breeding phenologies, and habitat preferences of the amphibians likely to be encountered in their areas. All other factors being equal, if only a single trip can be afforded, it should be timed to encounter the greatest species richness of larvae.

4. *Temporal variation may differ across regions.* The variability in breeding can become magnified in the southern United States, where seasons can last much longer than farther north. For example, in Florida it is not unusual for amphibians, especially anurans, to exhibit breeding behavior for more than half the year (Conant and Collins 1998). In contrast, some amphibians at the northern extent of their ranges or in arid regions of North America breed for a period of a week or less (Stebbins and Cohen 1995). To further complicate the situation, some species such as green frogs may metamorphose in the year that they were laid when breeding seasons are sufficiently long, but delay metamorphosis if laid late in the season or where breeding seasons are shorter (Conant and Collins 1998). It is often difficult or impossible to have monitoring schedules coincide with periods of activity in all species. Most adult ambystomatid salamanders in the Midwest and the Pacific Northwest are late-winter, early-spring breeders (Petranka 1998), and monitoring periods for adults of these species need to coincide with their life cycles. Many frogs in the Midwest, however, breed slightly later, and early- to mid-spring are the best times to encounter these adults. Thus, it is best to design a study that will allow monitoring of wetlands on two or more occasions during the breeding season.

5. *Multiple life stages should be considered.* A study design that monitors several amphibian life stages will provide valuable information for developing metrics and IBIs. Call counts of adult anurans provide early indication that frogs and, by extension, salamanders inhabit a wetland. Because of species specificity, calling males can be used to assess relative species abundance and richness. Counts of egg masses can also provide an index to breeding efforts. Frog and toad tadpoles and salamander and newt larvae may indicate a level of successful breeding or may be used in other ways in bioassessments. Metamorphosing juveniles provide a higher level of evidence for the suitability of a wetland than egg and larval censuses alone, and the frequency of malformations among juveniles may additionally be a useful metric.

6. *A variety of sampling methods may be necessary.* Investigators should be familiar with the various types of amphibian monitoring techniques that are available (Heyer et al. 1994, Bonin and Bachand 1997, Enge 1997, Olson et al. 1997, Crouch and Paton 2000, Mitchell 2000). Specific methods are appropriate for particular species and life stages but not for others. For example, adult treefrogs may be censused by inserting a 1 m long, 5 cm diameter PVC pipe partially into the ground whereas their larvae can only be censused with dipnets, seines, or aquatic funnel traps. Combinations of methodologies increase the likelihood of getting an accurate representation of the amphibians present. The type of monitoring protocols used must be weighed against the time and money available to conduct the study.

7. *Animal care and use concerns.* Although care should be taken with any living organism, invertebrates, macrophytes, and algae generally have sufficiently high numbers or reproductive potential

to tolerate moderate collection. However, amphibians are vertebrates with lower fecundity or total numbers than these "lower" groups, and extensive collecting may impact populations. Also, various States restrict the collecting of amphibians and may require permits. Animal care and use guidelines dictate that certain procedures (e.g., keeping animals in comfortable conditions, euthanasia through approved means) are followed to ensure humane treatment of amphibians. Every effort should be made to develop monitoring techniques that are noninvasive and nonlethal. Individuals to be released should be held for as short a period of time as possible. Researchers need to be sensitive to keeping the skin of amphibians moist but not allowing metamorphs and adults to drown in water. When taking vouchers or preserving individuals for later laboratory use, it is best that larvae rather than adults be sacrificed because removal of larvae will have less impact on populations. All of these precautions become more important when dealing with species that are federally, State, or even locally listed. Encounters with listed species should be well documented and reported to the appropriate Federal or State agency as soon as possible.

8. *Avoid spreading disease.* Whatever protocols are selected for monitoring, researchers need to ensure that they incorporate proper procedures to guard against spreading contamination and pathogens from one wetland study site to another. Monitoring equipment will need to be site-specific or treated between uses at different sites to ensure that the transmittal of any diseases, parasites, or other nuisance organisms is held to a minimum. Investigators should be thorough in their decontamination procedures and be aware that boots, waders, hands, and gloves are potential carriers of contamination. Detailed field practices to guard against contamination when working with amphibians can be found on the North American Reporting Center for Amphibian Malformations (NARCAM) website, http://www.npwrc.usgs.gov/narcam/techinfo/daptf.htm.

9. *Abnormalities in frogs (and other amphibians) are of critical interest.* Amphibian malformations are closely monitored and studied throughout the country. Any incidence of malformed amphibians greater than 2% or 3% of metamorphosing or juvenile frogs, or any incidence of bizarre malformations, should be reported to NARCAM (Plates 11–13). It is important that this information be relayed to the reporting center quickly to allow for any necessary followup studies. See the home page of NARCAM, http://www.npwrc.usgs.gov/narcam.html, for complete information and reporting procedures.

Plate 11. Blistering and edema in the heart region of a northwestern salamander larva.

Plate 12. Mouth malformation in the Pacific treefrog *(Pseudacris regilla)* is another example of a deformity.

Plate 13. Extra set of front digits in the rough-skinned newt *(Taricha granulosa)* is an example of deformities called fluctuating asymmetry.

Amphibian Monitoring Protocols

General Field Methods

Obligate wetland-breeding amphibians typically include four life stages: eggs, aquatic larvae, a rapidly developing metamorph stage, and terrestrial or semi-terrestrial adults. There are some exceptions. For example, some salamanders may be neotenic and become sexually mature without metamorphosing (Dent 1968) or, like the mudpuppy, *Necturus maculosus*, never exhibit a terrestrial phase at all (Plate 14). Collectively, amphibians exploit a diverse variety of wetland and upland habitats. These differences must be considered in monitoring if one wishes to inventory sites, determine relative abundance, and establish population health. For example, anurans may be highly vocal during the breeding season, whereas most caudates are silent. Therefore, well-designed calling surveys can be used to survey breeding anurans but are inappropriate for caudates. Conversely, cover board surveys may be ideal for surveying salamanders and newts but are generally deficient in establishing the distribution and abundance of frogs and toads. Some methods, such as aquatic funnel traps, and pitfall traps may capture both anurans and caudates, but all methods have some bias.

Plate 14. The mudpuppy *(Necturus maculosus)* inhabits a wide variety of permanent aquatic habitats, with clear as well as muddy waters that include large streams, lakes, ponds, and wetlands.

Overall Recommendations

Numerous methods are available for censusing amphibians (reviewed in Heyer et al. 1994, Fellers and Freel 1995, Halliday 1996, Olson et al. 1997). These include visual encounters, egg surveys, calling censuses, terrestrial cover boards, dipnets, seines, aquatic funnel traps, and terrestrial pitfall traps.

Regardless of monitoring method, several factors need to be considered to obtain reliable, unbiased data. These include:

1. *Regional modification and calibration*. Sampling designs need to be as specific as possible to region, local species, and habitats.

2. *Sample size*. The number of samples per wetland should be based on the total size of the wetland and on the diversity of vegetation within the wetland. Very large wetlands (> 5 hectares) can be divided into two or more units at constriction points. It is a good idea to sample vegetation communities in proportion to their approximate dominance within the wetland. Wetlands with diverse patches of vegetation and cover may require more sampling than those with simple communities and structure. When resources are very limited and only

a single sample of a wetland can be taken, do so when most anurans are calling or when most species have larvae present. Of these two, the period of peak larvae presence will probably record the most complete representation of species. However, identification of larvae and especially frog tadpoles may be problematic. Censuses conducted biweekly during spring and summer (March to July) will yield the greatest species richness and most accurate estimates of relative abundance.

3. *Timing of surveys*. Each of the methods described below has an optimal sampling window. Adult call counts produce their greatest species richness following rain or during a light rain that does not impair hearing. Cover board censuses and drift fence/trapline captures should take place during periods of annual migrations (e.g., late winter, early spring), under moist soil and cool temperature conditions. Aquatic funnel traps, seining, and dipnetting for larvae will be effective throughout the late spring/summer in most locations. Ultimately, the number and timing of surveys will depend on: (a) the goals of the survey (e.g., collect data on one life stage of a single species or several life stages of one or more species); (b) local and regional wetland conditions; (c) altitude of study site; and (d) wetland orientation (wetlands fully exposed to sunlight may be warmer and more advanced than those in deep shade at a given time of spring).

4. *Sampling location*. See Module #4: Study Design for Monitoring Wetlands of this series for descriptions of targeted and random sampling designs. A targeted design program selects habitats or other aspects of a wetland and assigns a sampling regimen to maximize the representation of these aspects. As the name implies, random designs develop from a sampling strategy that maximizes the representation of the entire wetland, regardless of habitat structure. Both types of designs are applicable to amphibians, depending on species and objectives. If an estimate of relative abundance or density is desired, random sampling designs are probably more appropriate. If the objective is to identify all the species inhabiting a site without regard to abundance, targeted designs may be more efficient and representative.

5. *Wetland maps*. Good maps such as United States Geological Survey (USGS) topographic maps; aerial photographs; National Wetland Inventory maps and other available State, county, or local wetland maps; or detailed sketches drawn to scale are invaluable (Figure 2). They help in identifying existing conditions such as wetland and habitat classifications, perimeter and area of wetlands, deep and permanently flooded locations, and plant communities. The location of amphibian surveys and other conditions through time can then be directly transferred to such maps and sketches to mark changes in these factors. The maps should be sufficiently detailed to show location of aquatic plant communities, the width and nature of immediate buffers around the wetland, and the adjacent upland cover and habitats beyond buffers. Because amphibians integrate wetland and upland features, details of land use within a species' range of dispersal, and hence gene flow to adjacent wetlands and amphibian populations (Dodd 1996, Richter 1997, Semlitsch 2000), should also be included.

Specific Monitoring Methods

Of the many methods available for sampling amphibians, some are specific for aquatic stages such as frog tadpoles or salamander larvae and, to some extent, adults that live in the water or at the shoreline. Other methods are more specific for adults, either in the water, moving between wetland and upland habitats, or in the uplands proper.

Aquatic surveys

Several techniques are available for sampling aquatic life stages of amphibians. These methods include funnel trapping, egg mass searches, call surveys, dipnetting, and seining. Each has advantages and disadvantages, and their use would depend on the objectives of the survey and available human and monetary resources.

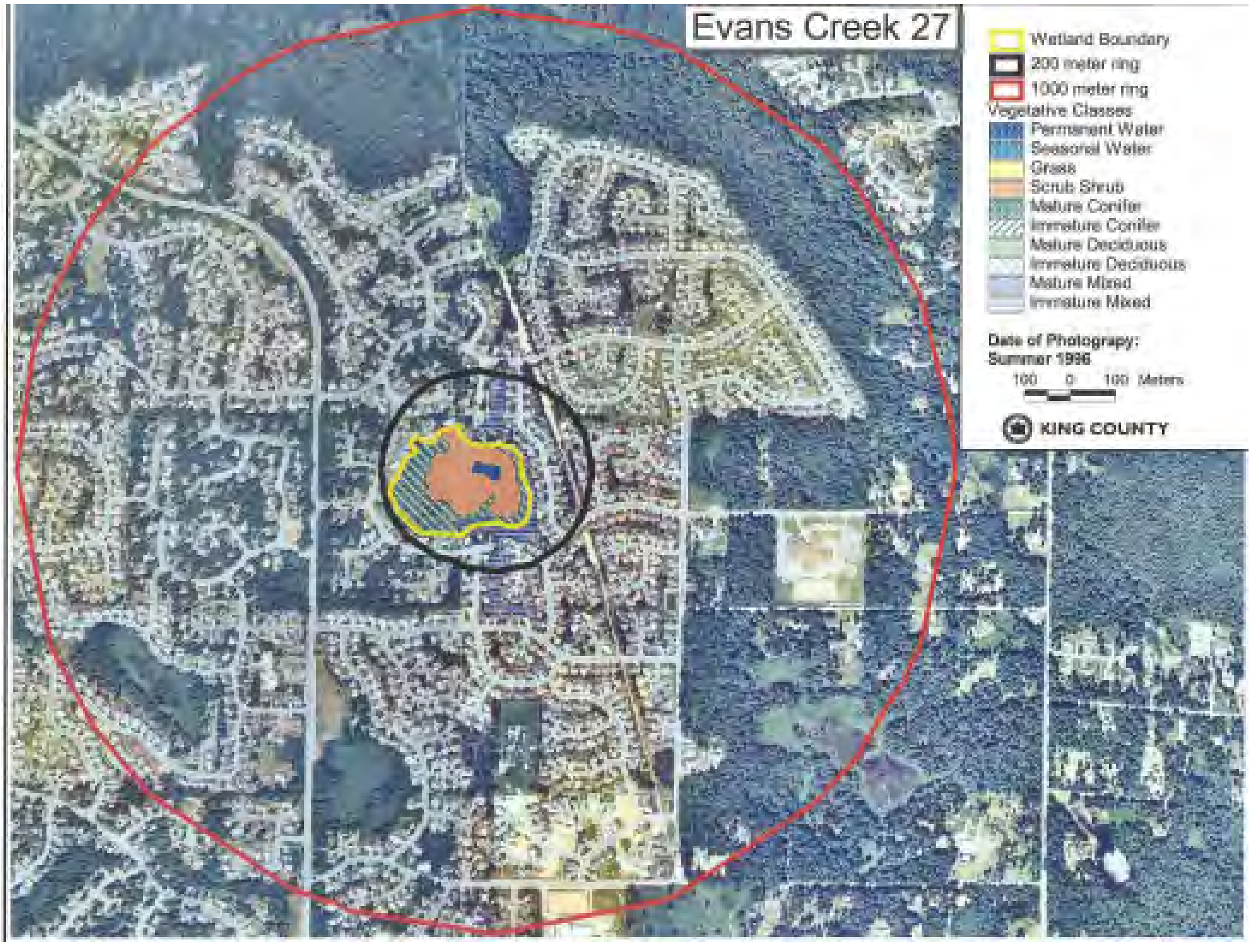

FIGURE 2: BECAUSE AMPHIBIANS USE BOTH UPLANDS AND WETLANDS, IT IS IMPORTANT TO DETERMINE LANDSCAPE LEVEL FEATURES OF THE ENVIRONMENT.

Funnel trapping

If there were only one method of sampling anurans to recommend, it would be funnel trapping. This method requires an additional visit to a wetland compared with some other techniques such as dip-netting or seining, yet it provides a reasonably economic method of sampling. This method captures most larvae, including cryptic forms that might go unnoticed with other techniques. When done correctly, it also has less potential for bias and a greater potential for obtaining information on the entire amphibian breeding assemblage than other aquatic methods (Adams et al. 1997). It is highly recommended for these advantages (Mushet and Euliss 1997). Traditional aquatic funnel traps are selective for larval amphibians (Griffiths 1985) as well as neotenes of some salamanders (Richter and Kerr 2001). Modifications for floating on the surface have additionally proven funnel traps effective for adult frog captures (Casazza et al. 2000). Aquatic funnel trap captures confirm successful breeding, egg development, and hatching. Clearly, when adults are captured, the traps are also valuable in directly determining their arrival times. Finally, with an appropriate study design, funnel trapping may also be used for determining abundances and population trends (Richter and Adams 1997).

Some funnel traps are easy to construct from inexpensive materials (Figure 3), making the method cost-effective (Griffiths 1985, Richter 1995). Even commercially manufactured nylon traps are relatively inexpensive (~$7.00). A great advantage of aquatic funnel trapping is that, like another passive method, pitfall trapping, but unlike the more active methods such as dipnetting, seining, or direct observation, the skill and experience of the surveyor have little impact on reliability of data obtained (Plate 15). Thus, trapline sampling can be consistently repeated over time, among wetlands, and with different crews. It is simple to use, requires minimal training of technicians or volunteers, and can be used for surveying and monitoring most aquatic amphibians (Adams et al. 1997).

One drawback of any sampling method that emphasizes larval forms is the difficulty of identifying

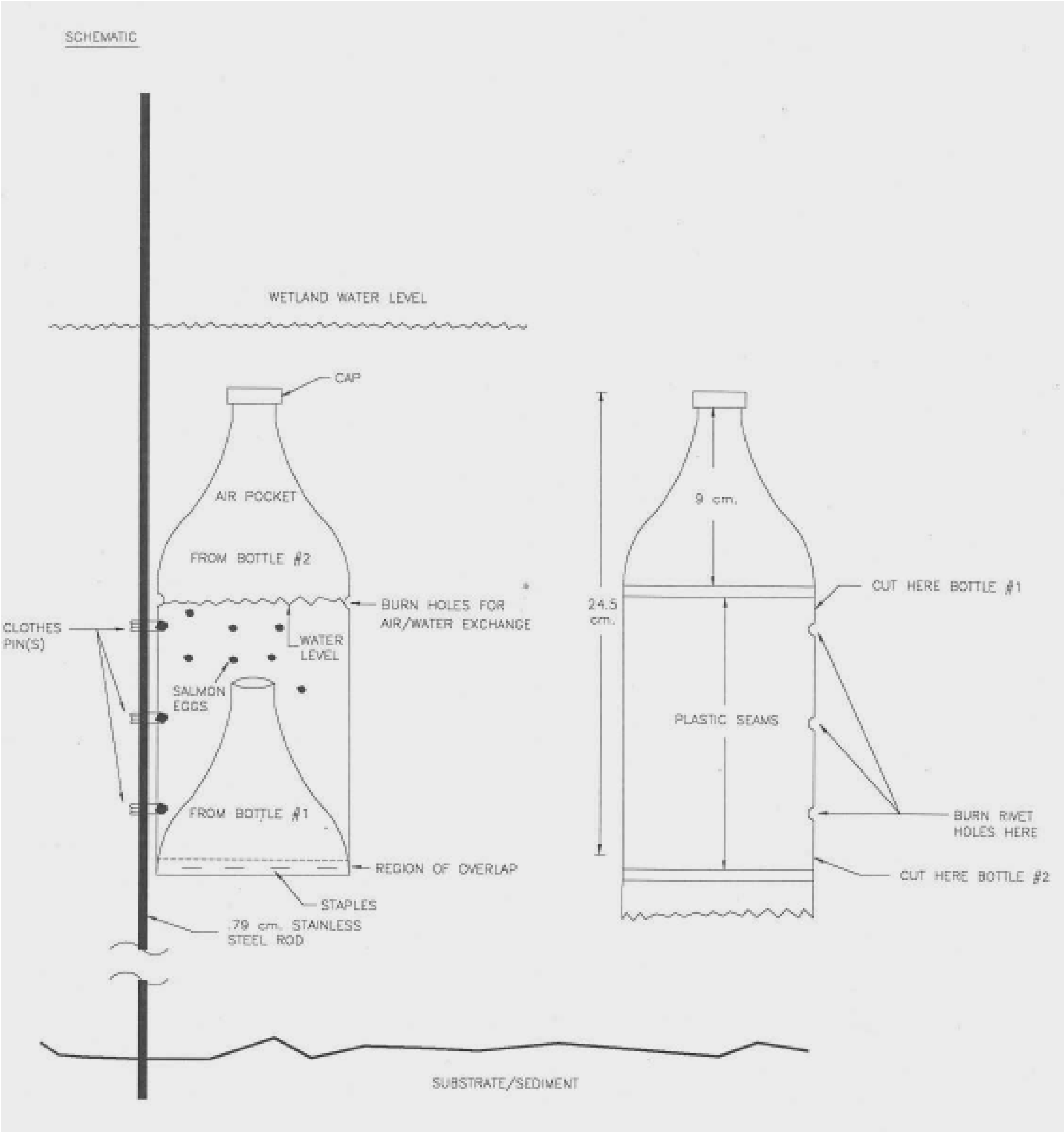

Figure 3: An economical funnel trap can be constructed from empty 2 L soda bottles.

Plate 15. Aquatic funnel trapping using recycled plastic pop bottles is one method of cheaply monitoring amphibians.
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captures by species. Taxonomic keys such as Altig et al. (1999), Petranka (1998), or several excellent regional keys (e.g. Pfingsten and Downs 1989, Hunter et al. 1999, Mitchell and Reay 1999) can be quite helpful in identification. With similar-looking species, culturing larvae through metamorphosis may be required. In other cases, voucher specimens should be preserved to ascertain identifications in the future. A second potential drawback is the interpretation of capture data. For example, what is the role of mutual exclusion/attraction among and between species (Wilson and Pearman 2000) and the impact of predation by conspecifics and other taxa (i.e., neotenes eat smaller captured larvae, Ditiscid beetle larvae destroy tadpoles, Richter pers. obs.)? Answers to these questions should be considered in the interpretation of capture data.

Because of the potential for mortality if animals are trapped too long, and species-specific differences in activity periods, funnel trapping normally should be conducted over a 24-hour period. Times shorter than this may be selected for some species. However, much longer periods may result in elevated mortality. Funnel trapping will always require at least two site visits within the sampling time span. Trapping within a variety of habitats and plant communities during breeding and larval development increases the probability of identifying all species using a wetland. Some statistical analytical methods such as power analysis (Gibbs 1993) can be used to estimate the number of trap-nights needed to provide a statistically valid sample of the population.

The number of animals caught per unit time can sometimes be used as a rough index of relative abundance, but more precise estimates of abundance require solid study designs to be sure that all assumptions required for population estimates are valid. Consequently, reliable population estimates (as contrasted to relative abundances) may be impractical. Most IBI analyses do not require the statistical precision or rigor of experimental hypothesis testing (see Module #6: Developing Metrics and Indexes of Biological Integrity). Besides, many amphibian populations exist as metapopulations and therefore show substantial variation in numbers from year to year, and presence and absence (i.e. failure to detect) data may be more reliable in reflecting changes in amphibian numbers over a broad area than absolute numbers (Fellers and Drost 1994). Presence data that are compared with expected species presence based on reliable regional guides might be an attribute for evaluating wetland condition.

There are several steps to a funnel trapping design, as outlined below. For more details see Adams et al. (1997), Fronzuto and Verrell (2000), or Mitchell (2000). Here we present enough detail to form a general understanding of the processes involved.

1. *Identify and map aquatic habitat types.* Identify and map areas characterized by water regimes, habitat classes/subclasses, and community types that occur between mean shoreline and open or deep (>1.5 m) water. Tadpoles and adult frogs seldom venture into deep or open water, where risks from predation by fishes may be greater. This mapping can be part of the overall assessment of a wetland and may be adapted to other assemblages in addition to amphibians. Habitat types vary by wetland classification (e.g., Cowardin et al. 1979, also Module #7: Wetlands Classification) and desired level of detail and may include:

i. Hydrology, especially permanently and semipermanently flooded areas

ii. Vegetation by major types of plant communities and including open areas

iii. Type of bottom material (gravel, silt, sand)

iv. Ecotones, the 1 m edge area where two different communities meet (see Richter and Adams 1997).

2. *Determine number, size, and location of sampling points.* If a priori methods to determine adequate sample size cannot be utilized, we recommend at least 10 randomly selected sampling points within each major habitat for funnel trapping.

i. Map to scale all habitat types, being certain to include 1 m wide ecotones at boundaries between habitat types.

ii. Use a planimeter or overlay scale to calculate the area (in m^2) of each habitat type, including ecotones.

iii. Divide total m^2 area of each unique habitat unit and ecotone by 10 to obtain a sample overlay grid size. Apportion these sample grids according to habitat block size.

iv. Identify sampling points within each habitat unit by sequentially sketching and numbering the total number of m^2 plots in each unit and use some method such as a random number table or random number generator to select sample points; repeat random selection until 15 sampling points are identified; the last five sampling points chosen serve as alternative contingency points. Set funnel traps at the crossing of diagonals on the map.

v. Randomly select new surveying locations for each monitoring activity every year to avoid trapping biases and to take into consideration yearly changes in hydrology and plant communities.

For wetlands with very simple structural complexity, sampling points can be randomly selected without regard to cover types. Start at a set point along the wetland's edge. Use a random numbers table or generator to obtain a fixed unit of distance in meters to space the funnel traps. A second random number may be used to determine whether the first trap is set to the left (e.g. even number) or right (odd number) of the set point.

3. *Sample the population.* Surveys should take place during comparable times and conditions of animal presence and development. For example, choose similar conditions of water depth, temperature, and initial set time (morning or afternoon) to standardize efforts. A two-person crew (one for placement, the other for carrying funnel traps and assisting) is essential for trapping; three persons are better. More than 100 traps generally require a 4-person crew. In hot weather, reduce trapping time to 18 hours to reduce mortality; set traps in the afternoon and remove them the next morning. Funnel traps should be set so that there is an air space at the top for breathing. They may be marked with brightly colored flagging and set about 2 m in a specific direction (e.g., north) of each trap to reduce the risk of predation. Lay traps on the substrate or diagonally attach to dowels with openings near the substrate. The sample data sheet for funnel traps (Appendix A) provides some ideas for data collection and recording.

4. *Egg surveys.* Counts of egg masses have served as another source of information on species

breeding in wetlands and their relative abundance (e.g., Richter and Ostergaard 2001, Crouch and Paton 2000). Unlike call counts that identify species using wetlands but do not provide data on breeding effort (see below), egg searches confirm breeding of amphibians and, with some training, provide a repeatable index to relative breeding effort (Richter and Ostergaard 2001). The abundance and health of eggs also may serve as indices of wetland and population health, in that a discrepancy between numbers of adults and eggs or an abundance of parasitized or diseased masses could denote less than optimal conditions (Plate 16).

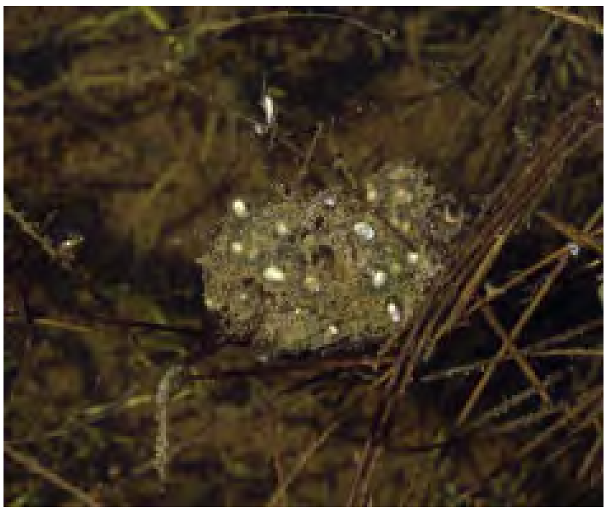

Plate 16. Abnormally high percentages of dead eggs within originally healthy egg clusters indicate potential problems with wetland conditions.

Although there may be some problems in identifying the species of egg masses where several species of the same genus are breeding concurrently, most egg masses can be identified by their shape, size, oviposition depth, substrate, and breeding chronology. Egg masses may be single small groups, grapelike clusters, or rafts; they may be free or attached to vegetation; or they may take on unusual forms (Stebbins and Cohen 1995). For example, bullfrog eggs and masses are larger than those of green frogs. Northwestern salamanders breeding in wetlands with natural conditions almost exclusively attach their orange-sized, clear, dense jelly egg masses to 3-6 mm diameter, thin-stemmed emergent plants and woody stems, whereas the long-toed salamanders attach their eggs in small clumps or long clusters. Consequently, they can be readily distinguished from each other (Richter pers. obs.). Breeding phenology may also separate species. In the Mid-Atlantic, southern leopard frogs (*Rana sphenocephala or R. utricularia*) breed earlier than either green frogs or bullfrogs. In the Puget Sound Lowlands of the Pacific Northwest, long-toed salamanders are the first to breed in late winter or early spring. Nevertheless, where species richness is particularly high, identification of frog egg masses or the separation of some frog egg masses from salamander egg masses may be problematic. Although some local field guides include keys to egg masses (e.g., Corkran and Thoms 1996), we are not aware of technically strong, peer-reviewed regional keys to identifying egg masses.

To get a total count, egg mass searches should be conducted soon after breeding has ceased for those species with relatively short breeding periods. For species with prolonged breeding (e.g., cricket frogs, *Pseudacris* spp., or bullfrogs), multiple searches may have to be conducted. Historical records of breeding may help to decide when to start searching, although annual weather conditions are important in initiating egg laying. In particular, rainfall often initiates breeding activity, and searches may be most productive a few days after a good thunderstorm.

There are three main zones for an egg mass survey: the *waterline*, *shallow water areas*, and the *shore zone* (Figure 4). The waterline is the physical line at which water and land meet at the time of the survey. This line will change seasonally and annually, and in some cases daily, through natural or manipulated fluctuations in water level. The shallow water zone includes all wadable water, from the waterline to a depth that can be safely walked

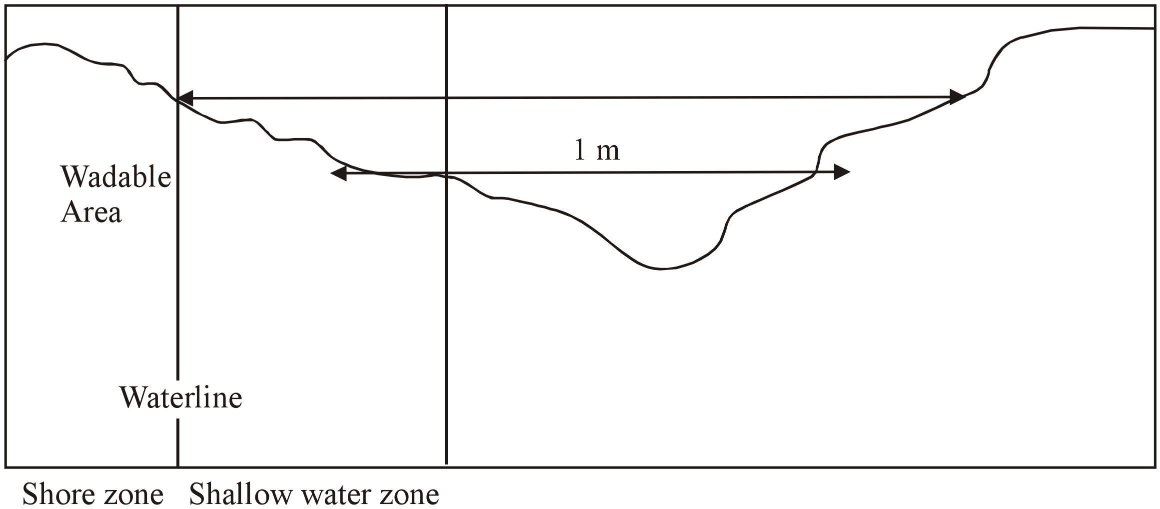

Figure 4: Side view of a wetland showing the different zones for surveying: shore zone (dry land to 3 m from water's edge), shallow water zone (to 1 m deep), and waterline (edge of water).

(usually less than 1 m deep). The shore zone is the land bordering the pond to within about 3 m of the waterline. The use of polarized glasses or an umbrella that casts shade over the immediate search area may help to see and identify eggs under water. Figure 5 shows one possible search method.

Recommended survey methods are described below for each zone; however, procedures may warrant adjusting based on site conditions. For example, a small vegetatively homogeneous wetland or flooded meadow may not readily be divisible into distinct survey zones and can be surveyed as a whole. Moreover, shoreline topography or vegetation affects accesses to or survey effectiveness of the near-shore zone, making it difficult or dangerous to survey at such locations. In some lentic habitats, deeper water (more than 1 m) can be sampled by boat or float-tube.

Search the waterline first. If the shallow water zone is narrow (less than 1 m), one person walks along the waterline scanning both the waterline and the shore zone while a second person walks in the shallow water zone about 2 m from the first. This allows both surveyors to search a 2 m wide strip. Where the shallow water zone is extensive, both members of the team can wade parallel to the waterline, one about 2 m from shore, the other about 2 m further out. We recommend that the pair stop every 5 m to more thoroughly search nearby areas and to scan ahead (see Figure 5).

Total egg counts can be accomplished in small wetlands for certain species; partial counts can be made for others. Sightings per unit area in large uniform habitats will provide a partial quantification of density. In situations where only a partial count can occur, a time-constrained sample can provide estimates on relative abundance. Search for a fixed period of time and count all egg masses identified during that time. Alternatively, the number of masses per linear distance of shoreline may be used. In wetlands with complex cover or a diverse habitat structure, counts should be in proportion to the major cover types. The method employed must be consistent across all wetlands sampled. At a minimum, identify, clearly mark, and map areas of

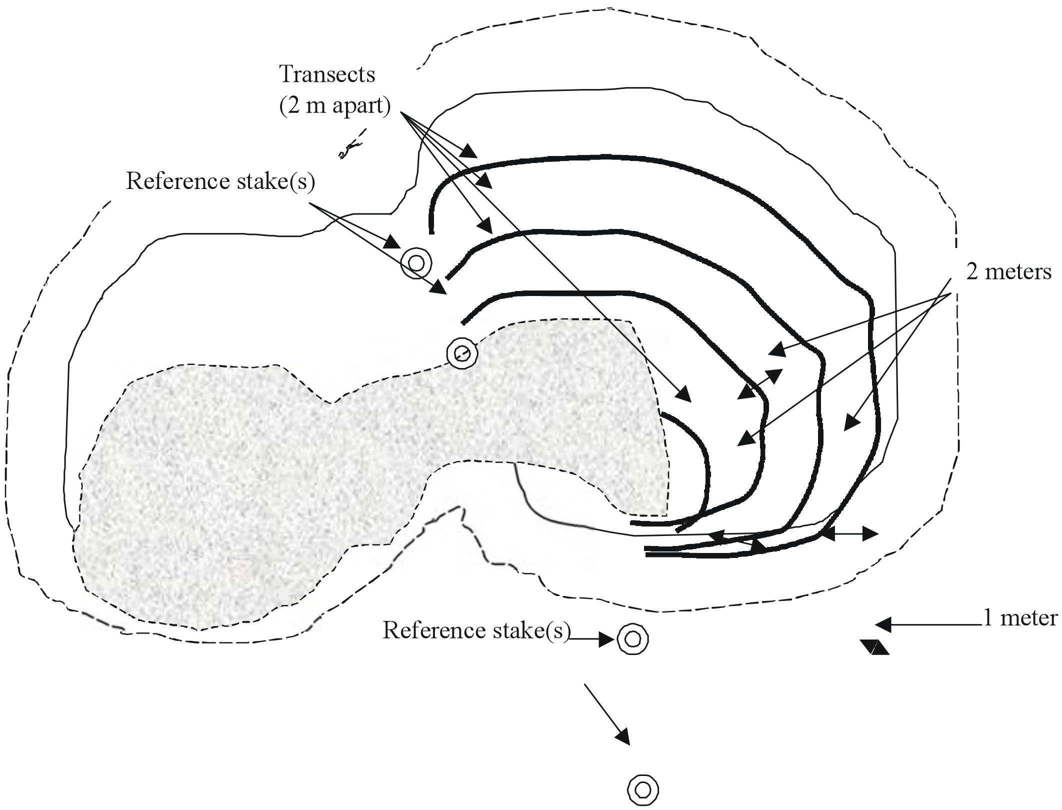

Figure 5: Top view of a wetland showing different zones for basic egg surveys.

greater concentrations. Egg masses can be marked by placing flagged dowels or poles at 2 m distance with the number of egg masses and date written on the flagging. Sometimes egg masses of different females can be laid in close proximity or even overlap each other. An estimate of the number of separate egg masses in such clusters can be made by careful inspection of the masses or by dividing the volume of the cluster by the typical volume of an individual mass.

5. *Dipnet sweeps.* Dipnetting is a fast, easy method of capturing and identifying slow-swimming amphibians and larvae in open water or along shallow shorelines within soft-stemmed emergent vegetation (Plate 17). Amphibians readily netted include most *Rana, Hyla*, *Bufo*, and *Pseudacris* species. It does not seem to be as effective on salamander adults, neotenes, or larvae as it is on anurans, although some species of *Ambystoma*, *Taricha,* and *Notopthalmus* can be collected by this method. It is inappropriate in wetlands or habitats where dense vegetation or woody debris limit visibility and inhibit sweeps.

Adult and larvae captures can provide information on the presence and health of some species. For some purposes, such as collecting metamorphs for examination of malformations, dipnetting may be a preferred method because of its efficiency. Dipnetting efforts can be standardized by consistently using the same net size (i.e., mesh and opening dimension) as well as pull length (usually 1 m), by using time-constrained sampling, and by standardizing for time of day, water depth, and microhabitat. Consequently, one can roughly determine volume of water sampled and make some judgment of density.

Plate 17. Dipnetting is a "quick and dirty" way of identifying amphibian species in wetlands. Both adults and larvae of slow-moving species can readily be netted. © Klaus O. Richter. Used with permission of the photographer.

6. *Calling surveys.* Males of many anuran species vocalize in wetlands. Sexually mature male frogs and toads call to attract mates and establish territories. Immatures may use warning and distress calls when disturbed. Consequently, vocal species can be identified by unique calls, and an approximation of their relative abundance can be determined by the number of calls heard. This information can be used to develop population trends based on the number of calls heard over repeated censuses. Such surveys can provide information on which species are breeding and a rough estimate of their abundance, but do not provide any information on the success of breeding. It is possible that adults may be attracted to wetlands that produce little recruitment because of contamination, high predator abundance, or other detrimental impacts. Call surveys are not good for salamanders, which generally are mute. Several good audio sources of frog and toad calls are available (e.g., Davidson 1995, Hunter et al. 1999) and should be consulted for species recognition. Moreover, standard procedures for call surveys have been developed by the NAAMP. We advise anyone starting a call count survey to obtain a copy of the NAAMP protocol available through their website: http://www.mp1-pwrc.usgs.gov/amphibs.html.

Typical surveys include 3 to 4 nights over the course of 4 to 6 months with a total of 1 to 2 hours per survey listening for calling frogs or toads. A typical call route has 10 stops. At each stop, several minutes are spent listening and taking notes on species heard and frequency of calling using the scoring criteria: 0 = no frogs and toads can be heard calling; 1 = individual calls not overlapping; 2 = calls are overlapping, but individuals are still distinguishable; and 3 = numerous frogs or toads can be heard, chorus is constant and overlapping. Surveys must be standardized for season, air temperature, wind speed, and occurrence of rain. If temperatures are too cool, winds too high, or rain too heavy, frog or toad calls may not be audible.

Terrestrial surveys

1. *Pitfall trapping.* Several studies have investigated ways of increasing the efficiency of pitfall trapping (e.g., Greenberg et al. 1994, Corn 1994, Dodd and Scott 1994, Enge 1997, Christiansen and Vandewalle 2000). The method is generally most appropriate for adult or juvenile amphibians. Pitfall trapping, in addition to providing a species inventory, can be combined with individual marking of amphibians to determine relative population densities, comparative estimates among wetlands, and population changes within wetlands. If an objective of a study is to obtain reliable estimates of these measures, surveys must be site-dependent and need specific multiple recapture methods and analysis (Donnelly and Guyer 1994). Consequently, they may require considerable planning and statistical expertise beyond the scope of this document.

Two trapline methodologies are suggested for descriptive rather than quantitative pitfall sampling.

The first and simpler "transect" method takes many forms but essentially is a line of pitfalls with or without drift fences. Pitfalls, which may be made with two #10 tin cans duct-taped together, plastic buckets, or other suitable containers, are buried level to the ground. Drift fences are typically constructed from plastic silt fencing and corrugated or smooth sheeting. In one configuration (Figure 6), pitfalls can be placed without drift fences at 10-, 25-, or 50 m intervals along a 100 m transect running parallel to shore. In another design they are placed at either end and in the center of a 25-50 m linear drift fence. In a third variation, drift fences with pitfalls spaced at 25-50 m intervals can completely surround small wetlands. Once installed, pitfalls are economical to operate and may last for several years in wet environments. Corrosion-resistant cans manufactured for the seafood industry are highly recommended for hydric soils and long-term studies. Pitfalls without drift fencing are suggested for wetlands with narrow (less than 10 m) buffers. Pitfalls without drift fences are also less conspicuous and may be useful in areas with heavy human visitation (and interference); however, they only collect animals that venture across the narrow gap of the trap opening. With drift fences, the effective trapping area can be increased considerably. In either case, linear transects only partially sample animals as they move into or out of a wetland, and a majority of animals may avoid being caught. Drift fences that completely surround a wetland have a greater probability of sampling more species than linear transects. In all cases, some species may have lower or higher probability of detection by remaining sedentary (e.g., some ranids), using tunnels under fences (newts), or climbing over the drift fence (treefrogs) (see Dodd 1991). Modifications of the tops of traps with plastic barriers can reduce even treefrog escapes (Murphy 1993). Clearly, the particular design will depend on study objectives and species of interest.

The second "array" method (Figure 7) captures amphibians within pitfall clusters or arrays located at randomly selected points along a transect. These may require more effort to install than linear transects because their placement and structure are more complex. The principal advantage of this design is that it samples mobile amphibians traveling in several directions rather than into or out of a wetland. Like linear formations, this method presents some bias in that the probability of detection is proportional to the degree of mobility displayed by a species; migratory salamanders and toads may have a greater probability of being sampled than more sedentary ranids (Dodd and Scott 1994).

When using pitfalls or drift fences, one should follow certain precautions: (1) The bottom of the drift fence must be directly contacting, even slightly buried below, the ground to prevent animals from crawling underneath the fence (Figure 8). In wet areas, pitfalls need to be secured in place with long stakes such as reinforcing rods hammered into the ground to prevent them from floating up. (2) The bucket or can pitfall should be topped with an overhanging lid, 6-10 cm above the top of the pitfall, to shield the interior from direct sunlight or rain. (3) A moistened, floatable sponge in the pitfall will provide moisture under most conditions and act as a raft should the pitfall flood. (4) A string suspended from the center of the lid provides an escape route for trapped mammals but also facilitates the escape of treefrogs. (5) The pitfalls must be checked regularly—daily whenever possible but certainly no longer than every 3 days (Corn 1994) to avoid dehydradation and excessive mortality. (6) The pitfalls must be sealed or inverted during periods of nonuse. (7) Pitfall transects and drift fences can be used for several years so they are most economical when studies call for repeated sampling of wetlands across time (Heyer et al. 1994).

Install pitfalls and drift fences in summer above the mean winter flood zone outside the hyporheic zone. For the transect method, lay out two 100-m transects across habitat types at each wetland and, if relevant, on opposite sides of the long axis of each wetland paralleling the shoreline. If drift fences are

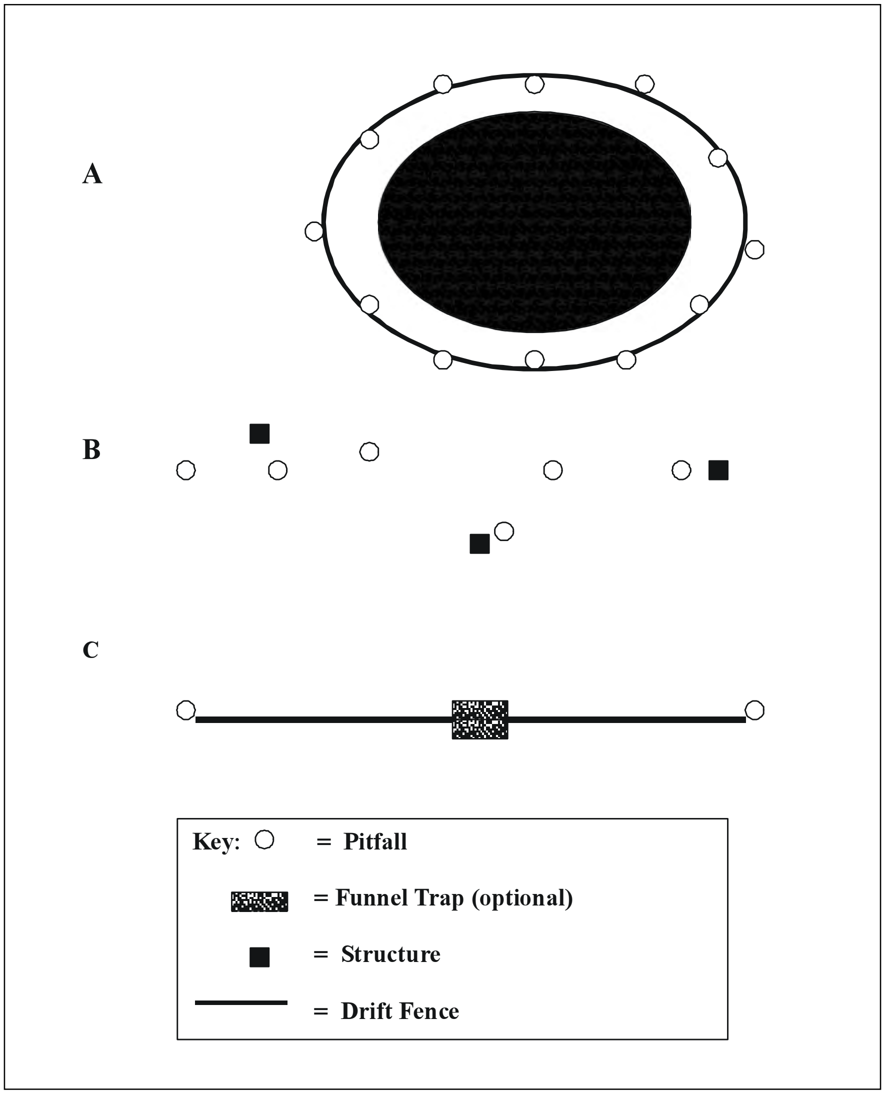

Figure 6: Pitfall traps can be placed in various configurations, with and without drift fences.

not used, excavate and install 10 pitfalls along the 100-m transect with their upper edges flush with the ground surface. Bury them within 2 m of each station, preferably next to logs, dense vegetation, and other locations likely to be catching amphibians. For short transects (50 m), pitfalls should be placed at either end and in the center of the drift fence. For encircling designs, pitfalls can be placed in pairs every 25 m on both sides of the drift fence. Alternatively, a terrestrial funnel trap made from window screen or other similar material can be placed on either side of the drift fence. Be sure to record if the animal was caught from the inside or outside of the drift fence; most researchers place the animal on the opposite side of the drift fence from which it was caught (e.g., Dodd and Scott 1994). For the array design, randomly select the starting point for each of the first of 10 trapping stations consisting of 3 arrays. Each of the 10 points is used for installing the central pitfall trap location of a 3-trap drift fence array (Corn 1994) (Figure 7). Locate the next two central traps at 10-m intervals along the perimeter baseline. Randomly rotate the "spokes" of each array by spinning a dial or with some other method. Install 0.5-m-high drift fences between traps.

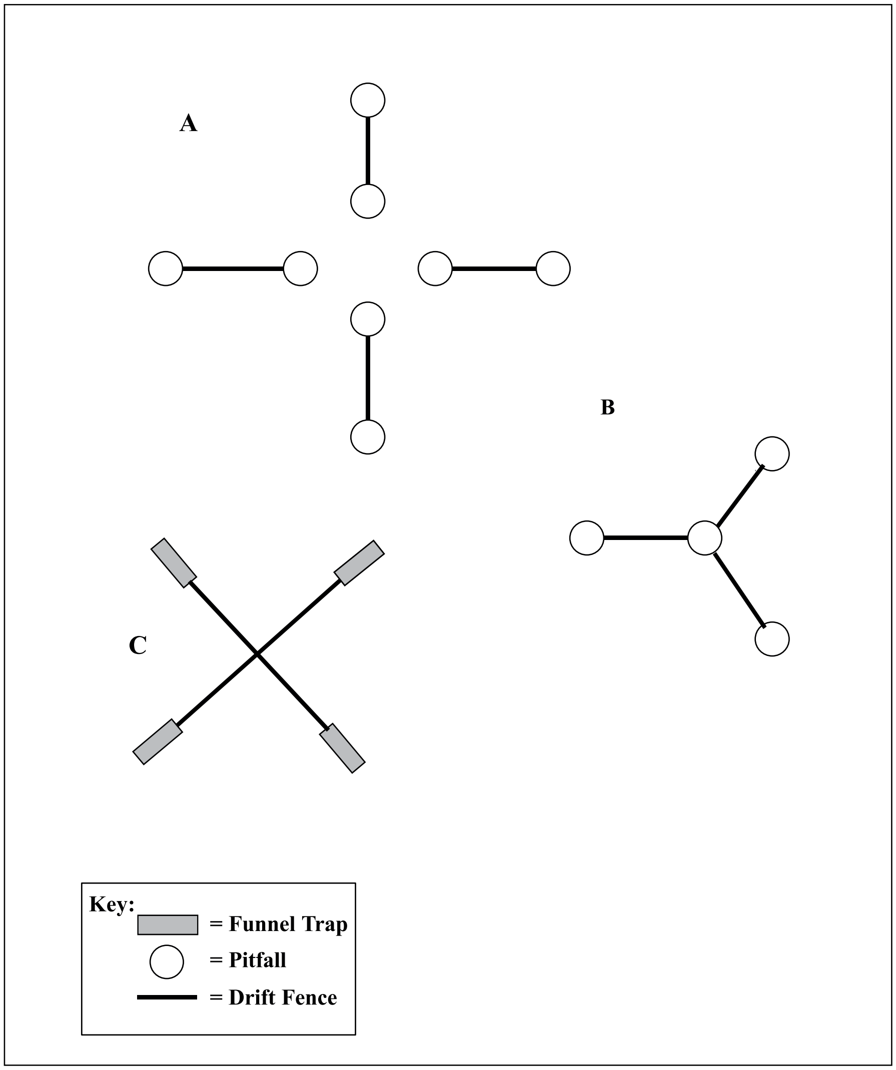

FIGURE 7: ARRAY CONFIGURATIONS OF PITFALLS ARE ALSO FLEXIBLE AND CAN BE ADJUSTED TO PARTICULAR NEEDS.

Ideally, repeated censuses during spring are recommended to maximize capturing the diversity of species migrating from upland hibernation habitats to wetland breeding sites. Drift fences are most efficient when survey plans call for repeated inventories of specific sites. Because of the labor involved, they may not be economical for one-time events. Census should commence at similar periods of yearly weather conditions. Amphibian movements are inhibited by freezing night-time temperatures and enhanced by warm temperatures in the 40's with rainy nights. Therefore, sampling periods may vary slightly from year to year based on annual phenology. Late winter censuses should be established for ambystomatid salamanders and early breeding anurans; early spring for tree frogs, bufonids, and some ranids; and mid-spring to early summer for other ranids. For example, in the Mid-Atlantic States, salamanders begin breeding in late January and February; grass frogs (*R. pipiens* complex), pickerel frogs (*R. palustris*), toads, and chorus frogs in March-April; and gray treefrogs (*Hyla versicolor*), bullfrogs, green frogs, and northern cricket frogs (*Acris crepitans*) from May into July. Of course, knowing regional variation in times is critical for maximizing the efficiency of trapping.

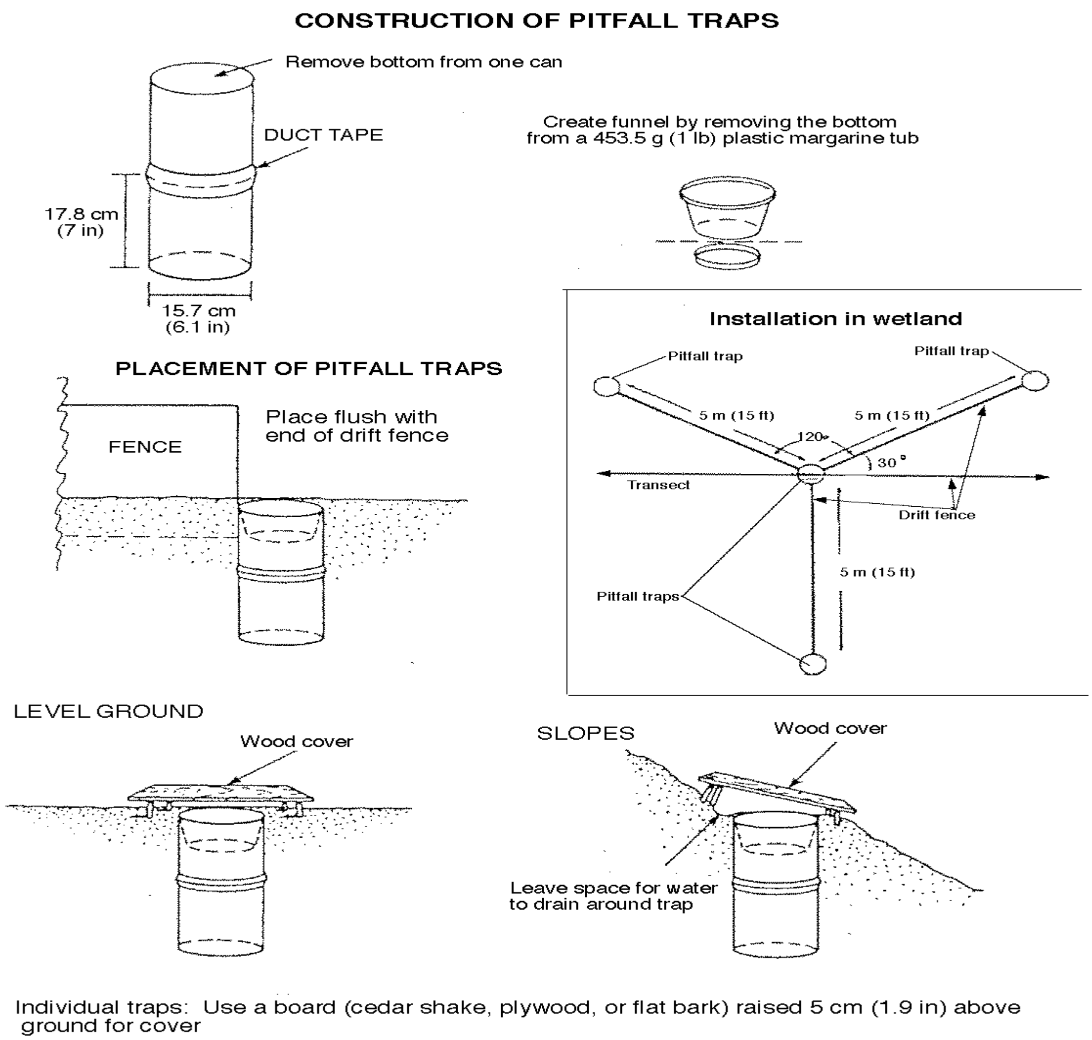

Figure 8: The proper setting of pitfall traps involves burying the pitfall to its lip, burying the drift fence at least slightly into the ground to prevent amphibians from crawling underneath, and careful placement of a lid over the pitfall to prevent excessive exposure to the elements.

Traps can be opened and closed as favorable weather permits for a total of 14 trap nights during each period. See Appendix B: Amphibian Pitfall Trapping Data Sheet for an example of how to record data.

2. *Artificial cover.* Studies that have evaluated sampling amphibians with artificial cover such as cover boards and other devices include Fellers and Drost (1994), Moulton et al. (1996), Bonin and Bachand (1997), and Monti et al. (2000). Cover boards are flat sheets of board such as half sheets of plywood or fencing that make use of the natural tendency of most caudates to seek shelter under flat objects during the day. They are useful in determining the presence and abundance of salamanders in wetland buffer and adjacent habitats, but are not very suitable for sampling anurans, although occasionally dispersing frogs and toads may

also be found by this nondestructive method. A general procedure is to place cover boards at random locations, but in proportion to habitat types along transects on a forested or grassland floor, and periodically lift the covers up and count the numbers and species of amphibians living there. Captures are released at the edge of the board so they can crawl back under. Monthly surveys in damp, cool spring and autumn weather adequately determine the presence of terrestrial breeders, provide indicators of densities, and establish ongoing habitat use. Be aware that snakes also use these artificial covers. Another form of artificial cover that is attractive to some amphibians is 5 cm diameter, 1-1.5 m high pipes made of polyvinyl chloride (PVC) pounded into the ground. Sometimes referred to as "canopy traps," these provide relatively safe and attractive shelters for tree and chorus frogs.

Field and Laboratory Methods of Assessing Health

Field methods

Eggs surveyed and animals captured in the field can be evaluated as to their health by visual observations and by several quick measurements. Abnormal egg mortality or the inability of larvae to hatch (escape) from eggs should be noted, as such traits may be indicators of high pH or chemical pollutants, injuries and sores, and abnormal coloration. Clearly, abnormal growths, multiple limbs and digits (fluctuating asymmetry), and other malformations are instant indicators that something with the animal and/or population is not normal and should be carefully recorded and if possible saved for pathological analysis. More importantly, as mentioned previously, if such incidences occur, they should be carefully documented in a standardized manner (Palmer 1994) and submitted to the NARCAM reporting center. Similarly, lethargy, poor or unnatural swimming or jumping ability, and other more subtle behavioral "abnormalities" can immediately suggest that something is amiss, should be carefully noted, and may warrant further assessment.

Standard counts and measurements often taken as part of regular field surveys may also suggest that a given animal and/or population is unhealthy. Animal lengths and weights that are outside normal ranges for the species or population may suggest wetland or upland perturbations, although predation, competition, and other density-dependent factors play an important role. Nevertheless, even these density-dependent influences can suggest abnormal environmental conditions. An index of health combining several measurements into one, such as described in Wilbur and Collins (1973), is another useful way of quickly evaluating the health of amphibians in the field. Total length and snout-to-vent-length of the animal can be measured readily with a small millimeter ruler or calipers (Plate 18). Weight can be determined by placing the animal in a small, thin plastic bag and using a Pesola spring or other appropriate scale. Standard techniques for such measurements are provided in Nussbaum et al. (1983) and other advanced field guides or in Petranka (1998) for caudates. More complex data manipulation and summary statistics of length, weight, and other readily measured data may be more suitably analyzed back in the laboratory or office.

Lab-assisted methods

Although several laboratory tools may be useful in assessing the health of individuals, these usually involve technical methodology and instrumentation

Plate 18. Measuring lengths of amphibians, such as bullfrog larvae, may provide an indication of yearly growth.

that can be costly and time-consuming. Some require sacrificing animals, others are invasive but nonlethal, and a few are noninvasive. Thus most laboratory investigations should be considered on a higher order or tier than routine bioassessments. If, after an initial investigation, it is determined that an amphibian population or community may be in poor health, these techniques can be valuable in determining the cause(s) of the problem so that appropriate remedial actions can be taken. Some of the methods also may be used to screen amphibian communities for adverse effects in landscapes where specific physical or chemical problems have been identified. The laboratory techniques summarized below have worked well with other vertebrate classes such as fish, birds, and mammals, but have not been used as extensively in amphibians and may require some adjustments for optimal results. This review is not intended to be comprehensive; rather its purpose is to increase awareness of some of the tools that are available.

The cost of such analyses can be expensive compared with other methods, and we have provided a rough guide for having the analyses conducted by contracting labs; obviously, direct costs might be reduced if one has in-house capabilities. Costs per sample are: $ = less than $50; $$ = $50-200; $$$ > $200.

Cholinesterase determination—$ to $$. Acetylcholinesterase (AChE) is an enzyme that blocks the action of acetylcholine at the synapse between two neurons and helps regulate the central and peripheral nervous systems. Organophosphorus pesticides including organophosphates (e.g. diazinon, chlorpyrifos, methyl parathion) and carbamates (e.g. carbofuran, methiocarb, aldicarb) work by binding with AChE and blocking its inhibition of acetylcholine. In target organisms (i.e., insects) and, unfortunately, in lethally exposed nontarget organisms (e.g., amphibians exposed to agricultural runoff or to broadcast spraying), death usually occurs because of nervous system failure leading to respiratory failure. Sublethal and recent lethal exposures can be detected by comparing AChE activities between suspect animals and those coming from a reference population. At least in birds, AChE activity depressed by ≥50% is forensic evidence that animals have been exposed to organophosphorus pesticides (Hill and Fleming 1982). There are also methods that promote reactivation of inhibited AChE and, by comparing activity levels before and after application of reactivation techniques, help determine if specific animals have been exposed to pesticides. Studies that have used cholinesterase inhibition to determine pesticide exposure in amphibians include Guzman and Guardia (1978), de Llamas et al. (1985), and Sparling et al. (2001). The most reliable tissue for AChE determinations is brain, because it shows the least amount of variance among individuals collected from reference sites. In larger amphibians, AChE can be measured in plasma without sacrificing animals, but in small amphibians such as most tadpoles, entire bodies less the gut coil may have to be used. Spectrophotometric measurement of AChE is relatively straightforward (Ellman et al. 1961, Hill and Fleming 1982), especially with multiwell spectrophotometers (Hooper 1988).

Fluctuating asymmetry—$. Under optimal conditions an animal may be expected to develop with minimal defects or teratogenic effects. As conditions deteriorate, for any of a number of reasons, developmental processes may be affected, resulting in quantifiable abnormalities. In many cases these abnormalities may affect the two halves of bilaterally symmetrical organisms unequally. In extreme cases, these abnormalities may be readily apparent, such as the widely publicized amphibian malformations. The concept of fluctuating asymmetry (FA) proposes that the quality of habitats can be assessed by comparing bilaterally paired morphological parameters. Greater differences in morphology indicate greater stress on developmental processes (Palmer and Strobeck 1986, Bailey and

Byrnes 1990, Leung and Forbes 1996). However, FA may respond to many different kinds of stressors, and the method may not identify specific causes for the difference in morphology. The method relies on very careful measurements of paired body parts such as length of hind feet or distance from one joint to another. Any difference due to injury or predation must be discarded. In theory, FA can be used on live animals, but if the subjects are very active, they may have to be quieted through sedation to achieve precise measurements. Palmer (1994) provides instructions for performing this analysis.

Flow cytometry—$ to $$. Exposure to certain clastogenic (i.e., mutagen-inducing) contaminants such as polycyclic aromatic hydrocarbons (PAH, e.g., benzene, anthracene) can be detected by using flow cytometry. Its principle is based on the observation that exposure to such chemicals can cause chromosomal damage, which causes variation in DNA content among cells. A flow cytometer (Otto and Oldiges 1980) is an automated, precise method of quantifying this variation in DNA content. The method has been used on birds and fish (Easton et al. 1997), turtles (Lamb et al. 1991, Bickham et al. 1988), and mammals (McBee and Bickham 1988), and has been proposed for amphibians (Bishop and Martinovic 2000). The technique uses a small piece of tissue, perhaps the tip of a salamander or tadpole tail or a digit from a frog taken during toe-clipping, and thus does not sacrifice animals. The method will not identify the specific cause for DNA variation, but can facilitate forensic investigations. Methodology is explained in Deaven (1982) and Otto and Oldiges (1980).

Blood elements and chemistry—$ to $$$ depending on analyses. The list of diagnostic factors that can be obtained from a sample of blood is extensive. They include the standard battery of enzymes (e.g., lactate dehydrogenase, alanine aminotransferase, hematocrit, and hemoglobin) that are often measured in routine health examinations for humans. In general, variations in these factors suggest similar health conditions such as impaired liver or kidney function or anemia. Although these factors have been used to assess health status of other vertebrates (see, e.g., Ritchie et al. 1994, Sparling et al. 1998), they have not been widely applied to amphibians. Experimental work may have to be conducted to obtain baselines and verify interpretations.

Some other plasma measurements are quite specific and point to certain conditions. For example, γ-aminolevulinic acid dehydratase (ALAD) and porphyrins can be used to assess exposure to lead in amphibians (Vogiatzis and Loumbourdis 1999, Steele et al. 1999). Elevated metallothionein activities can indicate exposure to other metals such as cadmium or zinc (Vogiatzis and Loumbourdis 1998). A high frequency of red blood cells with small nuclei can indicate exposure to clastogens (Fernandez et al. 1989). For descriptions of other bioindicators, see Huggett et al. (1992).

General Comments on Care and Preservation

All appropriate care and attention should be considered whenever handling live amphibians. Specific care recommendations are provided in the joint animal care and use committees of the major herpetological societies (Anonymous 1987). If tadpoles are to be examined in the field or laboratory and then released, they may be transported with pond water in 4 L or larger containers that have been rinsed with commercial bleach and distilled water to deter transmission of disease. If possible, carry ice in a cooler and use it to keep the water from becoming too warm. Containers should always be kept in a cool, shaded spot. For adults, soaked paper towels or sponges in a bucket with a tight-fitting lid are preferable to standing water because adults can drown. Handle the animals as little as possible, place inspected animals in a clean bucket, and return to the source as quickly as possible to minimize stress.

If animals are to be euthanized for purposes other than enzyme determinations, the preferred method is to overanesthetize them in tricaine methanesulfonate (MS 222) (0.5 g/L of water). The effects of MS 222 on physiological, especially neurological, processes in amphibians are unclear. When physiological measurements are prescribed, the preferred method is flash freezing in liquid nitrogen.

For most histological purposes including examination for parasite cysts, amphibians should be euthanized in MS 222 and fixed in 70% ethyl alcohol (10:1 ratio of alcohol to specimen volume). A small slit in the abdominal wall facilitates complete and rapid fixation.

Data Analysis

General Comments

We have mentioned that rigorous statistical precision is not as critical in surveys for IBI development as it might be for experimentation and hypothesis testing (see Module #4: Study Design for Monitoring Wetlands and Module #6: Developing Metrics and Indexes of Biological Integrity). Where time, expertise, and resources allow, the higher the quality of the data, the more certain are the interpretations, and it is ill-advised to cut corners if it is not absolutely necessary. We do not have the space to thoroughly cover statistical design and analysis here. Rather, we refer interested readers to sources such as Donnelly and Guyer (1994) or Hayek (1994). Consultations with good statisticians prior to collecting data are always recommended.

Possible Metrics

We are unaware of any study that has published IBIs using characteristics of amphibian communities in metrics, but there are several studies are in progress. A thorough examination of the ecological literature on amphibians, such as an upcoming literature survey by Paul Adamus of Oregon State University, would undoubtedly provide excellent leads for metric development in certain species. For example, Boward et al. (1999) reported that some amphibian species were not found along streams in areas with more than 3% impervious surface in the watershed basin, others disappeared at levels greater than 10%, and still others survived up to 25% impervious cover. Richter and Azous (1995, 2001) and Thom et al. (2001) found that species richness correlated to increasing water level fluctuations, which were attributable to greater runoff into wetlands because of increasing proportions of impervious surfaces in urban habitats. Pechmann et al. (1989) found a relationship between wetland hydroperiod and the species diversity and abundance of metamorphs. In a review of the literature, Sparling (1994) showed a clear relationship between pH and the tolerance of 26 species of amphibians. Many more examples can be gleaned from research publications. The following should be considered as potential metrics or attributes based on amphibian ecology, distribution, and sensitivity to anthropogenic stressors.

Suggested Attributes

1. *Species richness.* The diversity of amphibians in a given wetland is often much smaller than that of other taxa traditionally used in IBI development, such as plants, fish, or invertebrates. A very diverse region may have 20 endemic amphibian species (McDiarmid and Mitchell 2000), with only a fraction expected to inhabit any given wetland. In comparison, there may be far more than 20 inver-

tebrate or macrophyte species inhabiting wetlands in even fair condition. Thus, species richness may not be as useful a metric for amphibians as it is for other assemblages. Because species distributions show marked regional differences, the choice of specific metrics also may be regional. What works for New England, for example, may not apply to the Mid-Atlantic States.

2. *Comparison of species presence/absence with regional taxonomic lists*. There is an abundance of published State, regional, and national taxonomic lists of amphibians for many parts of the country. These lists can provide information on the species that might be expected to inhabit a wetland. One attribute could be a ratio of the number of species actually found to the number possible. A depauperate fauna would yield a low score whereas a diverse community relative to regional distributions would have a higher score. Also, the presence of rare species, especially ones that are listed for a State or the Nation, should be positive indicators of wetland health, whereas the absence of a ubiquitous species may signal problems. Because of the large annual variations in amphibian populations, an absence one year might not mean an absence in subsequent visits.

3. *Proportion of nonindigenous species*. Nonindigenous or invasive species generally are characteristic of impaired wetland conditions. In the South, the cane toad (*Bufo marinus*) may be a pest species, and there is some concern that introduced bullfrogs cause problems for indigenous species of anurans in the West (Lawler et al. 1999).

4. *Frequency of malformations*. Malformations in anurans take many forms. In addition to missing or extra hindlimbs, there are malformations of the mouth, extra webbing, body coloration, digits, and unabsorbed tails. Information on what to look for can be obtained at the website for the North American Reporting Center for Amphibian Malformations (NARCAM) http://www.npwrc.usgs.gov/narcam/. A 2% rate of deformities among metamorphosing frogs is considered acceptable; rates much higher than that may indicate serious problems in wetland health.

5. *Evidence of mass mortality*. Certainly, observations of large numbers of dead and dying frogs or tadpoles should be taken seriously. Such mortality demands further investigation.

6. *Number and condition of egg masses.* The number of egg masses and some indication of their status (i.e., percent of eggs with fungus, percent showing no embryonic development) should provide good estimates of amphibian breeding conditions. However, each wetland may have to be visited repeatedly to capture data across the breeding periods of several species, or to record data from species such as green frogs or bullfrogs that have extended breeding seasons.

7. *Ratios of the relative abundances of different life stages*. By repeatedly visiting a site and using different data collection techniques, an assessment team may be able to obtain information on the relative abundances of breeding adults, egg masses, tadpoles, and metamorphs. Ratios of these life stages may reveal information on population status. For example, wetlands with abundant breeding adults but very low abundances of subsequent stages could indicate a population sink.

8. *Percent tolerant and intolerant species*. What defines a tolerant or intolerant species depends on the stressor involved. Species with small tadpoles (e.g., northern cricket frog) may be less tolerant of predacious fish than those with tadpoles that can grow larger than the mouth gape of these fish (e.g., bullfrog). Sensitivity to contaminants varies across species and type of contaminant (Sparling et al. 2000). Some amphibian species are much more tolerant of habitat destruction than others. Sensitive species often have a very narrow set of habitat requirements whereas tolerant species tend to be generalists. Thus, a list of tolerant and intolerant

species should be developed by region based on the likelihood of encountering specific stressors. Wetlands in good condition would reasonably have a greater preponderance of intolerant species than highly impaired wetlands.

9. *Mensural characteristics*. In general, larger body weights relative to body lengths indicate good health. Thus, snout vent length (SVL) divided by body mass (or the square root of body mass because SVL is linear whereas mass is related to volume) by developmental stage and sex of adults may be useful information. Of particular interest is the ratio for recently metamorphosed individuals, because body size of metamorphs is related to survival (Wilbur and Collins 1973).

10. *Proportion of neotenic and metamorphosed adult salamanders*—Some metapopulations of salamanders show variation in the ratios of neotenic and metamorphosed adults. These forms are readily distinguished based on the presence of external gills in neotenes and sometimes color differences.

Some attributes can be very informative but may lie outside of routine bioassessments owing to the level of technical expertise or cost required. These include:

11. *Percent of individuals parasitized or diseased by trematodes, chytrid fungus, or iridoviruses*

12. *Presence and concentration of contaminant residues in bodies*

13. *Specific bioindicators (ALAD, hematocrit, etc.)*

See discussion under field and laboratory techniques.

Limitations of Current Knowledge and Research Needs

Current Knowledge

A few species of amphibians, especially northern leopard frogs and the African-clawed frog (*Xenopus laevis*), have been extremely well studied in terms of understanding vertebrate physiology. However, less research has been conducted on the ecology, habitat requirements, and limiting factors of amphibians than on mammals, birds, or fishes. Thus, there are many data gaps in our understanding of amphibian ecology. Obviously, we cannot summarize everything known about amphibian ecology and limiting factors, so here we direct readers to a few sources of information.

Major sources of information

Excellent compendiums have been written on amphibian environmental physiology (Feder and Burggren 1992), general biology and physiology (Duellman and Trueb 1986), natural history (Stebbins and Cohen 1995), and ecotoxicology (with reviews of ecology, physiology, population declines, diseases, and other issues, Sparling et al. 2000). Some of the information in these sources is somewhat dated, but they provide good starting points for understanding amphibians.

An eclectic mix of topics and associated scientific publications includes research on amphibian ecology has focused on understanding proximate factors driving community structure, including interspecific competition, predation, hydroperiod (Connell 1983, Sih et al. 1985, Hecnar and M'Closkey 1996c, Hecnar and M'Closkey 1997a, Chivers et al. 1999), presence of predatory fish (Sexton and Phillips 1986, Chivers et al. 1994, Hecnar and M'Closkey 1997b), exotic species (Lannoo et al.

1994), local environmental toxins such as nitrate or heavy metals (Berger 1989, Baker and Waights 1994), and water chemistry (Dale et al. 1985, Glooschenko et al. 1992, Rowe and Dunson 1993). Studies are typically short-term; emphasize local processes using seminatural field, laboratory, or mesocosm experiments; and often target larval stages (Morin 1983, McAlpine and Dilworth 1989, Biesterfeldt et al. 1993, Werner 1992, Skelly 1995, Anholt et al. 1996).

Limitations of Current Knowledge

Often information on species distributions is not complemented with long-term population data and information on life histories (Mossman et al. 1998). As a result, virtually no information exists on large-scale changes in the incidence of amphibians (Hecnar and M'Closkey 1996b). The North American Amphibian Monitoring Program was initiated in 1994 to address this gap.

Amphibian declines and malformations have not been easily explained. Some declines are tied to local phenomena whereas others are currently unexplained. Synergistic effects of environmental factors coupled with potential species-specific sensitivities further cloud the understanding of cause and effect. More research on individual species sensitivities is needed (Hecnar and M'Closkey 1996c). Lack of information on natural population fluctuations (Pechmann and Wilbur 1994,) coupled with a difficulty in distinguishing between true population declines and regional responses to landscape changes, makes assessment of species status difficult.

Whereas much current research has led to a greater understanding of some of the proximate factors that shape amphibian community structure, regional patterns of species diversity may not be understood adequately by reference to local conditions. An increasing number of studies suggest existence of metapopulation structure in amphibian species (Harrison 1991, Sjögren 1994, Bradford et al. 1993, Hecnar and M'Closkey 1996c). Thus the persistence of species within a wetland may be determined in large part by the dynamics of extinction, colonization, and migration at larger scales (Gilpin and Hanski 1991). Landscape attributes such fire, deforestation, and other land-use history (deMaynadier and Hunter 1995); current land cover including forests, agricultural fields, and developments (Richter and Azous 1995, Vos and Stumpel 1996, Kolozsvary and Swihart 1999, Skelly 1999, Werner and Glennemeier 1999); and wetland density and distribution patterns (Gibbs 1993, Semlitsch and Bodie 1998, Gibbs 2000) are important factors in regional species diversity and distribution patterns. Therefore, using amphibian species diversity or abundance in any one wetland as a surrogate for wetland health or as a tool for assessing the status of that species can be misleading without some knowledge about what is happening in adjacent wetlands. Colonization and extinction events may be quite frequent in one wetland within a given suite of wetlands that as a whole is stable for any given species (Hecnar and M'Closkey 1996a, Skelly 1999).

Important Research Gaps

We have enough information to begin using amphibians in bioassessments. However, to improve our capabilities to diagnose wetland condition through amphibian communities, we need to increase our knowledge of certain key areas and learn how to apply that knowledge to these bioassessments. Some areas that are most in need of further study are discussed below.

Expanding from local- to regional-scale assessments of amphibian populations. To assess the biological integrity of wetlands, researchers and land managers will have to look beyond the individual wetland using a multimetric approach *and* a multiscale approach (Hecnar and M'Closkey 1996b). Specifically, using amphibians as indicators of individual wetland health demands information on regional distribution patterns, life histories, and species stability based on this expanded perspective. This requires a solid base of reference wetlands within a region. Gibbs (1993, 2000) also emphasizes the necessity of evaluating wetland resources as a mosaic rather than as isolated entities. He found that as human populations shift from rural to urban landscapes, wetland spatial patterns go from many clustered wetlands (2-5 wetlands/km^2, 0.2-0.4 km apart) to fewer, more isolated wetlands (<1 wetland, >0.5 km apart). Gibbs predicted that wetland mosaics could withstand only modest losses and still provide wetland densities that are minimally sufficient to maintain wetland biota. Average dispersal distance is generally <0.3 km for frogs, salamanders, and small mammals (Gibbs 1993, Semlitsch 1998, Semlitsch and Bodie 1998). In Gibbs' model, wetland mosaics characterized by <1 wetland per km^2 and >0.5 km from other wetlands were not able to sustain metapopulations of wetland-dependent animals.

Gallego-Fernandez et al. (1999) looked at the effects of a 60% wetland loss in the past 50 years in Spain and reported a homogeneous, coarse-grained landscape characterized by low diversity and high dominance. Hecnar and M'Closkey (1996a) studied species richness patterns of amphibians in 180 ponds in southwestern Ontario. They emphasized the importance of a geographic perspective on the occurrence of amphibians. The high population turnover within the study area at the level of individual species showed the transience and spatial dynamism of amphibian populations even during a short time period. The researchers concluded that the pattern of species richness is more of a regional than subregional (or watershed) characteristic and that the most important processes structuring amphibian communities operate at the regional scale.

Regional species-specific information on tolerances and adaptability to specific disturbances. More information is needed on the tolerances and adaptability of each species to specific environmental factors so that their presence/absence or abundance in wetlands can be interpreted more reliably (Richter and Azous 1995, Skelly 1996, Hecnar and M'Closkey 1998, Skelly 1999). Individual species responses are variable depending on lifestage, geographic region, and type of disturbance (urbanization, agriculture, forestry). Some information is available that suggests, for example, that wood frogs and spotted salamanders (*Ambystoma maculatum*) rapidly disappear from agricultural lands (Minton 1992) and are sensitive to percent of forested habitat surrounding breeding pools (deMaynadier and Hunter 1995, Lemckert 1999). Kolozsvary and Swihart (1999) studied the effects of agriculturally induced fragmentation of forests and wetlands on amphibian assemblages and their distributions and observed nonrandom patterns, suggesting that amphibians respond to landscape-level attributes and that species differed substantially in the nature of their responses to fragmentation (patch size, isolation, or water regime of wetlands). Additionally, our current information is based largely on the survey approach (field observations) rather than a combination of field and experimental approaches. Hence it is often unclear what the field data are telling us other than that amphibians are observed and found under the conditions of our surveys. Such information is limited, as it demonstrates only the wide range of conditions under which amphibians are found but not necessarily their preferred conditions maximizing inclusive fitness or homeostasis. Moreover, most of our scientific data are correlational, and to improve the benefits of amphibians as bioindicators we need to establish specific cause-and-effect relationships.

Information is needed on amphibian movements (seasonal and dispersal) in different landscape settings. Several researchers (Windmiller 1996, Semlitsch 1998, deMaynadier and Hunter

1999) have studied amphibian movements in agricultural, urbanizing, and forest landscapes. More of this work, however, needs to be done.

Conclusions

- *Use of amphibians as indicators of the health of individual wetlands may be affected by their population structure.* Wetland ecosystems need to be assessed at the landscape scale if biological integrity is meant to convey functions that support metapopulations of wetland-associated wildlife. Also, many amphibian species are structured in metapopulations, and rates of local extinctions and colonization events, over a short time span, may be high. To overcome these limitations, the best studies must be broad in scope, either encompassing a large number of wetlands in an area or occurring over a span of several years.

- *Some amphibian metrics may prove useful in assessing wetland health.* Several possible attributes that can be developed from community structure, sensitivity to perturbations, malformations and growth rates, and other aspects of amphibian physiology and ecology. However, none of these have yet been tested in an IBI. Thinking regionally rather than universally will enhance the likelihood of successfully identifying amphibian metrics. An assessment of a wetland in Oregon that finds that bullfrogs are dominant would likely conclude that the wetland is degraded, whereas different interpretations could be valid in the Eastern United States where bullfrogs are native.

- *We are still in exploratory stages and should proceed to investigate amphibians as a useful taxon for monitoring the biological integrity of wetlands with care.* However, of all the vertebrate classes closely associated with wetlands, amphibians probably give us the best opportunity to develop bioassessments of landscapes in which wetlands play an important role. Knowledge of the natural history of the amphibians in an area can lead to judicious selection of attributes and species to monitor that ultimately may tell the investigator a lot about the wetlands in a particular landscape.

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Appendix A.
Sample Data Sheet for Funnel Traps

Wetland Name/ID Number: ______________________________ Survey Date: ______________

Biologists: ____

Date and Time Traps Were Removed or Checked: ____

Air Temperature (°C): ____________________ Water Temperature (°C): __________________

Weather: Clear Overcast Rain Snow

NOTE: Each individual is recorded on a single line of the table. Trap Ids, Habitat Types, and Species need to be entered only when they change.

Trap ID	Habitat Type	Species	Stage	Sex	SVL (mm)	TL (mm)	Mass (g)	Remarks

Appendix B.
Amphibian Pitfall Trap Field Data Sheet

Wetland Name/ID Number: ____________________ Survey Date: ______________________________

Habitat Type: ______________________________ Biologists: _________________________________

Habitat Unit: ____

Sampling Station: ____

Replicate Number: ____

Air Temperature (°C): ______________________ Water Temperature (°C): ___________________

Weather: Clear Overcast Rain Snow

Wind: Calm Light Strong

Soil Surface Condition: Dry Damp (dew) Saturated

Habitat Type	Habitat Unit	Transect Number	Species	Sex	Stage Neonate Juvenile Adult	SVL (mm)	TL (mm)	Mass (g)	Injuries