Chapter 6

Standard Techniques for Inventory and Monitoring

Selection of techniques

In this book we recommend 10 standard techniques that can be used for inventory and monitoring projects. Here we provide basic guidelines for selection among them. We emphasize that the questions being asked by the investigator will determine which technique or techniques are selected. Therefore, the first step in any project is definition of the research question(s) and identification of the kind(s) of information required to answer it.

Questions concerning amphibian biodiversity basically fall into two broad categories: (1) those related to habitats, sites, or areas and (2) those concerned with species or assemblages. The primary goal of habitat- or area-based questions is to inventory the species that occur in habitats or areas at a specific site. In some instances, a site will be visited only once. In most cases, however, if a complete species list is desired, a site must be visited several times because it is unlikely that all species occurring at the site will be encountered during a single sampling session (particularly in species-rich tropical assemblages).

Species-based studies may focus on one or more populations across space or over time. In the former instance, the goal is to determine the geographic distribution (e.g., counties in a state, states in a country, or countries) or the ecological distribution (e.g., habitat or microhabitat types) of a species. For such spatially oriented studies, an investigator may select an inventory method

appropriate for use across several habitats in a region or between regions.

If the study focuses on a species or species assemblage over time, then the goal is to determine the status of each species at a site and to look for population changes. In this instance, the species or assemblage is sampled several times (monitored) over a suitable period (e.g., years). If the goal is to determine general trends in the status of species (e.g., worldwide), then it is necessary to monitor many sites.

Amphibian biologists are likely to concentrate on organisms, that is, a single species, several species, or an entire assemblage of amphibians. Others will use the techniques in this book to determine amphibian use of specific habitats. For example, resource managers (e.g., persons in charge of parks, wildlife refuges, or federal or state lands) may be interested in the impact of successional or other habitat changes on amphibian species or on total amphibian biodiversity. Based on what is discovered during the inventory process, the investigators could decide that particular species (e.g., those that are endangered or suspected to be in decline) should be monitored.

Once an investigator has identified the question(s) and the kind(s) of information required, several important points must be considered prior to selecting from the 10 techniques. Foremost among these points is the biology of the amphibians targeted for study (e.g., are they aquatic, fossorial, or arboreal; are they prolonged or explosive breeders; and do they have aquatic larvae?). Time, funds, and number of field personnel available for the work also are of major importance in selecting techniques, as are the complexity of the habitat, the diversity of the fauna (e.g., number of species), and the size of the area to be studied. We have evaluated each of the techniques (Table 4) according to the amount of information it supplies, the time and number of persons required for its implementation, and its relative cost. For example, if the goal of the project is to determine the density of a species, then techniques 4, 5, 6, and 10 of Table 4 should be considered.

The values given in Table 4 change if the area being covered is large or if mark-recapture methods are used. For example, if an investigator uses a visual encounter survey (technique 2) and mark-recapture techniques to sample amphibians in a given area, then information gained will increase because density estimates as well as relative abundance and species richness will be provided. Time required also will increase because at least two visits to the site are necessary (initial marking sample and one recapture sample). Cost may also increase, depending on the marking system used and whether additional personnel are needed.

To summarize, selection of the appropriate technique or techniques depends on the question being asked, the information required, the nature of the organism(s) or habitat being studied, and the resources available for the project. For a successful project, all of these factors must be considered prior to initiation of the study. We also strongly recommend a careful reading (or rereading) of Chapter 4 prior to selection of techniques.

Standard techniques

We begin the description of each technique with a brief review of its purpose, followed by a discussion of the specific amphibians and habitats for which the technique is known to work and, if known, those for which it is inappropriate. A section on background information on the development of the technique and any inherent assumptions and limitations regarding its use is followed with an exploration of questions relative to the research design associated with the technique. Because executing a specific experimental design under field conditions is rarely a straightforward procedure, we also discuss the

Table 4. Factors to Consider in Selecting Standard Techniques

Technique	Information gained[a]	Time[b]	Cost[c]	Personnel[d]
1. Complete species inventories	Species richness	High	Low	Low
2. Visual encounter surveys	Relative abundance	Low	Low	Low
3. Audio strip transects	Relative abundance	Medium	Medium	Low
4. Quadrat sampling	Density	High	Low	Medium
5. Transect sampling	Density	High	Low	Medium
6. Patch sampling	Density	High	Low	Medium
7. Straight-line drift fences and pitfall traps	Relative abundance	High	High	High
8. Surveys at breeding sites	Relative abundance	Medium	Low	Medium
9. Drift fences at breeding sites	Relative abundance	High	High	High
10. Quantitative sampling of amphibian larvae	Density or relative abundance[e]	Medium	Medium	Medium

[a] Designations are hierarchical; techniques that provide a density estimate also give relative abundance and species richness. Those that estimate relative abundance also provide species richness. If a technique gives species richness only, then some other technique must be used to obtain relative abundance or density.

[b] Relative time investment.

[c] Relative financial cost: high = expensive; medium = moderately expensive; low = relatively inexpensive.

[d] Personnel requirements: high = more than one person required; medium = one or more persons recommended; low = can be done by one person.

[e] Some methods included in technique 10 give relative abundance only, and some yield density values.

reality of implementing the technique in the field, including personnel and materials needed. Guidelines on how to collect and organize data include sample data sheets where appropriate. We briefly discuss data interpretation and analysis and provide technique-specific information that supplements the general guidelines provided in Chapter 9. We also examine specific features of the technique, make recommendations about its use, and review other important technical points.

The 10 techniques are not mutually exclusive. For example, an audio strip transect could be treated as a special case of either the general transect or the quadrat sampling technique, and a drift fence encircling a breeding pond could be a special application of a straight-line drift fence and pitfall trap sampling array. Nevertheless, the focus of each technique is different, and each has sufficient importance unique to amphibians to justify separate treatment. Separate treatment also will better ensure standardization, repeatability, and quality results.

The first technique deals with how to assemble species lists. Such lists are critical for conservation-related decisions, among other applications. The approaches recommended sacrifice quantification in favor of maximizing numbers of species obtained. In this respect, technique 1 differs from the other nine recommended techniques. The field procedures recommended in the first section on assembling species lists should be

used only when time is limited and specific site inventory data are more important than comparing the data gathered with data from other sites.

1. Complete Species Inventories

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Several techniques are available for generating species lists or information on species richness for a site. For the most part, the field techniques are methods of general collecting, as historically practiced by herpetologists. Typically, they involve searching for and collecting amphibians in all possible (appropriate) microhabitats both during the day and at night and result in modest habitat modification, such as dismantling of rotten logs or removal of epiphytes. These general collecting techniques have been used for both long-term and short-term sampling projects, although long-term sampling often includes both data retrieval and fieldwork and thus is more eclectic.

I discuss three approaches to species inventories: (1) compilation of faunal lists; (2) short-term, time-constrained quantitative sampling; and (3) rigorous, short-term, number-constrained sampling, an approach that I call the Systematic Sampling Survey (SSS). The SSS has been used with birds (Terborgh 1989:75; see also Hurlbert 1971) and would appear appropriate for sampling amphibians. The three approaches provide an enumeration of the amphibian fauna at a site and, with some qualification, may be used to compare species richness among sites or to detect changes in faunal composition at one site through time. This type of information often serves to guide conservation efforts.

TARGET ORGANISMS AND HABITATS

The field techniques can be used for sampling any amphibian species in any habitat. Secretive, fossorial, canopy-dwelling, and deep-water species, however, are more difficult to inventory and may require specialized searching methods.

BACKGROUND

Species lists may be developed through long-term, gradual accumulation of records or by intense general collecting over a relatively short period. Numerous published checklists and herpetofaunal descriptions of specific areas attest to the usefulness of the long-term accumulation of species records. If the understanding of the systematic relationships among species and sampling of the faunas are comparable, site-specific lists can be used to compare species richness and details of faunal composition among sites, as was done by Duellman (1990) for five Neotropical rain forests.

General, nonquantitative, short-term collecting efforts cannot be used to estimate total species richness in complex faunas of more than about 25 species. Even for faunas of fewer than 25 species, I recommend use of quantitative short-term sampling techniques.

COMPILATION OF FAUNAL LISTS

ASSUMPTIONS. Faunal lists can be accumulated by integrating the results of general collecting by a few to many investigators with many research objectives; usually these collections are made using different techniques. The major assumptions are that differences in results caused by variation in technique and effort are smoothed over time and that the area does not change during the sampling period. Because most areas undergo change during long periods of investigation, however, these habitat changes must be documented. Most commonly, a few amphibian species disappear when water conditions change, as vegetational succession proceeds, or when habitats become insular (Myers and Rand 1969; Heyer et al. 1990; Rand and Meyers 1990). For example, on Barro Colorado Island,

Panama, at least three amphibian species disappeared from, and two others invaded, an original fauna of about 34 species over the 70-year period of record (Rand and Myers 1990).

LIMITATIONS. Long periods (usually in the scale of years) are needed to sample complex faunas (e.g., faunas with many species), areas with a highly seasonal climate, and areas where individual amphibians are scarce. For example, in a wet lowland forest in Ecuador, 90% of the species in an exceptionally diverse herpetofauna (185 species, including amphibians and reptiles) were taken after 500 collecting-days, and 97% after 800 days; the total was based on 1,300 collecting-days (Duellman 1978). Two to five times such effort may be needed in Southeast Asian wet forests where the amphibian fauna is equally species rich but individuals are relatively scarce (Lloyd et al. 1968a).

Long-term data accumulation is appropriate when a site (such as a field station) is visited irregularly by many collectors over many years or decades, as at La Selva, Costa Rica (Guyer 1990); Barro Colorado Island, Panama (Rand and Meyers 1990); and Boracéia, Brazil (Heyer et al. 1990).

SHORT-TERM SAMPLING

Short collecting visits to a single site cannot give much insight into the total number of species present. However, using time-constrained collecting techniques, rates of species accumulation in different habitats or sites can be compared if animal population densities are similar. If densities are dissimilar, scanty data suggest that samples derived from the protocol of Systematic Sampling Surveys (SSS) may be used to rank habitats and sites according to relative species richness. Quantitative short-term sampling techniques also can be used with other methods to gather more-detailed data on microhabitat variables for niche and assemblage analyses (see Chapter 5 and Inger and Colwell 1977).

ASSUMPTIONS. The results from short-term sampling are highly dependent on collecting and environmental variables. Some of these variables include weather (both prior to and during sampling), collectors' experience, level of sampling effort in each habitat, diversity of collecting techniques used, and phenology of the amphibian species. Before results from similar habitats at different sites are compared, any effects of these variables must be recognized and controlled.

Time-constrained searches (yielding a number of species collected per person-hour) must standardize collecting effort within habitat types. For example, Campbell and Christman (1982a:198) carried out a study in which each habitat type was sampled for 6 person-hours in the spring, summer, and autumn. In general, time-constrained sampling is a less robust form of the visual encounter survey (technique 2), which should be used when possible.

The SSS (number-constrained) method depends on the validity of another assumption: that more species are present in a limited sample of a species-rich fauna than are present in a similarly sized sample from a less rich fauna. This assumption seems to be valid for tropical herpetofaunas inhabiting forest litter (Scott 1976) but must be tested further to determine its general applicability for amphibians. As few as 100 specimens from each habitat, taken by a variety of techniques, may be adequate to rank a series of diverse faunas according to species richness. Cumulative plots of the numbers of species against numbers of individuals for litter-inhabiting reptiles and amphibians are available for wet tropical forests in Costa Rica (two sites), Borneo, and Cameroon (Scott 1982). The probably correct rank order of sites based on species richness was established after sampling about 80 individuals, and the order was preserved even after almost 200 individuals had been taken at the three most extensively sampled sites.

LIMITATIONS. Data resulting from time-constrained, short-term, general collecting can be compared among habitats at a single site but, given the large number of variables that potentially may influence composition of the samples, comparisons among sites usually are inappropriate. The number-constrained SSS technique, when applied to amphibians, enables an investigator to rank areas and habitats according to their species richness, but the actual number of species present will not be estimated accurately unless species richness is already known for one of the areas being compared.

RESEARCH DESIGN

There usually is no research design for long-term accumulation of faunal lists. In most cases, research museums are the major repositories of historic information on species presence, but other sources of information include private collections, published works, field notes, and station lists. Inventory lists can be augmented at any time by fieldwork specifically directed at discovering additional species. Time-constrained, short-term sampling should be stratified by major habitat type. The SSS methodology usually can be combined with time-constrained general collecting to provide a firmer basis for comparisons among habitats and sites. The SSS requires that an effort be made to record every animal encountered in each habitat, up to some preselected number (e.g., 100). Then, if the habitat has not been adequately sampled in the judgment of the investigator, efforts can be concentrated on the collection of additional species, not specimens. However, only data from the number-constrained collections can be used to compare sites.

FIELD METHODS FOR SHORT-TERM SAMPLING

The first step in time-constrained, short-term sampling is to identify and define the major habitat types at the study site. These habitats should be described in detail sufficient (see Chapter 5) to allow the identification of similar habitats at other sites or in other studies. All habitats should be sampled during the first few days of the sampling period. Information derived from this broad scale sampling can be used to plan how to distribute subsequent sampling among habitats.

Many factors influence the efficiency of short-term surveys, and they must be recognized and controlled if comparisons are to be made among different sites and habitats. Some of these variables are (1) total time spent on the survey and time spent using each type of collecting technique; (2) number and experience of fieldworkers; (3) topography; (4) area of the site; (5) local weather and climate; (6) season, date, and time of day; and (7) time required to sample each major habitat type.

Before each search, the exact locality, date, starting time, and observers should be noted along with vegetation, habitat, habitat disturbance, slope and aspect of the area, and temperature and weather at the time of searching and during the recent past.

The goal of the search is to collect as many species of amphibians as possible. Persons who live on or near the site often know where and when certain species may be found. A common method of organizing a search is to survey a habitat rapidly during the day, identifying possible amphibian breeding sites that can be investigated more thoroughly at night, and looking for tadpoles or egg masses. All accessible amphibian habitats should be searched, and as much area as possible should be covered. Ears are among the best tools for detecting amphibians. Some large breeding choruses can be heard up to 2 km away, whereas calls of males of some species are audible over a distance of only 1 to 2 m. Some frogs call underwater, and others from beneath the ground. Each distinct call should be traced to its source, and a voucher specimen captured. Many amphibians can be lo-

cated visually, as one walks along trails, streams, and lake margins during the day and at night.

Certain microhabitats often are unusually productive, including those in epiphytes, under loose bark, at the bases of buttressed trees, under and inside logs, under rocks, in rock crevices, in puddles and springs, along streambeds, and inside tree and bamboo hollows. Depending on the moisture regime, amphibians living in forest leaf litter are often highly concentrated. Accumulations of leaf litter around tree buttresses and moist spots, such as seeps and springs or dry streambeds, often harbor many individuals.

Every animal seen should be identified. Breeding choruses should be worked until the source of each different call has been located and identified. The decision to quit searching in one habitat and move to another is made by the investigator; searches within habitats are stopped either when all of the available habitat has been thoroughly searched or when no new species has been found for a predetermined period of time. Undue concentration on calling males of one species, to the exclusion of males of other species, will always be counterproductive when trying to characterize the entire fauna over a short period. The searcher records the time when the search period ends. The efficiency and comparability of short-term sampling efforts are enhanced if sampling is carried out at a time of year and during weather conditions when amphibians are most active. In most areas, that time is early in the warm, rainy season.

The SSS follows the above procedures but focuses on gathering data on (and usually capturing) specimens, not species. With the exception of members of breeding aggregations, individual amphibians are tallied as they are encountered, up to the previously determined number. Species occurring in aggregations, such as breeding choruses, should be counted only once in the specimen count. The decision to leave one habitat and proceed to another is dictated by the number of specimens tallied. The suggested sample size per habitat for diverse tropical faunas is 100 specimens. For less diverse faunas, fewer observations may adequately represent any single habitat.

PERSONNEL AND MATERIALS

General collecting and SSS can be performed by any number of workers, but one person should be in charge of describing the habitats and keeping the time records and should be responsible for the data collected by other workers. All of the collectors for an SSS should be experienced.

A machete or potato rake can be used to tear up logs, to turn stones, to pull down epiphytes, to rake through leaf litter, and to probe in holes and crevices. One should never use bare hands, especially in areas where poisonous snakes may occur.

If the fauna is complex (many species) or poorly known, amphibian eggs and frog larvae often cannot be identified reliably, and sometimes cannot be identified at all, unless samples are reared through metamorphosis or large larvae are reared from eggs obtained from known parents. Investigators should carry containers for rearing larvae and food for tadpoles (commercial tropical fish food, rabbit or trout chow, or leafy vegetables to be boiled) into the field with them.

Preparing for the field trip is time well spent. Many collectors, from lack of forethought, do not carry the specialized tools, such as seines, nets, machetes, rakes, and traps, that can make the difference between superficial and adequate sampling of a site.

DATA TREATMENT AND INTERPRETATION

Data derived from long-term historical records vary in quality and reliability. As a general rule, museum catalogue identifications should be verified by examining the specimens, and any unusual locality data should be questioned. Records not supported by voucher specimens, photographs, or recordings of calls should be clearly listed as tentative.

SYSTEMATIC SAMPLING SURVEY

OBSERVER(S) Kraus, J. Scott, Douglas C. Robinson DATE/TIME 12 October 1988 / 19:20

GENERAL LOCALITY Costa Rica: Heredia Province; La Selva

SPECIFIC LOCALITY Swamp, 100 m East of headquarters clearing

HABITAT TYPE Forest Swamp SEARCH TYPE General Collecting

HABITAT DESCRIPTION 1/4 ha. swamp with emergent aroids. Open water 20 x 20 m., 0.5 m deep. Ringed by forest.

SLOPE/ASPECT ± 20° / 180° CANOPY Complete except over pond

WEATHER Overcast, still. Vegetation still wet from rain in P.M.

TEMPERATURE
air 30.2 water at surface 28.3 water 50 cm deep 26.2

SPECIES ACRONYM OR ID

OLEL - Oleolyzon elaeochroa
HYEB - Hyla ebraccata
AGCA - Agalychnis callidryas
GAPI - Gastrophryne pictiventris
SMBA - Smilisca baudinii
LEPE - Leptodactylus pentadactylus
LEME - Leptodactylus melanonotus
BOCO - Bolitoglossa colonnea
ELDI - Eleutherodactylus diastema
CEPR - Centrolenella prosoblepon
AGSA - Agalychnis saltator
EL SP1 - Eleutherodactylus sp. 1
ELTA - Eleutherodactylus talamancae
PHVE - Phyllomedusa venulosa
EL RI - Eleutherodactylus rugosus

INDIVIDUALS OBSERVED
(C) = CALLING

OLEL (c), HYEB (c)
AGCA (c), GAPI (c)
SMBA (c), LEPE,
LEME (c), BOCO,
ELDI (c), CEPR,
LEPE, AGSA (c),
ELSP1, ELSP1,
ELTA, PHVE, ELRI

TOTAL "SPECIMEN" RECORDS (Species/chorus count as one) 17
APPROXIMATE AREA SEARCHED 1 ha ENDING TIME 23:20

Figure 7. Field data sheet used for adult amphibians in a systematic sampling survey (SSS) in a forest swamp in Costa Rica.

Observations made during an SSS should be recorded immediately in notebooks carried by each investigator. A sample data sheet for a hypothetical SSS in forest swamp habitat is illustrated in Figure 7. Different data sheets would be needed for other major habitat types. In the present example, a swamp and its surrounding vegetation were surveyed. Eight species of amphibians were congregated in breeding choruses; 7 species were not calling. For the purposes of SSS, a single entry was made for frogs (males and females) participating in each species' chorus. Otherwise, chorusing individuals would have overwhelmed the sample, and the data could not have been compared with those from other sites.

This sample contributes 17 "specimens" of 15 species to the target of 100 specimen records for this habitat type. Other forest swamps at the same site would have been sampled until data for 100 specimens had been gathered. The species richness of these 100 specimen records can be compared with that of forest swamps in other areas. In like fashion, samples of 100 specimens from other major habitat types (e.g., forest, forest stream, riverbank) can be compared with

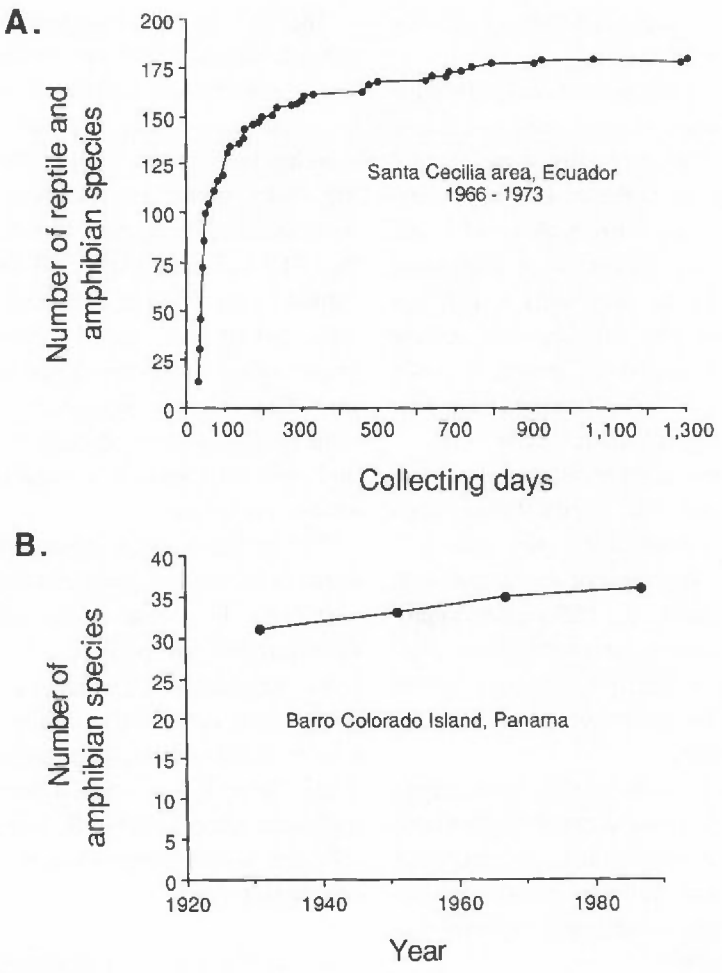

Figure 8. Species accumulation rates. A. Rate of accumulation of reptile and amphibian species in the vicinity of Santa Cecilia, Ecuador (redrawn from Duellman 1978). B. Rate of accumulation of amphibian species over a 56-year period for Barro Colorado Island, Panama (data from Myers and Rand 1969 and Rand and Myers 1990).

samples from other areas with similar habitats, and the characteristics of the entire site can be summarized in a species list derived from all of the samples. If the SSS procedure is valid, species lists and relative richness can be compared among all sites that have been summarized in the same way.

It is important to maintain up-to-date summaries during the actual fieldwork. They can be used to guide the remainder of the collecting effort.

The completeness of a species list derived from long-term records is evaluated by inspecting a graph of the cumulative number of species versus cumulative search time. Search time is usually expressed in days, months, or years. Curves plotting species versus search time rise sharply during the initial search periods but approach an asymptote as the species list nears completion. The asymptote approximates the total species richness of the site. Curves derived from the long-term studies in Panama (Rand and

Myers 1990) and Ecuador (Duellman 1978) are clearly asymptotic (Fig. 8).

The number of reptile species cannot be separated from amphibians in some published graphs (i.e., Duellman 1978). Typically, a greater proportion of the species collected late in the sampling period has been fossorial reptiles and snakes, with a greater proportion of amphibians sampled earlier. In the only study to compare long-term species accumulation rates among taxa, 95% of the amphibian species but only 90% of the lizards and snakes had been taken after 20 years of collecting (Myers and Rand 1969).

If the time-constrained technique is used in similar habitats and with similar faunas, then resulting species accumulation rates from general, short-term collecting can be compared. If the areas are dissimilar, the SSS may be appropriate. An SSS produces lists of the species present in the first x number of specimens collected in each habitat. The number of species then can be compared directly.

For comparisons among sites with nearly complete species lists, real data can be compared with randomly generated lists to determine whether among-site differences are greater, smaller, or the same as randomly expected differences (Guyer 1990).

SPECIAL CONSIDERATIONS

General collecting is the most efficient way for experienced collectors to take the largest number of species in the least amount of time. No other collecting method is as productive in amassing species for a list and in obtaining series of specimens.

The precision of indices derived from SSS depends on the sampling efficiency in different habitats and the accuracy of the species identifications. Even seasoned herpetologists are less efficient when sampling unfamiliar habitats. For this reason, data collected over time by the same collectors are more comparable than data taken by many collectors.

The SSS is most comparable among sites when a variety of collecting techniques is used and search times are well distributed among habitats and times of day and night. For example, searches based primarily on collections of calling males cannot be compared meaningfully with searches made only in forests during the day. The SSS collections used for data analysis should be the result of individual collecting efforts, not of some passive technique such as pitfall arrays. Collectors using an SSS can reduce bias in collecting effort. Species caught with passive systems depend on trap location and individual species susceptibility, so many species are missed.

The species richness index derived from SSS needs to be tested to determine its generality and usefulness. First, tests of the variation due to collectors and time period can be determined by a two-way analysis of variance of the data from independent collections made by different collectors working in the same habitat at different times. Second, data derived from SSS can be compared among sites with well-known herpetofaunas to determine whether SSS produces concordant results.

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2. Visual Encounter Surveys

MARTHA L. CRUMP AND NORMAN J. SCOTT, Jr.

A visual encounter survey (VES) is one in which field personnel walk through an area or habitat for a prescribed time period systematically searching for animals. Time is expressed as the number of person-hours of searching in each area to be compared. The VES is an appropriate technique for both inventory and monitoring studies.

The VES is used to determine the species richness of an area, to compile a species list

(species composition of an assemblage), and to estimate relative abundances of species within an assemblage. This technique by itself is not an appropriate method for determining densities (number of individuals per unit area) because not all individuals actually present in an area are likely to be visible during the survey. However, if repeated VESs are done in conjunction with a mark-recapture study, density can be estimated reasonably (Donnelly 1989).

Visual encounter surveys differ from transect sampling (technique 5). A VES can be done along a transect, in a plot, along a stream, around a pond, and so forth, and it samples all amphibians that are visible. Transect sampling uses lines of fixed length in fixed locations and focuses on surface-dwelling amphibians.

TARGET ORGANISMS AND HABITATS

The VES has been used most extensively for rapid evaluation of large forest areas, especially in uniform habitats where visibility is good. The VES works especially well for forest understory anurans that are active in the open (e.g., Toft et al. 1982) and for salamanders that live most or all of their lives in the forest litter but are on the surface after rains (e.g., Pough et al. 1987; Corn and Bury 1990).

Visual surveys also can be used effectively for target species that inhabit easily identified habitats, such as logs or riparian zones, or habitats that are widely spaced, such as talus slopes. They are also appropriate for target species that are highly clumped, such as frogs at temporary ponds; in these cases, the surveys are done in the restricted areas of interest. For example, a VES can be carried out at an aquatic breeding site by setting up multiple transects from the edge of the water into the center of the site.

The VES can be used to inventory aquatic assemblages under certain conditions (e.g., relatively shallow, clear pools with minimal vegetation), but generally such surveys are better for monitoring only certain target species, because not all species in an aquatic assemblage can be observed equally. Frazer (1978) and Griffiths (1984) surveyed aquatic newts at night by VES using flashlights. The VES can also be used effectively to monitor larval amphibians in small, shallow pools where the water is clear and the vegetation is sparse.

A VES is often the best way to survey species that are rare or unlikely to be caught with traps. The technique is not appropriate for surveying canopy or fossorial species.

BACKGROUND

Because the VES is simple it has been used for a long time. The technique has been formalized as the time-constrained technique by Campbell and Christman (1982a) and as the time-constrained searches by Corn and Bury (1990). The results of a VES search are measured against the time spent in the search.

ASSUMPTIONS. The VES is based on the following assumptions:

1. Every individual of every species has the same chance of being observed during a survey (i.e., each individual is equally conspicuous to an observer; there are no differential effects of coloration, size, behavior, activity, or microhabitat preference on the likelihood of being encountered).
2. Each species is equally likely to be observed during each sampling session (i.e., there are no seasonal effects of activity, weather, predators, or competitors on a species' likelihood of being encountered).
3. An individual is recorded only once during a survey (i.e., the observer can keep track of all movement so as not to record multiple encounters for the same individual).
4. Results from two or more observers surveying the same area simultaneously are identical (i.e., there are no observer-related effects).

Although these assumptions have never been rigorously tested and the validity of the results of a VES is unknown, we know intuitively that the assumptions will not hold in most instances. Species do differ in their conspicuousness, and people do differ in their abilities to see amphibians. The resulting potential biases should be recognized and minimized to the extent possible. For example, comparable training and expertise of the individuals involved in a VES are crucial. Some people can develop an excellent search image for amphibians; others never do. Most people improve with practice. If more than one person is required to carry out a VES, individuals should conduct independent surveys in the same test area simultaneously, and their results should be compared. Biases between individual observers may reflect differences in the amount of time spent looking up versus looking down or differences in walking speed. With effort, such biases can be controlled.

LIMITATIONS. Two obvious limitations are associated with a VES:

1. Not all strata or microhabitats within the habitat can be sampled with equal success.
2. Not all habitat types can be sampled with equal success. As a result, relative species abundances can be compared only among sites of the same habitat type.

Dissimilar habitats cannot be surveyed by VES with an equivalent degree of reliability because of differences in visibility; open habitats are surveyed more efficiently than are habitats with dense vegetation. Time of day can also affect a VES; most people find surveying the environment using natural light easier than surveying with a headlamp. Weather conditions can affect a VES; visibility generally decreases with rainy, misty, and cloudy conditions. Thus, surveys of areas to be compared directly should be done under comparable weather conditions, as much as possible, and at the same times of day or night.

RESEARCH DESIGN

The design for a VES will depend on the goals of the study (whether it is a one-time inventory or a long-term monitoring program and, if the latter, whether the intent is to determine phenology of species composition, phenology of species abundances, or both), the specific habitat and the size of the area to be surveyed, the desired periodicity of the sampling regime (diel and seasonal), and the species composition. For purposes of statistical analysis, censusing ten 100-m transects within a given habitat type is preferable to censusing one 1,000-m transect. Regardless of the experimental design, at some point early in the study the data and field methods should be evaluated and the methods modified as appropriate. Three basic sampling designs are used for the VES (Fig. 9): randomized walk, quadrat, and transect.

A *randomized-walk design* is appropriate when a large area is to be sampled. Prior to going into the field, the observer chooses at random a sequential series of compass directions (preferably at least 50); he or she also selects at random a number of meters (up to 50 m) to be walked in each selected direction. The start point can be determined by breaking the area into blocks, randomly selecting one, and starting from the middle of it. All amphibians observed within 1 m on either side of the path are recorded. This design (Fig. 9A) satisfies the assumption of randomized sampling, which allows statistical comparisons among replicated walks in different areas or habitats. Because the VES is a time-constrained field technique, the time spent per unit area must be specified for each investigator and for each area to be compared.

A *quadrat design* is appropriate for sampling a specific area thoroughly. A quadrat of given dimensions is established (we recommend 10 × 10 m or 25 × 25 m, depending on amphibian

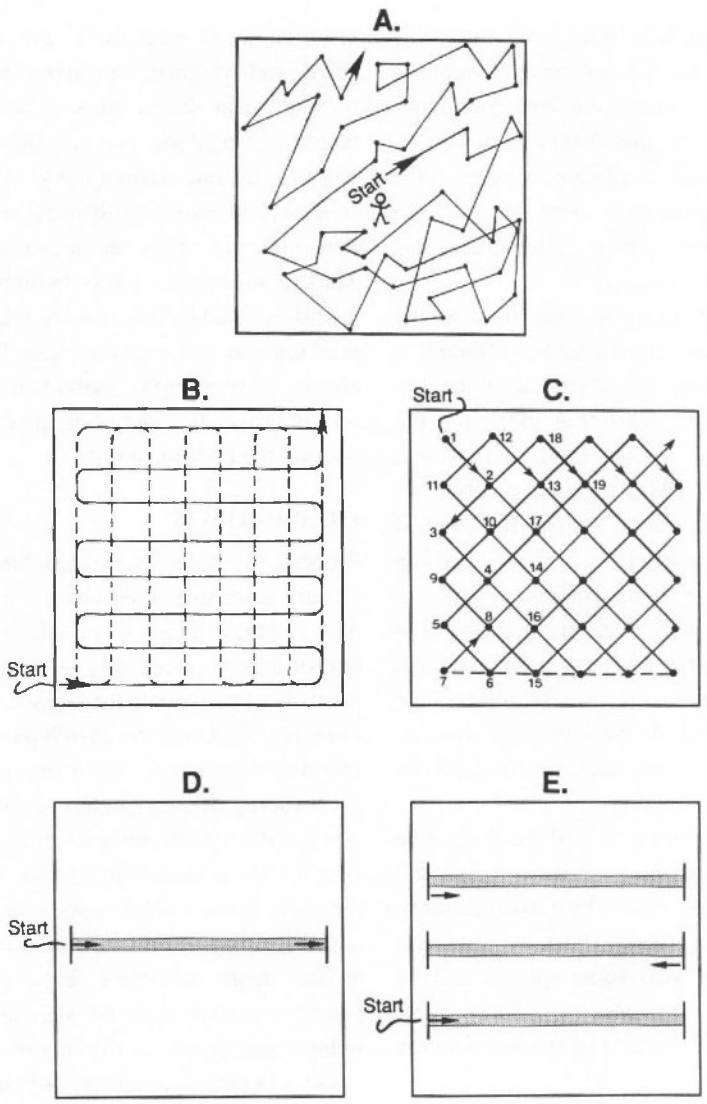

Figure 9. Experimental designs for visual encounter surveys. A. Randomized walk design. The observer chooses a series of compass directions at random and walks each in sequence for a given number of meters, also determined at random. B–C. Quadrat design. An area of given dimensions is systematically sampled either (B) by walking two sets, at right angles, of parallel adjacent paths across the plot or (C) by walking a zigzag pattern between numbered stakes (i.e., in this example, 1 to 13 in numerical order, then 10–14–8–15–6–16–4–17–2–18–19 and so forth). D–E. Transect design. A single transect (D) or multiple parallel transects (E) are set up, and areas on either side of the path are systematically sampled.

densities). The quadrat is then systematically sampled by walking parallel paths across the plot (Fig. 9B) or by walking a zigzag pattern between numbered stakes (Fig. 9C) (Hairston 1980a,b; Aichinger 1987; Donnelly 1989; Nishikawa 1990). If area grids have been established, the exact location of each individual encountered can be noted relative to the distance markers (this information could be used to examine spatial distribution patterns within the habitats). Multiple (at least 10) randomly placed plots can be used to test for changes in species richness or

relative abundances over time or to determine differences in species richness or relative abundances among sites at one time. Breeding ponds can be surveyed using this design by constructing a checkerboard of boardwalks or paths at the site (Fig. 9B). Again, time spent per unit area must be standardized among fieldworkers and among sites to be compared.

A *transect design* is appropriate for sampling across microhabitats known to be different or potentially so. In the simplest case, a single transect of preestablished length (Fig. 9D) is laid out and walked (Jaeger 1978; Pough et al. 1987; Crump and Pounds 1989); all animals observed within 1 m of the transect path are recorded. If desired, the exact location of each individual can be noted according to its position relative to previously established distance markers. For large areas, 10 or more transects 100 m long and spaced 20 m apart in the area of interest are appropriate (Fig. 9E). As with the other designs, time spent per unit area must be standardized among workers and areas.

If only one inventory is to be done, it must be scheduled for the time of year, time of day, and weather conditions in which the maximum number of species are expected to be active. Because at any one time of year some species will be inactive, a one-time inventory should be interpreted as a minimal estimate of species richness for the area.

For long-term monitoring programs, sampling periodicity is crucial. One must sample often enough to ensure that within-year variation does not obscure year-to-year differences. Because activity patterns of amphibians are greatly influenced by weather, VESs should be done during each season of the year. Time of day likewise greatly affects behavior patterns, and time of the survey can bias both species composition and abundance data if not taken into consideration.

An important decision to be made during the design of the fieldwork is whether to capture each amphibian encountered to obtain additional information. If individuals are captured, measured, and weighed, less time will be available for additional survey work. If animals are captured, investigators must also decide whether to mark each one individually. Mark-recapture studies yield valuable information on population dynamics, but, again, less area can be surveyed. Marked animals are likely to be recaptured frequently in studies that involve area searches, so marking may be worth the effort. In contrast, the chance of recapturing individuals along a transect is fairly slim and, thus, probably does not warrant the additional time.

FIELD METHODS

Procedures for a VES are straightforward. Habitats are searched, either along a transect or in a plot, and the number of animals encountered per unit of time is recorded. The length of time and intensity of the search, the boundaries of the area to be searched, and the search pattern should be specified in advance. For example, instructions for a survey of salamanders should specify the type(s) of substrate to be examined (e.g., every possible cover item or just logs), whether or not the cover items will be turned over or torn apart, and the maximum amount of time to be spent tearing apart substrates (e.g., a single log). Search methods must be standardized among fieldworkers to reduce bias in the results.

For a complete inventory, all possible microhabitats are searched: ground, water, tree trunks, stems, and upper and lower surfaces of leaves as high as the observer can see accurately enough to identify the animals. Time spent per unit area is standardized as much as possible within a given habitat type, but habitats with differing heterogeneities will require different survey times. When animals are encountered, they are identified and, if need be, captured and measured or collected as vouchers. The VES can be performed at several levels of intensity, as follows.

Among the least intensive surveys are counts of animals active on the surface (e.g., Hairston

1980b; Pough et al. 1987; Nishikawa 1990) or of animal-associated items (e.g., burrows of salamanders—Dodd 1990, 1991a). This type of VES is particularly useful for species that are active on the surface of leaf litter or that climb plants on rainy or foggy nights. Under such wet conditions, a large proportion of the population may leave underground retreats and move to the surface. This method is especially suited for inventory of habitats containing endangered species where habitat disturbance must be avoided.

The intermediate-intensity search is one in which the field crew, in addition to counting already exposed animals, turns over surface objects such as rocks and logs and counts the animals uncovered. The cover objects must be returned to their original positions to minimize habitat disturbance. This type of search generally yields higher return per unit time than a low-intensity search because many amphibians hide under cover objects when conditions are not suitable for surface activity.

At the most intense level of VES, surface objects are turned, decayed logs and bromeliads are torn apart, and litter is raked. These activities obviously change the habitat; long-term effects of this type of search have not been measured for any habitat or target species. Habitat disturbance increases with increasing search intensity, but more animals are encountered. Intense surveys are probably the most reliable in terms of sampling the most species, especially rare ones.

For some studies, it is useful to set up a transect or grid system with permanent distance markers before the survey begins, so that the exact location of every animal can be recorded at the time of encounter. This allows the investigator to calculate interindividual distances, record detailed microhabitat data for specific sites where amphibians have been found repeatedly, evaluate the homogeneity of the area searched, and if a mark-recapture study is done, obtain valuable information on individual animal movements through time.

Depending on the goals of the study, the animals encountered can be observed only (assuming that positive identification can be made without holding the animal), or they can be captured temporarily for positive identification and measurement (see Appendix 1) and then released at the site of capture. Voucher specimens should be preserved and deposited in an established research museum collection (see Chapter 5). Many groups of amphibians are undergoing significant taxonomic revision, and sibling species are often difficult to distinguish in the field. Museum collections provide a basis for verifying field identifications, which greatly enhances the reliability and usefulness of surveys.

PERSONNEL AND MATERIALS

The number of persons needed to execute a VES depends on habitat complexity, size of the area to be sampled, and level of survey intensity. If reasonably short forest transects are to be surveyed, only one person may be needed; surveying a large breeding pond may require several persons, each doing a transect from the shore into the center of the pond. All persons involved must be well trained in the same search techniques, and interobserver differences must be considered. If animals are common and data are taken for each individual, designating a person as data recorder can speed the operation and keep the pace of the survey more uniform.

A VES requires minimal equipment: data paper, pencils or pens with permanent ink, and a millimeter ruler and spring balance if body length and weight are to be taken. Plastic bags and a marking pen are needed if amphibians are to be collected. Potato rakes are useful for searching in leaf litter. If microhabitat measurements are to be taken, appropriate instruments are needed (see Chapter 5). If the transect is to be marked at regular intervals, numbered flagging tape or permanent stakes should be set in place prior to the survey. For nighttime surveys, headlamps are required.

DATA TREATMENT AND INTERPRETATION

The kinds of data to be collected depend on the goals of the study and the time and personnel available. Minimum data to be collected during an inventory of an area that does not contain grids with distance markers include (1) the number of individuals of each species encountered, (2) the total time searched, and (3) the size of the area searched. For inventories and monitoring surveys in areas with distance markers, location within the transect or grid system should be recorded in addition to microhabitat data and the minimum elements previously listed (Chapter 5) for each amphibian encountered. If desired, wet mass and reproductive condition can be recorded. If animals are taken temporarily to a laboratory facility, much of this information can be recorded after the survey is completed to avoid taking up valuable field time. Another option is for a second person to follow the primary VES searcher and record additional data while the first person continues the survey.

Well-organized data sheets that incorporate the minimum data elements (see Chapter 5) and include extra space for notes that do not fit preestablished categories are recommended for use in the field. Data sheets can be simple or complex, depending on the goals of the study (Fig. 10).

A main application of VES generally is determination of the composition of an amphibian assemblage. The list of species compiled for the area surveyed can be compared with species lists from other areas. Mean numbers of individuals can be compared statistically, and coefficients of association calculated (see Chapter 9). Before any data are interpreted or compared, however, effects of the biases noted previously must be estimated (see "Assumptions" and "Limitations," under "Background," above).

As discussed previously, if relative abundances are to be compared among sites, then habitat structure, weather, and search techniques must be similar for all samples. For example, if the project is designed to determine the effects of disturbance (e.g., logging) on amphibians, then separate VESs would be conducted in logged and unlogged parts of a forest. If, however, the researcher wishes to compare two forests, then all VES transects must be located in forest habitats; it would be inappropriate for some transects to include forest edge ecotones while others did not. Until VESs have been validated and we have some idea of their variability and their relationships to actual population levels, data gathered during VESs should not be used to report individual species densities. Dodd (1990) used a Fourier series to estimate salamander burrow densities along transects; this technique may have limited usefulness in the analysis of other transect data.

If long-term monitoring is done, phenology of presence and activity of various species can be presented graphically with histograms or frequency curves. If activity patterns are to be interpreted in light of climatic factors, then correlations, multiple regressions, and analyses of variance may be appropriate types of analysis. Tests of differences in species' activity levels under different weather conditions or among seasonal components of the year are also possible.

SPECIAL CONSIDERATIONS

Campbell and Christman (1982a) surveyed amphibian assemblages in four habitat types in Florida and compared results from intensive VESs with those from road cruising, litter removal–quadrat sampling, and drift fence–pitfall arrays. Fifteen of the 22 species known to be present were recorded with road cruising, and 12 were recorded with VESs. Quadrats and drift fence–pitfall arrays yielded 6 and 7 species, respectively. Bury and Raphael (1983) compared techniques and recommended that VESs be combined with a drift fence–pitfall array to

Visual Encounter Survey

Date _____ Name of obvserver(s) _____

Place _____ Area searched _____

Weather conditions _____ Air temperature _____

Time begin survey _____ Time end survey _____

Habitat description _____

_____

No.	Species	Sex	SVL	Substrate	Location	Activity	Time
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							

Figure 10. Sample data sheet for a visual encounter survey in which specific data are recorded for each individual encountered. If the survey requires only abundance data, the lower part of the data sheet can be redesigned so that individuals of each species seen on the survey can be recorded with tick marks; marks for each species are totaled later.

sample the herpetofauna more effectively. The VES can be validated by repeated sampling of the same areas or by comparison of results of VESs with those from surveys done in the same areas using different methods that estimate true population sizes. The need for validation is urgent. The VES done in conjunction with mark-recapture techniques is useful for studying population trends through repeated sampling of the same areas.

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3. Audio Strip Transects

BARBARA L. ZIMMERMAN

In the vast majority of frog species, males in reproductive condition use distinctive species-specific calls to advertise their position to potential mates and rivals (Wells 1977). The audio strip transect (AST) technique exploits this species-specific behavior. All calling frogs along a transect are counted. The width of the transect varies according to the detection distance of each species' advertisement call. The counts are then used to estimate or determine (1) relative abundances of calling males, (2) relative abundances of all adults, (3) species composition, (4) breeding habitat or microhabitat use, and (5) breeding phenology of species.

TARGET ORGANISMS AND HABITATS

Counting calling male frogs or aggregations of calling males (i.e., choruses) along strip transects can be the most effective way to inventory species composition, to provide a first approximation of relative abundances of breeding frogs, to determine breeding habitat use, and to map distributions of most frog species throughout a large area of several to hundreds of hectares. Audio transects are particularly efficient in tropical forests where species richness is high and frogs dwell at all strata and in many microhabitats. Most frogs are difficult to see in forest without time-consuming searching, which drastically limits the size of area that can be surveyed. The AST technique is generally inappropriate for sampling frogs in linear habitats such as streams and shorelines (see "Data Treatment and Interpretation," below).

Calling males of many species in tropical forest are widely dispersed or occur in groups small enough to be counted accurately by sound. It is usually impossible to count numbers of individuals in a large chorus by sound because calls overlap. Abundances of males in choruses have to be determined visually (see "Visual Encounter Surveys," above and "Surveys at Breeding Sites," below), although the choruses themselves can be counted aurally. Males of most temperate species aggregate at ponds to call. Therefore, the AST technique will probably be most useful in the temperate zone for acquiring species inventories and for mapping distributions of breeding populations (or choruses) and breeding habitats.

The AST technique should generate accurate estimates of the relative abundance of calling males and precise delineations of spatial distribution for species that call over a prolonged period of weeks or months. The method probably does not provide accurate density and distribution estimates for explosively breeding species (sensu Wells 1977) that call on only one day or very few days each year, because these species are encountered so infrequently.

The call count method for mapping distributions and estimating relative abundances of calling males is comprehensive and powerful. No time is spent searching, because within a certain strip width defined by the detection threshold of the species call, the probability of detecting a caller approaches 1. Therefore, hundreds of hectares can be surveyed quickly (Zimmerman 1991). More important, all habitats or microhabitats and forest strata are sampled practically equally; arboreal and fossorial species are counted as easily as ground dwellers, and concealed species are included as easily as unconcealed species. The only species not sampled are those with a short aural detection distance that call from high in forest canopy. We do not know how many, if any, such species exist.

Each person taking data must learn the advertisement calls and determine the detection distance for each species in each major habitat type at the study site.

BACKGROUND

Two transect methods are widely used for estimating terrestrial animal abundance. In *line transect sampling,* the perpendicular distance between each visually or aurally observed individual and the transect midline is estimated by the observer (Burnham et al. 1981). Line transect sampling compensates for the decrease in probability of detecting organisms with increasing distance by basing density estimates on perpendicular distances, so distant observations can be used. To estimate abundance, sample sizes are adjusted according to the detectability of a species (Burnham et al. 1981). An important assumption in line transect sampling is that all individuals on or near the center line are detected, although animals may go undetected away from the center line.

In *strip transect sampling,* all animals of interest within a fixed distance perpendicular to either side of the transect, the strip width, are recorded. The critical assumption of this method is that all individuals within the strip are detected. Because all individuals are counted, strip transects are treated as quadrats (Burnham et al. 1981; Seber 1982). Strip transect sampling has been used most widely to count birds, large mammals, and animal signs (Seber 1982, 1986).

Calling frogs satisfy the main criteria for strip transect analysis; they are readily detectable and identifiable (Eberhardt 1978). Members of a frog assemblage can be identified accurately by sound because species vocalizations are almost always species-specific and distinctive, vocal repertoires usually consist of only one or two call types, and normally fewer than 70 species of frog inhabit any site. If an animal calls within the observer's hearing range, it will be detected with high probability because the sound reaches the listener directly. In contrast, many variables affect whether a frog will be seen or not (e.g., size, movement, coloration, weather, position, observer search-pattern bias), even when it is well within the observer's visual range. In audio surveys, the animals advertise their presence; in visual surveys, the observer must find them.

Line transects have been widely used to sample singing birds (Ralph and Scott 1981) because distant observations can be used without worrying about changes in detectability. However, unlike the situation with many bird calls, the transmission distance of most frog calls is not great (Zimmerman 1991), and distance estimation is too inaccurate to be useful (Bart et al. 1984). Therefore, the main advantage of line transect sampling for birds, which is to extend the range of the survey, is not realized with frogs.

Emlen (1984) proposed a variable width strip technique for censusing calling birds that is essentially the same as the one outlined here for use on frogs. The strip width is twice the maximum distance the animal can be heard by the observer(s) and is determined separately for each species. All calls detected are recorded by the observer walking transects, and transects are surveyed repeatedly. Emlen (1984) concluded that densities calculated from average detection distances derived by the same experienced people working extensively and continuously in one area are more accurate than densities based on subjective observer-to-bird distances for all detections. Bart et al. (1984) compared line and strip transect methods for surveying calling yellow rails in Michigan. Their study is particularly relevant because the behavior of yellow rails resembles that of many rain forest frogs; that is, they are secretive and uncommon and call primarily at night (Bart et al. 1984:1382). The authors concluded that the strip transect method was superior to the line transect method for the following reasons: Estimating distances to calling individuals was not feasible; the length and

width of the transects could be adjusted to the terrain, weather, and yellow rail density; and all calling birds could be found if they were calling when the transect was sampled.

ASSUMPTIONS AND INTERPRETATION. Several additional assumptions are specific to the AST technique when used with frogs. The first is that the observer is fully knowledgeable of the species-specific calls. If information about frogs at a site (e.g., species list, tapes of calls) is not available, it could take an entire season to learn the calls. The time required will depend on the complexity of the fauna. If a site is relatively well known or if the fauna includes few species, the time required for learning calls will be reduced. A second assumption is that calling generally equates with breeding and that only males call (Littlejohn 1977; Wells 1977). This assumption was validated at a site in the central Amazon by the concordant distributions of calling males and their conspecific tadpoles (Zimmerman and Rodrigues 1990; Gascon 1991). Therefore, call counts can be used to locate breeding sites and to estimate abundances of breeding males; they cannot be used to determine distributions and abundances of females, juveniles, or silent males.

Because most frogs call obligatorily and practically exclusively while attempting to breed at a site, often an investigator can identify breeding habitats or microhabitats and, consequently, critical subsets of areas for conservation without ever seeing a female, juvenile, or larva (Zimmerman and Bierregaard 1986). Exceptions to this general rule include members of the Neotropical family Dendrobatidae, which lay eggs near call sites but later transport tadpoles some distance to aquatic sites where development is completed.

Use of AST data to estimate total adult abundance requires that maximum abundance of calling males correlate in a constant way with the abundance of noncalling adult males and the abundance of adult females. The violations of these assumptions are usually too severe to allow investigators to extrapolate male calling data to total population size.

The AST technique has several other assumptions. (1) Each calling frog is counted only once per sample (i.e., it does not move a significant distance). (2) Every male calling singly within the strip is detected. (3) The detection distance of a given call remains constant (i.e., an individual always calls at the same decibel level). (4) Mean detection distance of a species is appropriate for all microhabitats in which the species is usually found and for solitary individuals and choruses. (5) All habitats within the study area are sampled.

Several factors may affect the accuracy of assessments of abundance and habitat occupancy based on AST counts. For example, if counts of a species are not made during its peak breeding period, when the maximum number of males is calling, differences between samples in numbers of calling males may simply reflect differences in stage of the breeding cycle. Length of the breeding period also may affect accuracy. Males and breeding sites are most likely to be recorded for species in which the breeding period is prolonged and males call continuously (i.e., nightly for weeks or months). Relative abundance and distribution of species that appear sporadically over months (i.e., call anytime during weeks or months but not on most nights or even every week) can also be determined if transects are sampled frequently over several years. Even so, values for these species may be underestimated compared with those for continuously calling species. Long-term sampling also increases chances of encountering choruses of explosively breeding species, but accuracy of the count is probably lower than for species with longer breeding periods.

To determine relative abundance accurately, the distribution of a species throughout its available breeding habitat must be considered. The most reliable estimates of densities of calling

males are obtained for widespread, continuously calling, prolonged-breeding species, because sample sizes are greatest. Species with patchy or sparse distributions are encountered less frequently along randomly located transects, and mean densities are calculated from much smaller samples.

The error arising from differential phenology and spatial distribution of species reflects scale inequality. If calling males of a species nearly saturate their habitat spatially and/or temporally (by calling continuously), the number of individuals in the area and, therefore, the estimate of density vary little between transects, months, or years (Wiens 1981). If the species is rare, patchily distributed, and/or calls infrequently, then intertransect and intermonth density estimates will vary substantially even if the population is constant. A particular temporal and spatial scale may be too small to estimate densities of widely dispersed and infrequently calling species accurately, but adequate to measure densities of densely packed or continuously calling species. The implication is that only abundances of species that are sampled at roughly the same scale should be compared. Overall frog densities of continuously calling species cannot be compared with those of sporadically calling or clumped species, because populations of the former would be more evident and therefore better sampled.

OBSERVER EFFECT. Observers must have full hearing ability and be experienced (Emlen and DeJong 1981; Faanes and Bystrak 1981; Kepler and Scott 1981; Ramsey and Scott 1981). Even so, observer bias can profoundly affect the results of an acoustic survey, as has been shown with bird call counts. Methods proposed to reduce this bias in bird surveys (Ralph and Scott 1981; Bart and Schoultz 1984) apply equally well to frog surveys.

Observer effect is most likely to bias abundance estimates of species with high-frequency calls (4 kHz or more) and/or high call rates in large choruses. High frequencies attenuate more quickly than low frequencies, and high-frequency frog calls may be masked by insect noise in the same frequency band. Rapid call rates are a problem when more than a very few individuals are calling, and detection rates of such callers may vary among observers. It is nearly impossible to count individuals by call in large choruses because it is difficult to discern individual callers in the cacophony. Species with highly overlapping calls can be counted visually or, if callers are abundant and widespread, can be sampled aurally in small quadrats. Kepler and Scott (1981) recommended that observers perform simultaneous but independent trial surveys to identify species whose detection is inconsistent and to allow procedures to be corrected, where necessary, before the real survey.

To minimize potential observer bias from variable sound transmission, call surveys must be performed in similar habitats and under similar meteorological conditions (Emlen and DeJong 1981). Wind and rain are particularly disruptive because they reduce distances at which calls can be detected.

There is evidence that as the number of singing birds audible from a listening station increases, correct identification of the number of singers declines (Duke 1966; Bart and Schoultz 1984). Therefore, the number of animals recorded in a call survey may differ among populations, and the densities of abundant species and changes in their densities may be underestimated (Bart and Schoultz 1984). The importance of density-dependent bias in frog call samples is not known. It is likely that observer efficiency with frogs will decrease at caller densities somewhat higher than those measured for birds (Bart and Schoultz 1984). Densities of species in which aggregations of four or more callers occur regularly may be somewhat underestimated with this technique. Discerning number of callers in species with ventriloquial calls may be difficult with more than two individuals.

LIMITATIONS. The AST method has several limitations. (1) Numbers of calling males cannot be determined aurally for chorusing species or in situations of high call overlap (high densities of males calling at high rates). (2) Some reproductively active males of certain species do not call (e.g., Fellers 1979), so that even maximum counts probably underestimate the actual number of adult males present. (3) Explosively breeding species are acoustically evident for extremely short periods and are probably not sampled adequately. (4) Absolute population size cannot be estimated, because male and female survivorships may not be related and because maximum counts of calling males may not be constant proportions of the adult population.

RESEARCH DESIGN AND FIELD METHODS

Transects should approach a length of 1 km if they are to provide accurate estimates of relative abundances of rare as well as common species (Engel-Wilson et al. 1981; Hanowski et al. 1990). Transects should be spaced far enough apart so that frogs on one transect cannot be heard from another, ensuring independence of observations. Ideally, transects should be located at random with respect to frog breeding sites. In practice, observers usually use trails laid out for other purposes (e.g., hunting, travel between villages). Because such trails have been situated irrespective of frog breeding sites, the violation of criteria for random placement is minor enough to allow their use for this technique.[1] However, sampling order and starting points of the transects within each major habitat type must be determined randomly. It appears that 6 to 9 surveys during the breeding season of each of two to five 1-km transects are enough to provide accurate and consistent estimates of relative abundance for moderately abundant species (1–5 individuals/ha). At least 15 censuses are required for rare species or for 0.5-km transects (Engel-Wilson et al. 1981).

Observer calibrations for transect strip widths are determined separately for each frog species. The observer measures the minimum distance at which the frog can no longer be heard clearly (Zimmerman 1991). Measurements are made for at least six different frogs of each species to derive the mean detection distance and standard deviation. Detection distances should be measured in all the microhabitats in which calling frogs normally occur, and for frogs calling both alone and in groups. Sound transmission distances, and therefore detection distances, vary greatly with gross vegetation structure (Marten and Marler 1977; Marten et al. 1977), so mean detection distances must be estimated in each habitat (e.g., primary forest, dense secondary growth, grassland, swamps). Accurate detection distances are imperative because they strongly influence resultant density estimates.

Calling activity of nocturnal tropical frogs is generally greater before midnight (between 1800 and 2400 hrs) than after midnight (between 0000 and 0600 hrs—Zimmerman 1991). Diurnal species, in contrast, have pronounced calling peaks at dawn and dusk and after rain (L. O. Rodriguez, pers. comm.; pers. obs.). In general, the most productive time to walk transects is from dusk through the first two or three hours of darkness because this period encompasses peak calling activity of most species in both tropical and temperate zones. Observers simply walk transects and record the species, number of individuals, habitat or microhabitat, location, and time of each frog or chorus heard. Transects are sampled at a walking speed that depends on observer preference and terrain. It is most efficient to record observations verbally on a small tape recorder while walking and to transcribe the data later.

1. Some trails made by local people follow streambanks, and in some places streams are important breeding sites. In these situations the trails are not independent of breeding sites, and the results must be interpreted accordingly.

PERSONNEL AND MATERIALS

One of the strengths of this technique is that data can be taken by a single person. During the observer calibration phase, a second person can be helpful.

A good-quality tape recorder, a microphone, and a sound-level measuring instrument (Appendices 3 and 6) facilitate the calibration phase of frog detection distance and can be used to record frog calls unknown to the observer. It is imperative that the calling individuals, as well as tape recordings of calls, be preserved as vouchers for the study (Appendix 4). Other materials needed are data forms (or paper), pencils or pens with indelible ink, headlamps (Appendix 6) for night work, and, if affordable, a hand-size tape recorder for taking data in the field.

DATA TREATMENT AND INTERPRETATION

Counts used to calculate calling male or chorus density must be made during the species' breeding period when males are likely to call. For purposes of this technique, a species' breeding period is defined as that interval when most calling is observed throughout the study area. This interval is determined by plotting the mean number of callers or choruses against week or month of the study and identifying activity peaks. If males appear equally likely to call in any month, as is the case with some tropical species, the breeding period is considered to be all year, and counts from all months are included in the analysis.

To estimate density of calling males (or choruses) of a species along a transect, the maximum count of individuals (or choruses) during the breeding period is divided by the strip area for that species. The strip area is the strip width (i.e., twice the mean detection distance of the species call) multiplied by the length of the transect. The mean density of calling males of the species in the study area during peak breeding activity is the mean of the maximum densities recorded on each transect. This meticulous transect-by-transect analysis is facilitated by use of a computer if there are many transects, many species, and many months (Zimmerman 1991).

If breeding individuals of a species are found only in certain subhabitats within the study area (e.g., stream valleys or temporary pools within a tract of primary forest), then caller densities must be standardized with respect to the subhabitat (e.g., callers per hectare of stream valley or per pool rather than callers per hectare of primary forest). Subhabitat-specific densities can be converted to values representing the entire study area if the proportion of different subhabitats within a study area is known (e.g., from aerial photography, topographic maps, or habitat surveys).

SPECIAL CONSIDERATIONS

One can sample species in linear habitats (e.g., shorelines of ponds or lakes, along streams) by counting calling individuals. This procedure does not require determination of a detection distance and thereby differs from AST. With linear habitats, calling-male density is calculated as numbers of calling males per kilometer of linear habitat.

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4. Quadrat Sampling

ROBERT G. JAEGER AND ROBERT F. INGER

Quadrat sampling consists of laying out a series of small squares (quadrats) at randomly selected sites within a habitat and thoroughly searching those squares for amphibians. This technique can be used to determine the species present in an area, their relative abundances, and their densities. Because quadrats are placed at random in the area of interest, and because each quadrat constitutes an independent sample, statistical inferences can be drawn from the data, given that

the number of quadrats used is sufficiently large. Statistical inferences can be used either for monitoring (changes in population abundance in a given area through time) or for inventory (differences among areas of interest at a given point in time).

The strength of quadrat sampling, with a large number of randomly placed quadrats, is that for any area, effects of habitat heterogeneity do not compromise the results. This is true even if the area of interest contains many different kinds of habitat "patches."

Quadrat sampling has been used most effectively for sampling amphibians in the forest litter, where species often occur in high densities but are difficult to detect because of their secretive habits. It also can be used to sample aquatic amphibians (e.g., salamander larvae). A pond is divided into quadrats, and the quadrats are randomly sampled by a standardized dipnetting method (see "Quantitative Sampling of Amphibian Larvae," below). Open-area habitats can also be divided into quadrats, and at a later time the number of amphibians (e.g., frogs) visible in each randomly selected quadrat can be counted. The method loses effectiveness in habitats with dense ground cover and on irregular or steep terrain where it is difficult to place quadrats on the ground at random. Quadrat sampling should be used only when (1) animals do not leave the quadrat due to sampling disturbance before being counted, (2) quadrats can be randomly (not haphazardly) placed, and (3) quadrats yield independent data (not repeated measures).

TARGET ORGANISMS AND HABITATS

Quadrat sampling is particularly useful for studying forest-floor or streamside species of amphibians. Although the method has been used in relatively open vegetation, it is most effective in closed-canopy forests. In species assemblages having a significant proportion of riparian species, riparian and nonriparian components of the assemblage can be sampled by separate sets of quadrats. Quadrat sampling has been effectively employed in tropical forests to determine density, species diversity, and relative abundances (Lloyd et al. 1968a; Scott 1976; Inger 1980; Lieberman 1986), and in temperate forests to follow densities of salamanders over long periods (Jaeger 1980a,b; Mathis 1990).

BACKGROUND

When attempting to test for temporal or spatial differences in number, relative abundances, and densities of species, statistical testing of the null hypothesis that any differences detected are due to chance alone can be used. Thus, it is important to satisfy the statistical conditions of random sampling and independence of data sets. Correctly employed quadrat sampling techniques meet these requirements.

MONITORING. For measuring changes through time in a given area of interest, multiple quadrats should be placed at random in the area at each period to be measured. If animals are not removed from the area during sampling, it may be possible to reuse previously established quadrats in subsequent sampling. If animals are removed during sampling, then subsequent quadrats should be placed randomly with the restriction that previously sampled quadrat localities are not resampled. This technique is superior when sampling a given area before and after some type of treatment (e.g., perturbation of the habitat, such as biocide treatment). Quadrats, in addition to being placed randomly in an area, should be sampled in random sequence to minimize the effects of uncontrolled short-term temporal changes (e.g., weather).

INVENTORY. For measuring differences at a given time among different areas (or habitat types), multiple, randomly placed quadrats should be established in each area of study and sampled in random sequence. Ideally, all quadrats in all areas or habitats would be randomly

sampled sequentially, but this would require the observer to move back and forth among the different areas for sampling, expending valuable time.

ASSUMPTIONS. Effective use of quadrat sampling requires first that all animals be equally "available" to the researcher. If the study is concerned with a single species, it is best to lay out quadrats at a season or under weather conditions considered most favorable for that species, such as immediately after warm spring rains for terrestrial temperate salamanders. Repeated sampling in different years should be carried out at the same season and under similar weather conditions. If the study is concerned with many species in an assemblage, it is important to recognize that fossorial or semiarboreal species are not sampled as efficiently by this method as species remaining within or on the floor litter. Again, repeated sampling of an area (or of other areas) should be conducted at comparable times of the year.

Second, bias must not be introduced by changing observers during the study. Different observers have different visual quirks. If small quadrats (1 × 1 m) are used, it is best that a single person search all of the quadrats. If that is not practical, several observers should be assigned quadrats on a random basis. If large plots (8 × 8 m) are used, four or five persons are necessary to minimize escape of animals. The same team should be used for all quadrats.

STRENGTHS OF THE TECHNIQUE. Although quadrat sampling is labor-intensive, it has the advantage of bringing the eyes and hands close to the targets. Fossorial species, arboreal species that rest in the litter, and species that merely pass through the litter for short periods in the life cycle (e.g., juveniles) are all likely to be seen in quadrats, if sample sizes are large and plots are well distributed over the seasons. This technique is excellent for dealing with habitat heterogeneity when sampling for multiple species with different microhabitat preferences. It also allows for powerful statistical analyses.

RESEARCH DESIGN AND FIELD METHODS

Ideally, the area of interest should be visualized as though covered by a rectangular grid, and sampling quadrats should be located within the grid by use of a table of random numbers (Appendix 7). Local topography may make such a scheme impractical, but any necessary modification should depart as little as possible from the ideal. Quadrats should be separated by enough distance to avoid presampling disturbances.

On a map of the sampling area of interest, relatively large squares (e.g., 100 × 100 m, although this particular size is not critical) are identified or located. Each square is assigned a number for use in randomized sampling. Two methods for quadrat sampling are now possible: point sampling and broad sampling. *Point sampling* (small quadrats) should be used when studying single species with small, densely distributed individuals (e.g., the salamander *Plethodon cinereus,* in which density can reach 3 individuals/m^2 and adult total length can range to about 9 cm). *Broad sampling* (large quadrats) should be used to sample species in which individuals are widely dispersed, large-bodied, or both and to sample multispecies populations. In either case, all quadrats in the study are the same size. Comparability among samples is limited to quadrats of the same size. We recommend that small quadrats measure 1 × 1 m. We recommend that large quadrats measure 8 × 8 m rather than 10 × 10 m, because in most of the previous studies of amphibians, large quadrats have measured 25 × 25 feet, which approximates 8 × 8 m.

POINT SAMPLING. A priori, one chooses the exact number of large squares (or units) to be sampled; 25 to 30 will provide sufficient data for statistical analysis. Using a random numbers table, the investigator identifies these squares

and the randomized sequence of sampling. A sequential series of compass directions and distances (up to 20 m, although this may vary with the dimensions of the grid) are also determined. It is easiest to complete this phase of the study design prior to going to the field. Once in the field, the investigator goes to the center of the first square to begin a randomized walk. He or she walks in the compass direction and for the distance previously determined, to arrive at the first point-sampling spot. He or she drops a 1 × 1 m quadrat-frame on the ground, quickly removes every stone, piece of wood, and leaf in the quadrat, and counts the number of individuals of each amphibian species present. The debris and the amphibians (if resampling is to be done at a later time) are replaced, and the investigator walks for the next number of meters in the next compass direction in the sequence, again dropping the quadrat frame and sampling as before. This procedure is repeated for a total of 10 sample points. A restriction is that a given spot can be sampled only once in a particular sampling period. The investigator moves to the second randomly chosen large square and repeats the point-sampling procedures. The process is repeated until all of the large squares have been sampled.

The 10 point samples in each large square can be averaged to yield a mean density of each species in that sample unit. If the research design includes 25 to 30 large squares, then the procedure yields 25 to 30 independent data points. Two-sample statistical tests can be used to compare two areas (or habitats) or the same area (or habitat) at different times. Multiple sample statistical tests are used for comparisons among several areas or several samples through time.

BROAD SAMPLING. A priori, the investigator chooses the exact number of large quadrats to be sampled; 50 to 100 are sufficient. Using a table of random numbers, he or she determines the position of each quadrat. If, for example, the area to be sampled is 1,000 × 1,000 m, the first 3-digit number determines the position on the x-axis and the second 3-digit number determines the position on the y-axis for the corner of the first quadrat. An 8 × 8 m quadrat is laid out using stakes and twine. One person on each side of the quadrat removes all litter from a 30-cm swath along the outer perimeter in order to make an escaping animal more easily visible and to minimize possible bias due to edge effect. Each person removes the litter and ground cover from strips inside the quadrat and parallel to the boundary twine, working successive strips from the outside toward the center, until the entire area is covered. Appropriate information for all amphibians encountered is recorded. If the area is to be resampled later, all litter, ground cover, and amphibians should be replaced. This process is repeated, in a randomized sequence, for all quadrats.

Although statistical variances for average densities may differ from area to area, they usually approach an asymptote for means based on between 50 and 100 quadrats even where densities of species are low (Lloyd et al. 1968a). A sample from a single season or a single site should include at least 50 quadrats.

For valid estimates of species densities, investigators must take extraordinary care to follow completely randomized procedures, not an easy task under field conditions. The temptation to look for amphibians of interest in areas deemed "good" for such animals always exists. Acting on such temptation leads to sampling bias (see Chapter 4) and may invalidate any generalizations possible from statistical analysis (or the analysis itself), or it may undermine the goal of the study, which is to obtain accurate estimates of species number, relative abundances, and densities. Sampling biases usually result in incorrect estimates of species densities.

To illustrate, say that an investigator wishes to compare the densities of two species of salamanders in two forested valleys. In valley 1, species

Table 5. Sample Data from Broad Sampling of an Area Using the Quadrat Sampling Technique[a]

Quadrat number	Number of individuals of each species					
	Species A	Species B	Species C	Species D	Species E	Species F
02	1	0	0	0	4	8
05	1	0	0	0	10	2
11	0	0	0	0	8	2
15	1	0	0	0	16	4
19	2	0	0	1	11	10
21	1	1	1	0	7	4
22	0	0	7	0	8	21
24	10	1	1	1	9	15
32	0	1	0	1	0	11
35	1	4	1	0	7	20
37	0	0	0	0	9	9
40	1	0	1	0	4	32
49	13	5	1	0	8	14
52	1	0	0	0	5	19
56	1	2	1	0	7	27
60	0	0	0	0	4	1
61	1	1	3	2	9	18
62	1	1	1	0	7	45
68	1	2	2	0	15	17
73	0	0	0	0	0	0
77	9	3	2	1	9	9
78	1	0	0	0	7	23
82	1	1	1	0	9	23
85	1	1	0	0	9	23
91	0	1	1	0	1	10
96	0	0	0	0	8	16
97	1	1	0	0	10	14
Total	49	25	23	6	201	397
Abundance						
Average	1.81	0.93	0.85	0.22	7.44	14.70
Relative (%)	7.0	3.6	3.3	0.9	28.7	56.6

Species richness = 6 species

n = 27 quadrats

[a] Data are provided for 27 quadrats measuring 8 × 8 m. An actual data set should include samples from a minimum of 50 quadrats.

A is patchily distributed under logs; in valley 2, species B is evenly distributed in the forest leaf litter. The two methods of quadrat sampling described above will allow an unbiased sampling of individuals and species in both valleys, because a reasonably large number of randomly placed quadrats will include both patchy and more homogeneous areas of habitat. The need for biodiversity studies based on randomization cannot be overemphasized.

We recommend that the following information be recorded for each quadrat sampled: (1) location of the quadrat within the grid; (2) date, time at which sampling begins and is completed, and general weather conditions during sampling; (3) temperature and relative humidity; (4) vegetation type in the quadrat; (5) slope of area on which the quadrat is located (using a clinometer); (6) canopy cover (as a percentage of area directly above the quadrat); (7) leaf litter cover (percentage of the quadrat covered by leaves, and depth of the leaves—the latter can be estimated by pushing a sharp stick into the litter at the four corners of the quadrat and counting the number of leaves pinned to the stick); (8) herb cover (estimated percentage of quadrat covered by herbs and seedlings < 1 m tall); (9) shrub cover (estimated number of multistem plants > 1 m); (10) tree numbers and sizes (measured as diameter at breast height); (11) rock cover (estimated percentage of area covered, and size range of the rocks); and (12) logs (number and size).

PERSONNEL AND MATERIALS

Point sampling is minimally biased if a single person samples all quadrats. Broad sampling usually requires a minimum of four persons.

Quadrat sampling requires few materials: random numbers table, map of the sampling area(s), meter measuring tape, bags in which to place amphibians, watch, compass, and either string and stakes or a 1 × 1 m quadrat frame.

DATA TREATMENT AND INTERPRETATION

For comparisons of species numbers, relative abundances, and densities, only the number of individuals of each species in each quadrat needs to be recorded. Data from broad sampling may be recorded easily, as shown in Table 5; for point sampling only two columns would be used, one with the heading "Quadrat number" and another with the heading "Number of individuals in quadrat."

Species richness can be obtained from a simple tally of the number of species found in the target area, just as with other sampling methods. However, this total is the accumulation over all quadrats sampled. Species densities and relative abundances can also be determined (e.g., see Table 5).

Quadrat sampling can provide suitable data for investigation of spatial patterns. A variety of statistical distributions have been used to describe the scatter of different taxa in target geographical areas. The approach to fit and estimation is well described by Krebs (1989).

When data from more than one study are evaluated, locality or time comparisons can be made by descriptive and inferential statistical methods. Regardless of the procedure selected, the problems of zero entries and the so-called ties in ranking will need to be evaluated as possible sources of bias and reduced precision (see Chapter 9).

Quadrat sampling methods have been applied in several studies (e.g., Heatwole and Sexton 1966; Barbault 1967; Lloyd et al. 1968a; Toft 1980; Scott 1982; Lieberman 1986; Fauth et al. 1989), although not always precisely as we have defined them here.

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5. Transect Sampling

ROBERT G. JAEGER

Amphibians frequently respond differentially to environmental gradients, especially gradients that reflect moisture. Transect methodology can be used to sample either across these habitat gradients or within habitat types. Randomly located narrow strip transects (e.g., 2 m) are laid out, within which portions of the habitat are searched thoroughly for amphibians.

The strength of transect sampling, using a randomized design, is that it effectively tracks species numbers, relative abundances, and densities across habitat gradients. That is, the method is very useful in determining intraspecific and interspecific changes in amphibian populations across some continuously changing environmental feature. Thus, transect sampling is the best technique for studying elevational gradients (on mountains) or habitat gradients from lowland (e.g., streambed) to upland (e.g., forest floor), and is preferred over visual encounter survey techniques for such studies (see "Visual Encounter Surveys," above, for further discussion of differences between the two techniques).

Because transects are randomly placed and because transects constitute independent samples, statistical inferences can be drawn from the data, given that the number of transects used is sufficiently large. Statistical inferences can be used either to monitor changes in a given area through time or to evaluate faunal differences between areas at a given time.

TARGET ORGANISMS AND HABITATS

Transect sampling has been effectively employed in open temperate forests to determine the patchy distribution of the salamander *Plethodon cinereus* on the forest-floor (Jaeger 1970) and to determine the distributions of genotypes (i.e., relatedness of individuals) of this species in the forest (J. Neigel and R. G. Jaeger, unpubl. data). Transects also have been used for precise mapping of distributional and habitat discontinuities between parapatric species (Jaeger 1970) and for tracking changes in numbers and densities of salamander species with elevation on a mountainside (Hairston 1951) and along gradients from streams to upland areas (Hairston 1949, 1980b). Transect sampling also can be used with anuran species that exhibit low mobility during the sampling period (i.e., do not move out of the transect due to sampling disturbances).

BACKGROUND

To measure changes through time in a given area of interest, multiple transects should be placed at random in the area at the first sampling period. In subsequent sampling periods, transects are placed at random with the stipulation that previous transects are not resampled. Depending on the length of each transect, either the entire transect (for shorter transects) or randomly chosen subsections of it (for longer transects) can be sampled. To measure differences among two or more areas at a given time, the same procedure is used, except that each area is sampled (with replicates) only once.

The configuration of the multiple transects depends on the question being asked. If an investigator wishes to sample continuously—crossing a known gradient of habitats (e.g., up the side of a mountain; from a stream onto the forest floor)—then parallel transects should follow the gradient, and each should start at a randomly chosen point along a predetermined starting line (e.g., at a given elevation on the mountain; in the center of the stream). However, if the investigator wishes to compare differences at specific places on the gradient (e.g., within different habitats or ecotones), then parallel transects should be oriented perpendicular to the gradient, and each should start at some randomly determined point.

Whatever the configuration of the transects, they should be sampled in a randomized sequence to dampen the effects of short-term temporal changes in the sampling areas. For example, weather conditions may change over a short time, and this may influence the number of amphibian species or the density of each species observed in different transects. A thoroughly randomized design will allow for reasonably unbiased estimates. Each of the multiple transects should be sampled in random sequential order. Within a particular transect, at the very least, the end at which sampling begins should be determined randomly. If each transect is partitioned into subsections, those to be sampled should be selected randomly and sampled in a random sequence.

Multiple transects in each area to be sampled are preferred. It is difficult to obtain the replicated samples needed for statistical testing from a single transect. A single, very long transect certainly provides the easiest route to sampling in the field, but this method should be avoided whenever possible. However, a single transect will be necessary in certain circumstances, such as when sampling the species in a single stream or on the floor of a very narrow canyon.

Randomized placement of transects is important for preventing biased sampling of an area. Thus, establishing transects in areas that "look like good places" to find the species of interest must be avoided. Unbiased sampling procedures will estimate populational changes along the gradient, rather than in a particular location suspected to be favorable.

Interobserver differences also can lead to sampling errors. Such differences are reduced by a randomized sampling design. This point is discussed extensively in the section "Quadrat Sampling," above. Randomization can be implemented if each person samples the same number of transects in each area and if those transects are randomly assigned to each person.

RESEARCH DESIGN AND FIELD METHODS

Different approaches are used for comparing transect samples taken across an obvious habitat gradient and for comparing samples taken from among discrete subsets of the habitat gradient.

CROSSING THE GRADIENT. This approach is most commonly used to compare differences across habitats, such as sampling from a stream to uplands or sampling along an elevational gradient. In this technique, multiple transects are placed in parallel.

To sample from a stream to uplands, the stream itself becomes the starting line, where all transects begin. The portion of the stream of interest is marked at uniform intervals (e.g., every 5 m) to provide known points of origin for the transects.

To sample along an elevational gradient (e.g., a mountainside), the investigator chooses an elevational starting line. This line can be at the bottom of the mountain (if the entire mountain is to be sampled) or at any contour line on the mountain. Again, the starting line is marked at uniform intervals.

Transects used in gradient studies can be placed in various ways. With an arithmetical progression, transects begin at equal intervals along the starting line. This is a poor design, because it lacks randomization. Yet it is the technique easiest and fastest to use in the field. With a geometric progression, each transect is separated from the next by a geometrically increasing distance. For example, transects 1 and 2 might be separated by 2 m, transects 2 and 3 by 4 m, transects 3 and 4 by 8 m, and so forth. This design does not satisfy statistical randomness and is inappropriate for the kinds of studies of interest here. The preferred design is a randomized one. Here, randomly chosen points along the starting line, selected from a table of random numbers, define the origins of the transects.

The length of each transect and the number of sampling points on each will depend on the question being asked (or the hypothesis being tested) and the area to be sampled. The length of the transect is determined by the minimum distance that traverses the entire range of habitats in the gradient. Clearly, transects running from the bottom to the top of a mountain will be quite different from those running from a stream into the surrounding forest. Whether one area is to be sampled at several different times or several areas are to be sampled at one time, the shortest transects possible should be sampled. Sampling short transects allows for more replicated transects to be included in the survey. In general, the greater the number of replicates, the less the chance of committing a type 2 statistical error (see Chapter 4).

An excellent design for small-area sampling involves random location of the origins of 25 to 30 parallel transects on the starting line; using this many transects will provide sample sizes adequate for statistical testing. Transects should be 100 m long and 2 m wide and partitioned into 100 subsections measuring 1 × 2 m. Ten subsections are randomly chosen from each transect for sampling. Within each subsection, every rock, piece of wood, and leaf is turned, and the number of individuals of each species is recorded. This sampling procedure provides 250 to 300 blocks of data over all transects in the area. If shorter transects are established, it is possible to sample each along its entire length, but the risk that the observer will drive animals just ahead, out of the transect, increases.

SUBSETS OF THE GRADIENT. The technique described above is not designed to reveal changes in species parameters along transects. Rather, it is designed to estimate parameters for the entire given area of interest. Thus, a transect running from a stream to uplands will treat all species and individuals encountered as in the area of interest, despite the habitat gradient. In contrast, certain types of studies may focus on just such parameter changes, as from a streambed to uplands. It is tempting to treat each transect described above (see "Crossing the Gradient") as a measure of change in species parameters along that transect. I do not recommend this treatment, because there is no information on distributions of individuals within the habitat types encountered along the gradient. An alternative method is to rotate the direction of the transects by 90° such that the first transect to be randomly subsampled lies in the center of the stream, the second at a given distance toward the edge of the stream, and each succeeding one at the same distance from the previous one and parallel to it, until the uplands is broached. Subsections of each transect provide independent data points to test for clinal changes in species and population parameters. Thus, each transect essentially surveys a different "habitat" along the cline, and the randomly chosen subsections of a transect become replicated data points for that habitat. The same approach can be used on elevational gradients (such as mountainsides), where transects can be placed, in arithmetical progression, along contour lines.

TRANSECTS IN HOMOGENEOUS AREAS. Relatively short transects are sometimes used within a relatively homogeneous area. Because habitat gradients are neither severe nor predictable (i.e., they do not occur in a straight line, such as up a mountainside), parallel transects are replaced by randomly positioned transects. I discourage use of this technique because the quadrat sampling method is far superior for determining species numbers, relative abundances, and densities in relatively homogeneous areas. Quadrat sampling also is more likely than transect sampling to uncover patchiness within an otherwise relatively homogeneous area. Finally, randomly placed transects tend to run into each other, causing problems of replicated samples (i.e., sampling the same place twice). Transects

should be reserved for studies of known habitat gradients.

Ideally, every aspect of a sampling design should be randomized, including the placement of transects, the selection of subsections to be sampled, and the order of sampling. For a study in which 10 subsections are sampled from each of 30 transects, a total of 300 subsections must be visited in random order. If two or more areas are sampled in the same time frame, the order of visits to all subsections among all areas should be randomized, but travel and time restrictions often make this approach impossible. However, such thorough randomization would ameliorate the effects of short-term changes in the observed number and densities of species that are due to short-term changes in the environment (e.g., weather).

Each transect should follow a straight line. This can be accomplished by following a given compass direction and running a string (anchored at one end) the length of the transect. The observer also must be careful not to disturb sections of the transect yet to be sampled, either while establishing the transect or while sampling other subsections. All transects should be laid-out using a tape measure, before the first sampling begins, so that the transects can be explored in random order.

PERSONNEL AND MATERIALS

The best results are obtained when a single person samples all transects or subsections of transects. When several individuals are involved, procedures should be used to minimize inter-observer bias.

Transect sampling requires only a random numbers table, a map of the sampling area(s), a compass, a 100-m measuring tape, and string, stakes, and flagging to mark the transects.

DATA TREATMENT AND INTERPRETATION

For comparisons of species numbers, relative abundances, and densities through time or space, only the number of individuals of each species in each transect or subsection of each transect need be recorded. Data sheets used for quadrat sampling (Table 5) can be adapted easily for use with transects, merely by replacing "quadrat" with "transect" or "transect subsection."

If each transect is sampled entirely, then the data are analyzed by transect and can be presented as units (e.g., mean density of a given species, number of species) per square meter. When subsections of transects are sampled, each subsection can be used as an independent data point. However, it is preferable to collapse the data for the sampled subsections into a mean for the entire transect (i.e., mean density per square meter, as above) to provide area-wide data sets. Final data analysis, then, is conducted on a set of data points, including one for each transect.

A transect can be envisioned as a long, rectangular quadrat; therefore, if all amphibians are seen and counted along the entire transect, the analytical methods appropriate to quadrat sampling apply. The probability of observing an animal may vary with its perpendicular distance from the path walked by the observer. Krebs (1989) provided a lucid examination of the use of detectability functions as an aid in minimizing this possible source of bias.

When transects are subsampled, the subsection results can be used as the sampling units in any of the inferential or descriptive statistical procedures suitable to answering the research question. Alternatively, a mean per transect may be used as the focus of analysis.

Eberhardt (1978), among others, provided details of statistical analysis appropriate for line transect methods. Models for population density estimation have been and continue to be developed under a varied set of conditions and assumptions. Seber (1973) and Burnham and Anderson (1976) provided methods for density estimation under general conditions of transect sampling. Burnham and Anderson (1984) and

Burnham et al. (1985) discussed the problems of incomplete counts, the need for distance data, and the bias and efficiency of strip transect methods. Skellam (1958) provided a general method for estimating density that allows for individual specimen mobility, assuming that the observer's presence does not affect it. Smith (1979) derived a model to eliminate this latter constraint. Rao et al. (1981) described a sequential program in which sampling is continued until a prescribed number of target organisms has been included in the sample. With a combination transect method (Rao 1984), sampling stops when either a defined number of animals has been sighted or when observations have been completed along a defined length of transect. Seber (1986) provided a readable review of estimation methods and important methodological improvements developed after 1979.

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6. Patch Sampling

ROBERT G. JAEGER

Amphibian density commonly varies within habitats. High densities are often associated with specific microhabitats or patches (i.e., logs, tree buttresses, bromeliads) that can be identified and randomly sampled. Patch sampling can be used to determine the number, relative abundances, and densities of species present in discrete subunits of an area of interest. Because patches are sampled at random in an area, and because each patch constitutes an independent sample, statistical inferences can be drawn from the data, given that the number of patches sampled is sufficiently large. Statistical inferences can be used either for monitoring (changes in a given area through time) or for inventory (differences between areas of interest at a given time).

TARGET ORGANISMS AND HABITATS

Patch sampling can be applied to any organism that is known or suspected to be confined to discrete microhabitats that can be considered as patches within a broader environment. For example, *Plethodon cinereus* in the eastern forests of the United States defend territories under rocks (Mathis 1990). Each rock on a section of forest floor can be considered as a patch. The technique also can be used to study the amphibian fauna of a particular patch type or habitat subunit.

BACKGROUND

Patch sampling is merely a modified form of quadrat sampling (technique 4). In quadrat sampling, the researcher studies all of the amphibians in a given area independent of whether individual species occupy patches, live in homogeneous areas between patches, or are found in both. Patches in the environment are randomly sampled along with the rest of the environment, and the area of interest is considered to be homogeneous.

In patch sampling, the researcher focuses on species of amphibians that inhabit patches and disregards species or individuals that occur between patches. Thus, the patches themselves can be treated as quadrats in a statistical sense. In quadrat sampling, the quadrats are placed at random in the environment; in patch sampling, the patches are fixed in space (but not necessarily in time), are treated as independent units, and are assigned numbers that can be used for purposes of randomization. Because patches can be treated as quadrats, the reader should consult the section on quadrat sampling for particulars about monitoring versus inventory and the necessity for a completely randomized design.

Several assumptions, in addition to those discussed for quadrat sampling, are basic to patch sampling. First, it is assumed that each patch has an unambiguous border and can be defined precisely. A bromeliad, for example, is a definable

patch, whereas a particular elevation on a mountainside may not be so definable in a biological sense.

A second assumption is that patches are operationally definable. A log "ranging from 1 to 5 m long and 10 to 80 cm in diameter" is operationally defined, whereas "a log" is not. Operational definition is important if one wishes to compare attributes of a species within patches between areas, rather than of species within the areas as a whole. For example, if one forest has many logs that are 5 × 0.8 m, whereas another forest has few such logs but many that are 10 × 1.5 m, samples from the second forest should not include large logs, because they were not included in samples from the first.

A third assumption is that in areas to be compared statistically, observers can locate all patches, or at least can locate the same proportion of patches, in an unbiased way. This means that patches need to be visible to the observer; for example, logs must be visible in a forest containing many shrubs or brambles.

Finally, it is assumed that observers can count all individuals of interest in a patch once it has been located. If individuals escape from a patch before being counted, then estimates, particularly of relative abundances of species, may be biased.

RESEARCH DESIGN

The procedure is quite simple. First one identifies all of the patches in the area of interest and assigns a number to each, in sequential order of discovery. If the number of patches is small, all patches are sampled. If patches are too numerous for all to be sampled, or if some patches must be left undisturbed as habitat for patch-inhabiting species, patches are selected randomly for inclusion in the study.

The number of patches required for statistical treatments will depend on the variance in the data, which is not known a priori. I recommend a minimum of 30 patches per area (or per sample period for monitoring). The 30 patches and the sequence of sampling are selected randomly. It is tempting to sample the patches in some sort of linear sequence, such as in a minimum-distance walk through the study area, but this is a poor option. Randomizing the sequence of sampling provides a degree of control for extraneous environmental variables.

FIELD METHODS

How a patch is actually sampled will depend entirely on the type of patch, and most patch sampling requires individualized techniques. It is important to detect every individual of every species that occurs in each patch. Wake and Lynch (1976) and Wake (1987) described procedures for sampling bromeliads and logs for salamanders. Heyer and Berven (1973) provided an example of sampling tree buttresses for amphibians and reptiles.

PERSONNEL AND MATERIALS

The basic tool for patch sampling is a table of random numbers (Appendix 7). Specific types of patches (e.g., logs, potholes) will require specific sampling materials. It is possible and desirable for a single person to sample all of the patches, to reduce interobserver sampling error.

DATA TREATMENT AND INTERPRETATION

To estimate species numbers, relative abundances, and densities in patches, only the number of individuals of each species in each patch need be counted. By substituting "patch" for "quadrat," data can be listed as shown for quadrat sampling (Table 5).

When one size or type of patch is sampled in only one target area, the results can be examined only in descriptive ways. For example, an estimate of richness or evenness may be obtained, as with quadrat sampling. If each patch's location is recorded (e.g., latitude and longitude), nearest neighbor and clustering techniques can be used to determine spatial distribution patterns. Data

on environmental and microhabitat conditions in each patch can help to explain microhabitat sharing or avoidance patterns among species.

When patches are sampled across time or space, species or specimen data may be compared with inferential techniques in which the sampling unit is the individual specimen.

RECOMMENDATION

Randomized patch sampling is a particularly good approach for inventorying or monitoring species that are restricted to particular microhabitats.

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7. Straight-Line Drift Fences and Pitfall Traps

PAUL STEPHEN CORN

Straight-line drift fences typically are short barriers (5–15 m) that direct animals traveling on the substrate surface into traps placed at the ends of or beside the barriers. Traps (described below) can be pitfalls, funnel traps, or a combination of the two.

Drift fences with pitfall or funnel traps and pitfall traps without fences are used commonly to inventory and monitor populations of amphibians and reptiles. For example, 9 of 17 field studies reported for management of terrestrial vertebrates (Szaro et al. 1988) used these techniques to sample amphibians. Drift fences with pitfall traps can be used to determine species richness at a site and to detect the presence of rare species. They also can yield data on relative abundances and habitat use of selected species.

Pitfall traps arrayed in a grid without fences can also be used to study the population ecology and habitat use of selected species. Population density can be estimated with this latter technique if it used in conjunction with mark-recapture techniques (see Chapter 8). Drift fence arrays or pitfall grids can be left in place for long-term monitoring.

In this section, I discuss the use of this technique to obtain data on amphibians away from breeding ponds. Use of drift fences and traps to monitor amphibian activity at breeding ponds is discussed in the section "Drift Fences Encircling Breeding Sites," below (technique 9). Some materials and procedures are common to both techniques. Investigators contemplating the use of drift fences and traps in any context should read both accounts.

TARGET SPECIES AND HABITATS

Both arrays of drift fences and grids of individual pitfall traps have been used to sample amphibian assemblages in a variety of temperate habitats, including deciduous forests (Pais et al. 1988), coniferous forests (Jones 1988a), riparian woodlands (Friend 1984; Jones 1988b), wetlands (Beauregard and Leclair 1988), and sandhills (Campbell and Christman 1982a,b). Aquatic salamanders are difficult to trap with pitfalls, but drift fences with funnel traps at the ends have been used successfully to trap *Siren* and *Amphiuma* in seasonally flooded stream bottoms (D. E. Runde and K. M. Enge, unpubl. data).

Drift fences and pitfall traps capture some species more easily than others (Karns 1986; Corn and Bury 1990; Dodd 1991b). Anurans that are strong jumpers or climbers (e.g., *Acris, Gastrophryne*, most *Rana*, most *Hyla*) are more difficult to trap than terrestrial species (e.g., *Bufo, Scaphiopus*) that lack these abilities (Franz and Ashton 1989; Dodd 1991b). Accordingly, numbers of the former either should be omitted from an analysis or should be reported with caution. For example, several studies report the capture of large numbers of the eastern narrow-mouthed toad (*Gastrophryne carolinensis*) using drift fences (Campbell and Christman 1982a; Enge

and Marion 1986; Mengak and Guynn 1987). Because of the climbing ability of this toad, however, the numbers of individuals captured probably do not accurately reflect its relative abundance.

Drift fences and pitfall traps usually sample terrestrial salamanders very well but undersample species closely associated with specific microhabitats. For example, in forests of the Pacific Northwest, the primary habitats of the plethodontids *Aneides ferreus* and *Batrachoseps wrighti* are large pieces of fallen trees, whereas *Ensatina eschscholtzii* and *Plethodon vehiculum* commonly are abroad on the forest floor. *Aneides* and *Batrachoseps* are seldom caught in pitfall traps (with or without fences), but *E. eschscholtzii* and *P. vehiculum* are captured in large numbers (Bury and Corn 1987; Corn and Bury 1990).

Some groups—for example, caecilians or tropical arboreal salamanders—normally cannot be sampled with conventional drift fences. However, Vogt (1987) captured the arboreal salamander *Bolitoglossa platydactyla* with funnel traps suspended between branches of trees on a plastic walkway.

Drift fences and pitfall traps are also effective at capturing ground-dwelling organisms other than amphibians, including insects (Greenslade 1964; Luff 1975), reptiles (Jones 1981, 1986; Campbell and Christman 1982a; Vogt and Hine 1982), and small mammals (Spencer and Pettus 1966; Beacham and Krebs 1980; Williams and Braun 1983).

BACKGROUND

Drift fences intercept amphibians moving on the surface of the ground and redirect them into a pitfall or funnel trap. Pitfall traps without fences act in a similar manner, but individual traps intercept only a few centimeters of ground versus several meters for a fence. Therefore, large numbers of traps are needed if fences are omitted (Corn and Bury 1990).

If pitfall traps are used as live traps and population estimates are derived from mark-recapture data, biases from trap avoidance or trap attractiveness must be considered. Franz and Ashton (1989) observed only one recapture in drift fence arrays of *Gastrophryne carolinensis* tagged with radioactive (^{60}Co) wires. Conversely, Shields (1985) observed preferential use of pitfall traps by southern leopard frogs (*Rana sphenocephala*), possibly in response to warm or moist conditions inside the traps.

Pitfall trapping alone is insufficient if comparison of relative abundance among species within an assemblage is the objective. Drift fences with pitfall traps, however, effectively capture some individuals of most species, at least in temperate areas. Therefore, unequal capture rates are less of a problem for determining some indices of species richness. If one accepts the untested assumption that capture rates do not vary among habitats, trap data can be used to compare relative abundance of individual species among study areas.

If animals are released from traps, they must be marked to eliminate recaptures from calculations of relative abundance. If animals are not released, the researcher must consider the consequences for subsequent samples, especially if an area is to be sampled repeatedly. Drift fence arrays can decimate populations of small mammals (Bury and Corn 1987), but this effect has not been observed for amphibians. Corn and Bury (1991) operated the same grids of pitfall traps for 50 days in 1984 and 30 days in 1985 and removed all animals captured; captures of amphibians did not differ between the two years, except that one species was more abundant in the second year.

RESEARCH DESIGN

The objectives of a study determine the sampling design. Installation of arrays or grids of fences and traps is labor-intensive, and running the system can require significant funds and per-

sonnel time. Inventories of species present in different habitats may require less effort than comparisons of species' abundances and densities among habitat types. The objective of many inventories is to sample as many habitats as possible. Therefore, each habitat type may have only one array or grid. This methodology, however, reduces the probability of detecting rare species. Operating traps for a longer time may compensate for fewer arrays or grids.

Quantitative comparisons of species' abundances or densities among habitat types require replication—that is, multiple arrays or grids in each habitat type. This methodology makes detection of all species present in each habitat most likely.

Selection of the locations for arrays or grids should have a sound statistical basis. If a researcher is surveying different habitat types and more than one unit of each habitat type exists, habitats sampled must be selected at random from the larger pool, and arrays or grids must be placed randomly within them. To determine whether stratification is appropriate, a researcher must have fairly detailed knowledge of the habitat(s) in the study area. Decisions regarding stratification must be made before trap systems are installed.

The timing of trapping may also vary, depending on the study objectives. Vogt and Hine (1982) recommended operating arrays of drift fences opportunistically, after rainfall, to maximize captures. In other studies traps have been operated continuously for from 30 days (Corn and Bury 1991) to nearly two years (Campbell and Christman 1982a; Raphael 1988). Both sampling strategies have drawbacks. Opportunistic trapping may be logistically difficult, so that different sampling efforts are applied in different study areas, and short periods of trapping may not be adequate to verify presence of all species (Jones 1986; Bury and Corn 1987). Continuous trapping requires more personnel and may have a greater effect on resident animals, but continuous trapping can be scheduled to accommodate known seasonal variations in amphibian activity. Bury and Corn (1987) trapped in forests of the northwestern United States continuously for 180 days, beginning at the end of May, but captured few amphibians until the onset of rainy weather in October. Subsequent trapping was conducted for a shorter time (30 or 50 days) and was begun on 1 October (Corn and Bury 1991).

Investigators have seldom used the same design for arrays of drift fences or grids of pitfall traps. Shields (1985) operated one hundred 3-liter pitfall traps spaced 10 m apart in a 10×10 grid; Raphael (1988) used ten 8-liter pitfall traps placed 20 m apart in a 2×5 grid; and Corn and Bury (1990, 1991) deployed thirty-six 8-liter pitfall traps 15 m apart in a 6×6 grid. D. B. Wake (pers. comm.), who uses 1-liter traps to live-trap *Ensatina eschscholtzii*, has 176 traps spaced 10 m apart in an 11×16 grid.

Several array designs for straight-line drift fences with pitfall and funnel traps are possible (see Vogt and Hine 1982). In most studies, three or four fences with pitfall and funnel traps are used (Fig. 11). An array is preferable to a single straight fence. Arrays intercept animals from any direction, whereas animals moving parallel to a single fence probably are not captured. Because arrays with three fences (Jones 1986; Bury and Corn 1987) use less material than those with four (Campbell and Christman 1982a), they are less expensive and less time-consuming to install. D. E. Runde and K. M. Enge (unpubl. data) found that arrays of different design, when tested side-by-side, yielded comparable results.

The length of the drift fence influences the number of animals captured, and the optimum length probably varies by habitat type. Vogt and Hine (1982) observed that single drift fences less than 15 m long captured fewer amphibians and reptiles than 15-m and 30-m fences, but the component fences of most arrays are either 5 or 7.6 m long (Fig. 11). Bury and Corn (1987) compared arrays of 2.5-m fences with those of 5-m

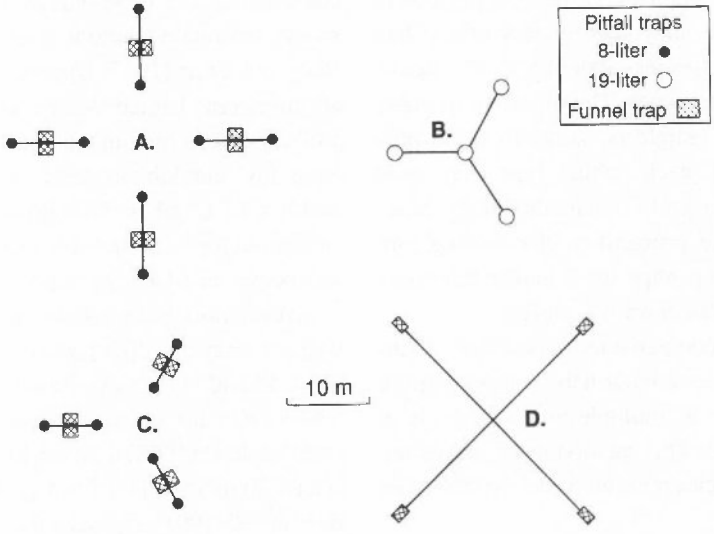

Figure 11. Designs for arrays of drift fences. A. Array used by Campbell and Christman (1982b). B. Array used by Jones (1981). C. Array used by Bury and Corn (1987). D. Array used by Dalrymple (1988). Fences and spacing are drawn to scale; trap sizes are not.

fences and found both adequate for sampling amphibians in coniferous forests in northwestern North America.

The pitfall traps that are used most often are 19-liter plastic buckets and 8-liter cans. Sometimes both are used in the same array. Funnel traps also may be effective for capturing amphibians (Campbell and Christman 1982a; Beauregard and Leclair 1988; D. E. Runde and K. M. Enge, unpubl. data), particularly in areas with saturated soils, where pitfall traps tend to fill with water. Some investigators have caught a variety of amphibians using a standard Campbell and Christman (1982a) four-fence array (Fig. 11A), in which the terminal pitfalls of each fence have been replaced with funnel traps (Vickers et al. 1985; Enge and Marion 1986). Indeed, K. M. Enge (pers. comm.) suggested that pitfall traps are not necessary if amphibians are the primary target animals.

Too little experimentation on the efficacy of different array designs has been done, and too much variation exists among amphibian assemblages for me to recommend a single design for all situations. However, a three-fence array with funnel traps (e.g., Fig. 11C) is probably suitable for most studies. Individual fences should be at least 5 m long. This length is convenient because fences for one array can be cut from a single roll of aluminum (see below).

FIELD METHODS

CONSTRUCTION. Large pitfall traps are made from 19-liter plastic buckets. Smaller pitfall traps (8-liter) are constructed by removing both ends and one end, respectively, of two number-10 tin cans (i.e., 3-lb coffee cans) and fastening the open ends of the two cans together with duct tape (Fig. 12). Single number-10 cans or 4-liter plastic jars may be used if the ground is particularly difficult to dig and the target organisms are small. Traps are buried in the ground, with the opening flush with the surface. For 8-liter cans a plastic collar is constructed by cutting the bottom out of a 1-lb plastic margarine tub, which is then inserted at the top (Fig. 12). This collar

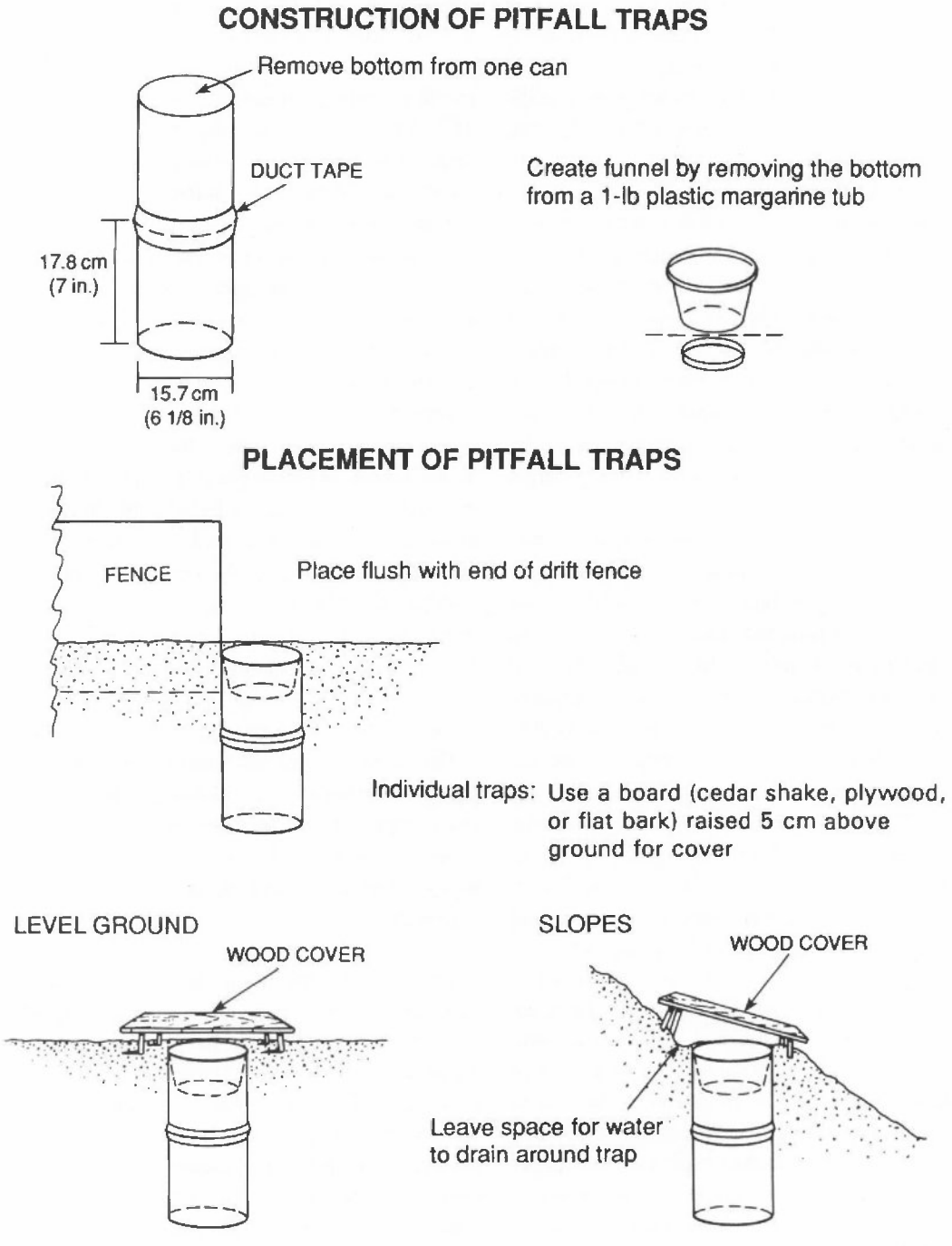

Figure 12. Construction of pitfall traps from two number-10 tin cans (reprinted with permission from Corn and Bury 1990).

keeps animals from crawling out of the trap (Vogt and Hine 1982). Pitfall traps should be closed when not in use. Plastic buckets come with lids, and although the shape of the buckets is often distorted after buckets are placed in the ground, lids will cover them effectively. The plastic lids from the margarine tubs can be used to cover 8-liter traps.

The hole for the trap is dug most easily with a posthole digger, which creates a hole with the correct diameter for 8-liter traps. A tile spade can also be used. Traps also may have a wood cover (Fig. 12). When the trap is open, the cover is raised above the opening. In hot, dry weather the cover protects trapped animals from desiccation and may inhibit predation by birds. The cover may also attract target animals.

Funnel traps consist of rounded tubes (or rectangles) with an inwardly directed funnel-shaped opening at one or (usually) both ends. They are constructed from window screen (Karns 1986) or rigid hardware cloth (Vogt and Hine 1982). Window screen can be purchased in rolls 76 cm wide. The body of the trap is constructed from a piece 90 cm long, and the cut ends are stapled together along the length of the tube. If the cut edges are folded before stapling, the tube is about 25 cm in diameter by 76 cm long. The funnel part of a trap is made from a square piece of screen rolled into a cone and stapled. The diameter of the large opening of a funnel matches that of the tube. A funnel is placed in one end of the tube, and the distal margins of both are attached to each other with staples. If only a single funnel is used, a piece of screen is stapled to the other end of the tube to close the trap. If two funnels are used, the funnel at the other end is attached to the tube with paper clips, so that it and animals can be removed easily from the trap. Funnel traps are placed parallel to the drift fence, midway along each side. Traps should be shaded with loose bark, palm fronds, litter, or plywood.

Drift fences can be constructed from a variety of materials, including hardware cloth, tar paper, window screen, or plastic. The preferred material is 50-cm-wide aluminum valley flashing (weatherproofing material), which comes in 15.2-m rolls. Desired lengths can be cut with tin snips. A mattock or hoe is used to dig a trench 20 cm deep for the length of the fence; the fence is placed in the trench, which is backfilled with soil. Occasionally an ax is needed to cut large roots. Loose dirt is tamped down and smoothed alongside the fence to create a runway, and small obstacles (twigs, rocks) are removed. In forests, aluminum fences 5 to 7 m long usually are self-supporting for a few months. Fences in open areas, fences left in place for several years, longer fences, or fences made from other materials need supporting stakes. Pitfall traps are placed at the ends of the fence so that no gaps occur between the fence and the rim of the trap. If desired, the edge of the trap can be slit and the fence run a short distance into its mouth (Jones 1986). An individual number is affixed to each trap for data recording purposes. Trap numbers can be written on the drift fence with a permanent marker.

For safety, fieldworkers should always wear gloves when handling the aluminum, because of sharp edges. In wet weather, tools quickly become coated with slick mud, so fieldworkers should exercise extreme caution when handling a mattock or an ax.

OPERATION. Ideally, traps should be checked daily, before noon, but with a large number of study areas, this schedule may not be possible. Traps should always be checked at least every three days. If the number of study sites is such that all traps cannot be opened on the same day, traps must be closed in the same order in which they were opened. This procedure ensures the same trapping effort for each area. Because traps can contain dangerous snakes and invertebrates, either long forceps or a small, stout aquarium net should always be used to check them.

If captured animals are to be released, they must be marked. Stockwell and Hunter (1989) released all animals but apparently did not mark them. They then reported total captures for each species. Because numbers of recaptures were unknown, their numbers of captures are difficult to interpret. Animals should be marked and processed in the field. If that is not possible, marking and processing can be done in the laboratory. Dead mammals, live amphibians, and dead amphibians should be placed in separate small plastic bags; all specimens from a single array or grid should be placed together in a larger bag. A cooler with reusable ice containers is best for transporting specimens from the field. Each day's catch should be processed on returning to the lab to reduce the likelihood of specimen and information loss. Processing of individuals to be marked and returned to the site should begin immediately. Live amphibians can be kept for a day or two in a cool place or refrigerator if processing must be delayed, but they must be checked frequently. Dead amphibians should not be frozen (Scott and Aquino-Shuster 1989; Appendix 4). Specimens should be processed by the person(s) who checked the traps, to minimize the introduction of inaccuracies into the data. See Appendices 1 and 4 for information on handling and preserving amphibians.

PERSONNEL AND MATERIALS

Installation of drift fences and pitfall traps is simple but labor-intensive. A large crew (4–6 people) can install three to six arrays or two grids per day. Fewer people are needed to check the traps once they are open. One person can check an array or a grid of 36 traps in an hour or less, depending on the number of animals captured. Several sites can be checked in one day, depending on the travel time between study areas.

Construction materials are expensive. Required items include posthole diggers, 15-m tape or measured nylon rope, plastic flagging (1–2 rolls), waterproof ink markers, aluminum flashing (in rolls 15 m long × 50 cm high, 1 roll per array) or suitable alternative for the fence, 19-liter plastic buckets, number-10 tin cans, 1-lb margarine tubs, and wood covers. Most items can be obtained from building supply stores.

Materials required for operation include a waterproof notebook and paper, large and small plastic bags, large forceps, a plastic cup or long-handled spoon, a small dipnet, and a small cooler with reusable refrigerant.

DATA TREATMENT AND INTERPRETATION

The species and the array and trap numbers of all individuals caught are recorded in the field. This record is important for quality control and should become a permanent part of the data set. It provides critical information during the initial processing of specimens and is a valuable reference for the questions that inevitably arise even after the data have been processed. The study area, date, and array and trap numbers are written in pencil on a small piece of waterproof paper and placed in each bag of specimens.

If animals are released, information must be recorded on formal data sheets at the time the animals are handled. If animals are retained, formal data sheets are completed when the animals are processed. Formal data sheets can be drawn by hand as needed, but preprinted forms are more convenient (Fig. 13). Several software packages can be used to design forms, and many word processing programs have table generation capabilities.

Proper identification of animals is essential, especially if animals are released. Identifications of preserved animals can be verified later. Discarding badly decomposed specimens from traps after field identification is risky. For example, a field crew in southern Washington captured more specimens than it was prepared to handle (Corn et al. 1988). Many small mammals were discarded in the field; the rest were preserved as skulls and deposited in the U.S. National Museum of Natural History. When the skulls were

Locality: Oregon, Lane County, H.J. Andrews Experimental Forest

Technique	Province	Stand Number	Habitat	Day	Month	Year	Trap Night	Collector's Name
PIT	COAST RANGE	737	OLD GROWTH DOUGLAS FIR	22	10	85	22	P.S. CORN

Catalog Number	Species	Trap Column	Trap Row	Snout-Vent Length (mm)	Total Length (mm)	Mass (g)*	Sex	Age
155244	PLVE	A	1	80	38		M	S
155245	PLVE	A	1	68	35			S
155246	PLVE	F	2	95	48	1.9	F	A
155247	PLVE	B	1	94	47	1.6	M	A
155248	ENES	A	4	103	50	3.2	M	A
155249	ENES	C	6	98	54	3.1	M	A
155250	ENES	E	5		19	2		J

* Right column is tenths
Trap Night: Number of nights since traps were opened
Sex: Male, Female (if unknown, leave blank)
Age: Adult, Subadult, Juvenile
PLVE: Plethodon vehiculum
ENES: Ensatina eschscholtzii

Figure 13. A sample data sheet used to record amphibians collected from a pitfall grid. Note that species are identified by a four-letter abbreviation using the first two letters of the genus followed by the first two letters of the species. Data are from an inventory of forest vertebrates in Oregon (Corn and Bury 1991).

cleaned and identified, up to 10% of the field identifications were shown to be wrong, even though they had been made by experienced biologists familiar with the vertebrate fauna of the Pacific Northwest. This finding threw into question the identifications of most of the discarded specimens and severely limited the data analyses.

Species richness of ground-dwelling forms is the minimum information obtained by pitfall trapping. Numbers of animals trapped most often are converted to rate values by dividing by the trapping effort, either trap-nights (i.e., 1 trap-night is 1 trap open for 1 night; a grid of 36 pitfall traps open for 30 nights is 1,080 trap-nights) or array-nights (the array is treated as the trapping unit, and individual traps are ignored). These values can then be used as a measure of relative abundance. Grids of pitfall traps can provide data for estimates of density of selected species if used with mark-recapture. A computer program for estimating density from trapping grid data, CAPTURE (Rexstad and Burnham 1991), is available, but to my knowledge, it has not been used for amphibians.

Data can be used to generate simple species lists, or they can be analyzed with complex

multivariate ordinations. Various measures of species diversity and association are reviewed in Chapter 9. Gauch (1982) and Pielou (1984) described classification and ordination techniques and the use of multivariate statistics. Relative abundance can be compared among habitat types with analysis of variance (e.g., Corn and Bury 1991), but proper application of parametric statistics requires rigor (e.g., randomization) in selection of study sites and placement of arrays.

Diversity measures that include abundance (or anything but species richness) should be used with caution, because of species-specific capture rates. The numbers of each species trapped may bear little relation to real population sizes. The diversity index or abundance curves, therefore, are peculiar to the sampling scheme employed and may have quite limited biological meaning. Comparisons among habitat types (e.g., Stockwell and Hunter 1989) are probably not appropriate if the amphibian species assemblages differ. Determination of species richness can be enhanced by combining results from pitfall trapping with those from other techniques (Corn and Bury 1990; Bury et al. 1991), but abundance values are not comparable among different sampling methods.

SPECIAL CONSIDERATIONS

After collecting the required voucher specimens (see Chapter 5 and Appendix 4), the investigator must decide whether to collect or release the remaining animals captured. Release requires that all animals trapped be positively identified. Identification in the field may be impractical, particularly in areas where the fauna is poorly known. Collection of all animals trapped requires that arrangements be made for verification of identifications and deposition of specimens in a museum. Also, permits may be required for collecting.

In some habitats, large numbers of small mammals, especially shrews, die in pitfall traps (Bury and Corn 1987). These specimens are an important resource and should be saved. If project personnel are unable to process mammals, arrangements for outside help should be made. All applicable data should be recorded for mammals as well as amphibians. Pitfall traps also capture invertebrates, another important scientific resource. Generally, the collection of mammals, but not of invertebrates, is regulated by law.

Each time a trap is checked, debris and excess water must be removed. A small amount of water should be placed in traps when they are opened, but in wet weather, most traps accumulate more water than is desired. Previous workers (Raphael and Barrett 1981; Williams and Braun 1983) have recommended that water be placed in pitfall traps, as the quickest and most humane way to kill small mammals. Current guidelines for trapping small mammals with pitfall traps (American Society of Mammalogists 1987) specify drowning as the only acceptable method of kill-trapping. However, drowning is a slow and inhumane way to kill amphibians, and it is prohibited in the current guidelines for field methods for amphibians and reptiles (Committee 1987). A compromise between these apparently incompatible recommendations is to keep a small amount of water (2–5 cm) in the traps and to check the traps frequently. Small mammals, particularly shrews, will become hypothermic and drown in this amount of water, but most amphibians should survive. Daoust (1991) placed a $10 \times 5 \times 7$ cm piece of saturated sponge in funnel traps and improved survival of trapped *Rana sylvatica*. Dodd and Scott (technique 9, below) recommend using synthetic foam rather than sponge, which disintegrates rapidly.

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8. Surveys at Breeding Sites

NORMAN J. SCOTT, Jr., AND BRUCE D. WOODWARD

Many amphibians are most conspicuous at breeding ponds. Therefore, surveys conducted at breeding sites are especially effective. Sampling at the breeding site involves counting the animals in some predetermined fashion. Generally, adults are counted along visual or aural transects. Techniques for counting larvae, which can also be used to document breeding populations, are treated separately (see "Quantitative Sampling of Amphibian Larvae," technique 10).

Data from surveys at breeding sites can be used to estimate species richness or abundances of breeding adults or larvae at one or several sites. Across-site comparisons are useful for identifying areas most suitable for development or preservation, studying the effects of acid precipitation or pollution from point sources (e.g., factories), and determining the presence of predators. The techniques can also be used to monitor changes in population levels of species, to detect changes in species assemblages through time, or to carry out detailed autecological studies.

TARGET ORGANISMS AND HABITATS

The techniques described here can be adapted for the study of any amphibian that breeds in communal aggregations in temporary or permanent ponds, lakes, or streams. This criterion excludes viviparous species, species with terrestrial nests, and those that breed in very small groups or that use small, ephemeral, widely scattered breeding pools (e.g., bromeliads and tree holes).

Breeding-site surveys can focus on adults or larvae. Adults are usually more conspicuous and easier to sample and identify than larvae. However, larvae are typically present at the breeding site for longer periods than adults. Sampling both adults and larvae is the best approach.

Monitoring adults at a breeding site is easiest when breeding is concentrated in a narrow, well-defined period, but it can be done also when the breeding period is extended. At some temperate zone sites, where water availability is predictable and freezing temperatures constrain activity, most amphibians breed in a relatively few weeks in spring and early summer. In arid areas most breeding is rain-dependent, and developmental times of larvae are often short; investigators must be ready to take advantage of the proper weather conditions whenever they occur (Low 1976; Wells 1977). At the other extreme, some tropical amphibians may breed at any time of year.

For short, infrequent surveys, larval sampling yields more complete species lists than adult surveys do (Wright 1914; Wiest 1982). However, if there is any doubt as to larval identification, larvae should be reared through metamorphosis. Larval densities can be strongly influenced by local factors (e.g., climate and co-occurring predators—Woodward and Mitchell 1991) and can vary greatly over short periods. Larval densities are not good predictors of adult population size.

Breeding-site studies are most thorough in small, shallow bodies of water that are free of emergent vegetation and that can be surveyed by investigators in a relatively short period.

BACKGROUND

The sites used by amphibians that congregate for breeding encompass nearly the entire spectrum of aquatic habitats (Crump 1974). Each type of habitat presents its own sampling problems, but the general objectives remain the same. A basic assumption in breeding-site studies of single species is that all individuals or all members of some population subset, such as breeding males, are equally available for sampling. For studies of whole amphibian faunas, this assumption implies that all species can be sampled equally well. This latter assumption is usually false.

Some species are more secretive than others, and some are present at breeding areas for a longer time than others and are thus more susceptible to observation. Breeding aggregations also may vary in structure (e.g., the percentage of satellite [noncalling but reproductively capable] males—Perrill et al. 1978). Violations of these assumptions typically pose problems for estimates of relative abundance; rarely should they interfere with compilation of species lists.

Many factors contribute to the configuration of an amphibian population at any single breeding site (Alford and Wilbur 1985; Wilbur and Alford 1985; Morin 1987; Woodward and Mitchell 1991), and many different sites must be surveyed if the investigator wants to understand the "typical" condition. Savage (1962) surveyed *Rana temporaria* breeding sites in England over a 10-year period and found that frogs did not breed in any specific pond every year. The number of ponds used also varied among years; in 1937, spawn was found in 11 of 78 ponds, but 22 of 86 ponds were used for breeding the following year. Large year-to-year differences in densities and number of breeding areas used are common in amphibians, probably because of the strong influences of environmental parameters and because of the boom-and-bust cycles typical of these fecund organisms. Investigators need to describe conditions under which they collect their samples and attempt to interpret the effects that these conditions may have on their results.

RESEARCH DESIGN

The sampling design must conform to the special rhythm of each species' breeding cycle. Different species may be active at different times. Surveys may need to run in 4-hour blocks of the 24-hour day, or nocturnal surveys may suffice. If the species of interest are erratic or explosive breeders (Low 1976; Wells 1977), sampling protocols must take into account the conditions (usually weather) that induce breeding, or the species must be studied by observations of eggs or larvae. Most individuals of species with prolonged breeding periods spend only a fraction of the breeding season in the breeding area (Fellers 1979; Woodward 1982; Godwin and Roble 1983; Ryan 1985). Density estimates for these species require some sort of mark-recapture procedure (see Chapter 8), preferably over several samples. Unless a mark-recapture study can be done, investigators may have to rely on counts of larvae or recently transformed individuals. Larval densities drop rapidly throughout the larval period; thus for comparative purposes, samples must be collected when the larvae are at approximately the same ages. Even then, larval numbers are poor predictors of adult population size. If the habitat is extensive, populations can be subsampled either aurally (adult males only) or visually (adults and larvae under certain conditions) along randomly located transects (see "Visual Encounter Surveys" and "Audio Strip Transects," techniques 2 and 3).

The basic data obtained at a breeding site, for either larvae or adults, are species richness and abundances. These data also may be recorded for microhabitats within each breeding site to allow tests for differences across microhabitats. If the study area is large enough, the proportion of the total breeding habitat occupied by each species should be determined.

FIELD METHODS

Breeding-site monitoring involves counting animals in some preestablished manner. If the surveys are visual or aural, precise survey conditions, such as time of day and year, weather conditions, walking speed, the exact locations to be searched, and the time spent on each major habitat subdivision, should be specified. Characteristics of aquatic habitats vary with time, so descriptions should be detailed enough to allow interpretation of the effects of year-to-year variations, as well as within-year changes (see "Data Treatment and Interpretations," below).

With practice, investigators can recognize all possible anuran calls in an area. Aural surveys of calling anurans along predetermined routes are performed annually throughout Illinois and Wisconsin, and regular surveys are planned for Iowa and the Upper Peninsula of Michigan. Such surveys are especially efficient if species composition rather than abundance data are required.

In many breeding aggregations, total counts are possible; in larger aggregations, subsamples of the population should be counted by visual or aural transects. Sometimes adults migrate a few meters from the surrounding habitat to the breeding site (e.g., pond, lake) each night, migrating back late in the evening. Surveys must be restricted to those times of the day or night when most adults are present at the site.

Habitats differ in complexity. Therefore, spending equal amounts of time in different habitat types is usually not appropriate, because the effectiveness of the searches per unit of time in each area are not equal. In visual surveys the investigator must search until all frogs or salamanders have been counted and, if necessary, captured. This approach works only if sizable numbers of amphibians are not moving into or out of the breeding area. If they are, then numbers will be biased according to search time. For aural surveys, equivalent time spent per unit area in each habitat type is the appropriate approach.

Differences in the effectiveness of sampling amphibians in different habitats have seldom been examined. One way to address this problem would be to mark, release, and resample individuals in several habitat types to see what proportion of marked individuals in each habitat is resighted.

Three examples—the regional survey, the single-area survey, and the survey along a stream or river—will demonstrate the range of approaches that can be used for breeding-site surveys.

REGIONAL SURVEY. The regional breeding-site survey, an annual inventory of a series of breeding ponds, is one common approach. The question raised is usually whether amphibian populations are stable, increasing, or declining. The approach is exemplified by the frog and toad survey program of the state of Wisconsin, which was started in 1981 as a survey and was expanded in 1984 into a monitoring program (see "Group Activities and Field Trips," in Chapter 7). Survey routes consisting of up to 57 km of road with 10 preselected anuran breeding sites are assigned to volunteers. Each year, each route is surveyed one night in early spring, one night in late spring, and one night in summer, when the weather is calm and water temperatures are above stipulated minima for each season. Observers spend 5 to 10 minutes at each site recording data for all calling frogs. Call intensity is ranked according to number of individuals calling, and observations are entered on a data sheet (Fig. 14). The survey data are filed with the sponsoring state agency.

SINGLE-AREA SURVEY. A more detailed approach focuses on a single breeding area, such as a large pond, in order to determine the density of each amphibian species breeding in each habitat. A diagram of a hypothetical breeding area is given in Figure 15. If the resources are available to survey the entire area repeatedly, a mark-recapture program is appropriate. If the area must be subsampled, mark-recapture methods probably cannot be used, and the data will yield relative instead of absolute abundance.

Sites for subsamples should be stratified by major habitat type and located randomly within each, in and around the pond. However, if the area is too small for random placement of sampling sites (e.g., transects), then they should be placed wherever possible within each habitat. The order in which the selected sites are sampled is chosen randomly. If the data are to be analyzed statistically, at least three subsamples should be taken within each habitat type or stratum. Data analysis is facilitated if there are the same number of replicates in each habitat type.

A.

WISCONSIN FROG AND TOAD SURVEY -- Field Data Sheet
Bureau of Endangered Resources
Department of Natural Resources
Box 7921, Madison, WI 53707

Observer name(s), (Add address and phone on back.) RUN 1 _____ RUN 2 _____ RUN 3 _____

Route No. _____
Year _____
County _____

INSTRUCTIONS: Use this form for new or established survey routes. Each route consists of 10 listening sites, and is repeated 3 times during the breeding season, according to the minimum water temperatures and approximate range of dates given below for each survey period. Run surveys after dark, when wind velocity is less than 8 mph. Listen 5-10 minutes at each site and record a call index value of 1,2, or 3 (see below) for each species calling. See back of sheet for wind and sky codes and additional comments. Return to above address by 15 August.

	FIRST RUN Water 50°F; 15-30 April	SECOND RUN Water 60°F; 20 May - 5 June	THIRD RUN Water 70°F; 1-15 July
	BEGIN: Date; Time; Wind; Sky; Air temp. (F) — END: Time; Wind; Sky; Air temp. (F)	BEGIN: Date; Time; Wind; Sky; Air temp. (F) — END: Time; Wind; Sky; Air temp. (F)	BEGIN: Date; Time; Wind; Sky; Air temp. (F) — END: Time; Wind; Sky; Air temp. (F)
SITE NAME	CALL INDEX*	CALL INDEX*	CALL INDEX*
	Site Number; Water Temp (F); Wood frog; Chorus frog; Spring peeper; Leopard frog; Pickerel frog; Am. toad; E. gray tree frog; C. gray tree frog; Cricket frog; Mink frog; Green frog; Bullfrog	Site Number; Water Temp (F); Wood frog; Chorus frog; Spring peeper; Leopard frog; Pickerel frog; Am. toad; E. gray tree frog; C. gray tree frog; Cricket frog; Mink frog; Green frog; Bullfrog	Site Number; Water Temp (F); Wood frog; Chorus frog; Spring peeper; Leopard frog; Pickerel frog; Am. toad; E. gray tree frog; C. gray tree frog; Cricket frog; Mink frog; Green frog; Bullfrog
1.	1.	1.	1.
2.	2.	2.	2.
3.	3.	3.	3.
4.	4.	4.	4.
5.	5.	5.	5.
6.	6.	6.	6.
7.	7.	7.	7.
8.	8.	8.	8.
9.	9.	9.	9.
10.	10.	10.	10.
For office use only: Mean			
For office use only: Freq.			

*The call index is a rough estimate of the numbers of calling males of a particular species, according to the following index values:

1 Individuals can be counted; there is space between calls.
2 Calls of individuals can be distinguished but there is some overlapping of calls (intermediate between "1" and "3").
3 Full chorus. Calls are constant, continuous and overlapping.

Form 1730-8
6-86

B.

Please provide names, addresses, and phone numbers of all observers.
Place asterisk by name of cooperator who should receive materials next spring.

Route No. _____
Year _____
County _____

Name				
Address				
Phone				

Enter sky and wind codes on front of data sheet.

Sky code no.	Sky condition
0	Clear or a few clouds
1	Partly cloudy or variable
2	Cloudy (broken) or overcast
4	Fog
5	Drizzle
6	Showers

Wind code no.	Wind speed (miles per hr)	Indicators of wind speed
0	less than 1	Smoke rises vertically.
1	1-3	Wind direction shown by smoke drift.
2	4-7	Wind felt on face; leaves rustle.
3	8-12	Leaves and small twigs in constant motion; wind extends light flag.
4	13-18	Raises dust and loose paper; small branches are moved.

Comments (difficulties, background noise levels, uncertain calls, habitat changes since previous run or previous year, etc):

Site	Run 1	Run 2	Run 3
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Misc. comments:

*** IMPORTANT: Documentation required for records of cricket frog and species outside known range--see instructional materials

Figure 14. Field data sheet used in the Wisconsin frog and toad survey. A. Front. B. Back.

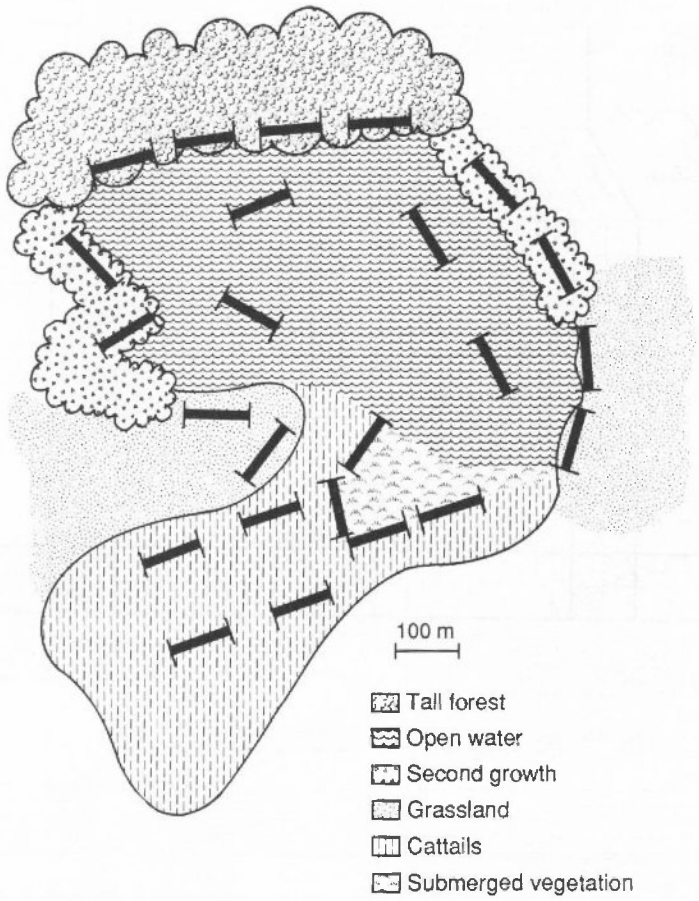

Figure 15. Diagram of a hypothetical amphibian breeding pond showing the distribution of habitats. Four survey transects are shown in each habitat except submerged vegetation. Four transects are located along an ecotone between the cattails and the submerged vegetation.

The sampling protocol should recognize that amphibian breeding choruses may be distributed in different ways. Transects should always be located within the chorus, regardless of its shape. If the interface between two habitats appears to be especially important to breeding amphibians, the ecotone should also be subsampled as if it were a separate habitat (Fig. 15).

SURVEY ALONG A STREAM OR RIVER. A detailed survey of breeding amphibians along a stream or river may pose special sampling problems, depending on the complexity and accessibility of the shoreline. Figure 16 illustrates the placement of transects in habitats, including a swampy backwater, along a deep, wide river with a relatively simple shoreline.

Inger and Greenberg (1966) used mark-recapture methods to estimate sizes of both breeding and nonbreeding *Rana* populations along a stream in Borneo. Their data provide actual density estimates. Where mark-recapture is not possible, visual and audio transects can provide data on relative abundances. In some rivers, it may be possible to carry out audio transects from canoes, although to our knowledge this has not

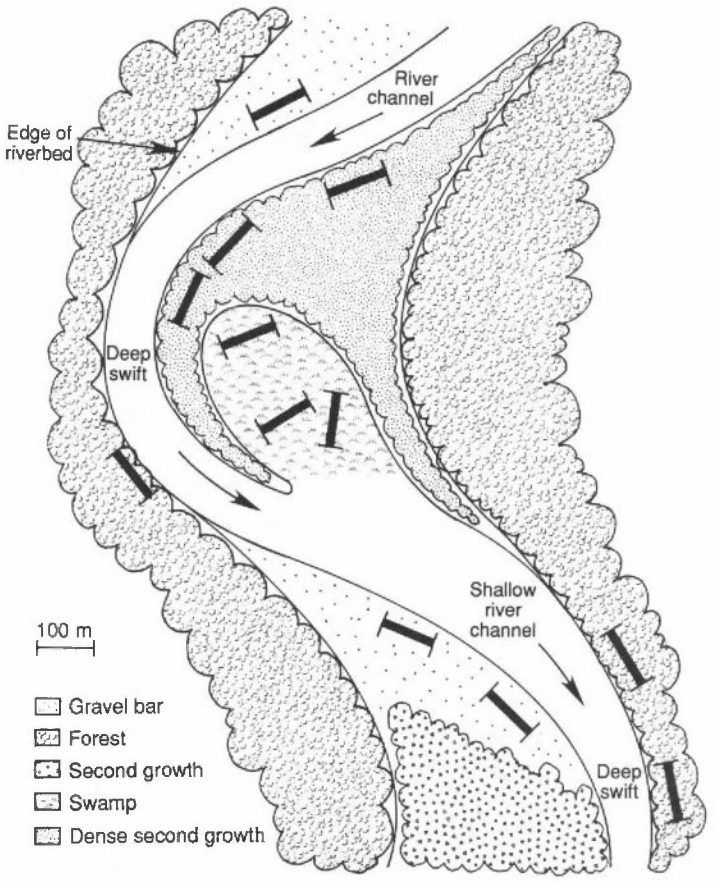

Figure 16. Diagram of a hypothetical section of river showing the distribution of habitats and three survey transects in each habitat except second growth.

been tried. Centrolenids and hylids breeding along larger streams might be especially amenable to canoe-based surveys.

Equal numbers of transects should be randomly placed (if possible) within each habitat type and should be sampled in random order.

PERSONNEL AND MATERIAL

The number of people required to survey a breeding site depends on the desired intensity of the survey and the sizes and numbers of the areas to be sampled. One person can survey a number of small sites if presence or absence of calling males is the only information needed. However, two or more people usually provide more reliable and more consistent data, because each person can check the other's results and maintain continuity if one person is unable to survey on a particular occasion. Sampling periods should be as short as possible to reduce temporal variation, and more people should be used if breeding sites are large or numerous. For nighttime surveys, good-quality headlamps and extra batteries are required (see Appendix 6). Some investigators prefer red light because it may be less disturbing to the animals.

Other materials needed for breeding-site surveys are thermometers, watches, hip boots or waders, wet suits, long-handled dipnets, waterproof data sheets, writing materials, plastic bags

for captured amphibians, and colored flagging for marking the habitat.

DATA TREATMENT AND INTERPRETATION

The data recorded in a breeding-site survey depend on the goals of the study. In addition to the minimum data required with any technique (Chapter 5), the following information should be recorded: surface-water and deep-water temperatures, the presence or absence of calling sites (bushes, trees, floating and emergent vegetation), reproductive activity of adult amphibians, and, if possible, developmental stages of larvae. (Additional information that may be of interest can be found in the section "Sampling with Artificial Pools," in Chapter 7.) Data should be recorded by breeding site or, preferably, by transect and microhabitat within each breeding site. The data from breeding-site surveys can be used to produce a list of amphibian species encountered, or they can be combined with other information to form a basis for detailed ecological and population analyses.

Species lists can be compared across sites, although we caution against attributing too much to species absences if only a few breeding sites are examined per area. Not all species of amphibians in an area breed at any one site every year (Savage 1962; Bragg 1965; Heyer 1976). If several breeding sites are examined in each of two or more study areas, then frequency of occurrence can be compared across study areas. In a similar manner, species richness or abundance can be compared across study areas or across time in the same study area. Any estimates of abundance are referable to the breeding population present during the period of study and not to the entire adult population. In numerous species that live in cold (and possibly arid) environments and have short activity seasons, individual females breed only as often as they can store enough energy to produce a clutch, which may be every second or third year (Bragg 1940; Blair 1943; Turner 1960; Metter 1964).

Data can be pooled across many breeding sites to yield area-wide species lists and relative abundances. Data from several sites can be used as replicate samples under one treatment type (e.g., an area near a source of airborne pollution or an area subject to agricultural runoff) for comparison with sites from a control area.

Breeding-site surveys can be used to estimate effective population size and operational sex ratio, two parameters that are important for conservation work (Gilpin and Soulé 1986; Falconer 1989). For these purposes, surveys must be made over an extended period because breeding populations vary widely from night to night at a single pond (Fellers 1979; Woodward 1984; Ryan 1985).

Data derived from the Wisconsin survey routes (see "Regional Survey" under "Field Methods," above) have been analyzed in two ways and the results disseminated by M. J. Mossman in an unpublished newsletter (dated 3 April 1990).[2] In the first analysis, a regression of percentages of total sites occupied by a particular species against year was calculated. The slopes of the regression lines that were significantly different from zero were interpreted as indicating an increase (positive slope) or decrease (negative slope) for that species over time. In the second analysis, a trend for each route for the period 1984–1989 was computed using the call index values for each species (Fig. 14), summed over each year. The yearly sums were used in a regression against years. The resulting line was compared with the regression resulting from the averages of all routes compared among years. Trends that were significantly different from the average were interpreted as showing increases or decreases.

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2. Available from Michael J. Mossman, State of Wisconsin, Department of Natural Resources, Southern District Headquarters, 3911 Fish Hatchery Road, Fitchburg, WI 53711-5397, USA).

The data from the second and third examples in the section "Field Methods" ("Single-Area Survey" and "Survey Along a Stream or River," above) are either absolute densities from mark-recapture studies or relative abundances resulting from visual or audio surveys. Unless all species are equally susceptible to the sampling methods, relative abundances cannot be determined across species except at a very coarse, qualitative level. If repeated samples are taken within habitat types, a one-way analysis of variance (ANOVA) may distinguish differences in relative abundances among habitats; a two-way ANOVA can be used to analyze patterns among habitats and sampling periods (such as years).

SPECIAL CONSIDERATIONS

Survey results can be affected by the experience of those conducting them. Observers must be well trained, but they still may differ subtly in walking speed, ability to find animals, disturbance caused, and concentration. With effort, such differences can be minimized for a group of observers working at the same time at one site. They can cause major problems when comparing data from different geographical areas or different years. It is important to design the sampling protocol to minimize differences among observers when making comparisons among sites or across time. Ideally, each person should sample each habitat an equal number of times.

One last caveat: Calling in frogs does not necessarily mean breeding. Many species, such as American bullfrogs (*Rana catesbeiana*) and some hylids, call well outside of the breeding season (Salthe and Mecham 1974). If precise information on the breeding season is desired, observations of more-explicit indicators, such as amplexus, egg masses, or larvae, are needed.

CONTRIBUTOR: MARTHA L. CRUMP

9. Drift Fences Encircling Breeding Sites

C. KENNETH DODD, Jr., AND DAVID E. SCOTT

Drift fences are typically used to sample species that move to aquatic breeding sites. A barrier fence with traps on either side is installed around a pond, and amphibians are monitored as they enter and leave the area. Straight-line drift fences and pitfall traps (technique 7), in contrast, are used to sample individuals away from breeding sites. Although procedures differ in many respects between the two techniques, investigators intending to use drift fences and traps can benefit from reading both accounts.

Drift fences at the breeding site are best employed for long-term population studies and assemblage monitoring, but they can be used in conjunction with short-term species inventories (e.g., during well-defined breeding seasons) and field experiments (e.g., Cortwright and Nelson 1990; Scott 1990). However, the efficiencies with which species are captured when this technique is used differ. Generally, only a subset of the amphibian assemblage using the pond is censused. Therefore, the technique should not be used alone if species richness information is needed. In addition, estimates of amphibian species richness, diversity, or evenness based on data gathered with this technique must be interpreted with caution, especially for among-site comparisons (see Chapter 9; Magurran 1988; Noss 1990). Investigators should be especially careful to distinguish replication from subsampling (Eberhardt and Thomas 1991).

This technique can also be applied to questions not involved with biological diversity per se (e.g., activity patterns—Gittens 1983a, Pechmann and Semlitsch 1986; homing—Gill 1978a; migration—Hardy and Raymond 1980, Gittens 1983b, Semlitsch 1985; orientation—Shoop 1965, Phillips and Sexton 1989).

TARGET ORGANISMS AND HABITATS

Small temporary or permanent ponds often are the foci of amphibian breeding activities and are particularly amenable to the drift-fence technique. Larger aquatic sites may be fenced, but the benefits of sampling larger areas often are offset by increased costs of materials and labor. Doubling the area to be sampled increases the cost of construction materials and the time for construction, maintenance, and daily operation by an exponential factor of two.

One of the major problems of the drift-fence technique is trespass (Gill 1985, 1987; Dodd 1991b), when an individual amphibian enters or exits a pond without being captured. Surface-dwelling species that breed in aquatic sites and that have limited climbing, jumping, or burrowing abilities, such as mole salamanders (*Ambystoma* spp.) and some anurans (*Pseudacris ornata, Bufo* spp.), are best sampled by the drift-fence technique. Sampling of species with good climbing abilities (*Notophthalmus* spp., *Eurycea quadridigitata, Gastrophryne carolinensis, Hyla* spp.) and jumping abilities (many *Rana* spp., *Acris* spp.) is far less efficient. The extent to which fossorial species (e.g., *Scaphiopus holbrooki*) trespass by digging under a fence is unknown but may depend on depth of the fence and soil structure and may be site-specific.

BACKGROUND

The principal assumption made when using an encircling drift fence is that an animal has a reason to enter or leave the encircled area. For many amphibians, reproduction and metamorphosis provide the appropriate motivations. A related assumption is that the behavior of the target species is not altered by encountering a drift fence. In theory, the animal walks along the fence until it falls into a pitfall trap, enters a funnel trap, or is otherwise captured; it does not leave and go elsewhere. Many amphibian species apparently prefer specific sites for breeding, but the potential for non-site-specific reproductive behavior should be kept in mind.

Additional considerations may be important, depending on the type of question being asked. For example, the assumption of equal catchability among species or individuals probably is not valid. Trespass rates may vary spatially and annually (both intraspecifically and interspecifically) with size (juveniles versus adults; large females versus small males) and, perhaps, with reproductive condition (gravid versus nongravid females). In addition, some animals avoid traps after an initial encounter, whereas others deliberately seek out traps as cover from harsh environmental conditions (Shields 1985). Laboratory and field experiments and observations may assist in determining the likelihood of trespass as a threat to the validity of results and their interpretation.

Comparisons of species richness, reproductive output, relative abundance of breeding and transitory individuals, and population structure among sites should be made cautiously, particularly where the species assemblages among sites differ.

RESEARCH DESIGN AND FIELD METHODS

The basic methodology is to capture and process (e.g., measure, weigh, determine sex, mark) animals at the fence and release them on the opposite side of the fence. If possible, the fence should completely encircle the breeding site; interpretation of data from partially fenced ponds is hampered by ignorance of movement corridors used by different species, individuals, and age classes. The shape of the fence may conform to the shape of the water body. However, if orientation studies are included, a circular or near circular fence is necessary, because all statistical tests are based on circles, not ellipses or other shapes. The fence should be placed above the anticipated high-water mark. Drift fences may be constructed of a variety of materials: aluminum flashing, plastic sheeting, hardware cloth, highway filter fabric, or tar paper. Aluminum

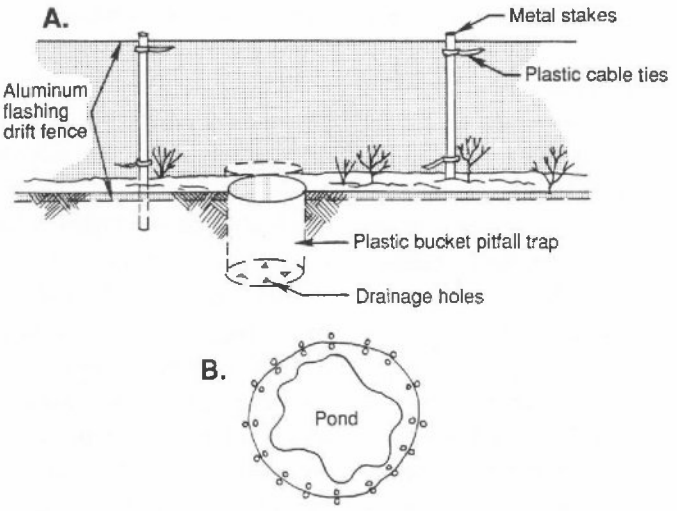

Figure 17. A drift fence with pitfall traps around a breeding site. A. Placement of stakes and pitfalls in relation to drift fence. B. Continuous fence around breeding pond. Reprinted with permission from Gibbons and Semlitsch (1982).

flashing is highly recommended for long-term studies despite its high cost. If possible, the lower edge of the fence should be sunk into the ground 20 cm below the surface (a mechanical trencher greatly facilitates installation). The fence should extend at least 35 to 40 cm above the ground surface.

When the substrate is too hard or rocky for the fence to be buried, plastic sheeting can be curved outward flush with the ground and covered with soil to close gaps under the fence. Wooden, metal, or plastic stakes placed on both sides of the fence as required keep the fence stable and upright, which minimizes trespass. Stakes are anchored to the fence by bolts or plastic electrical ties (Gibbons and Semlitsch 1982) and should extend as little as possible above the top of the fence. Each pitfall should be identified with an individual number painted or otherwise affixed to the adjacent fence for unambiguous future reference.

Amphibians are captured in open containers (pitfalls) placed along the fence (Fig. 17). Hard plastic buckets (19-liter capacity) are most effective because they resist collapse and do not disintegrate after prolonged exposure to water. However, availability of cans or buckets, nature of the substrate, or other requirements of the study may dictate alternate choices. Buckets should be the same color. We recommend using dark buckets, although the effect of bucket color on capture rate is unknown. A hole (slit) that will allow excess rainwater, but not captured animals, to escape should be cut in the bottom of the bucket. If shrews are common in the area and frequently captured, however, then the bottom of the bucket should be left intact. Numerous small holes (3–5 cm) should also be drilled in the bucket wall 4 to 5 cm from the bottom for drainage. In arid regions, an absorbent material (preferably a small square, 5 to 8 cm thick, of synthetic foam used for seat cushions; natural and synthetic sponges disintegrate rapidly) should be placed in the bottom of the bucket and saturated with water to provide moisture and cover. Buckets should be buried straight up, flush with the substrate surface; no gaps should exist between the bucket and the fence.

Buckets are paired on opposite sides of the fence at 10 m intervals. In areas with direct sun,

buckets should be partially shaded. Shades made of pegboard are particularly effective. They can be slanted over the pitfall opening to allow room for transit beneath, while preventing an animal from climbing up the outside (a trespass). Vegetation should be removed outward from the base of the fence for at least 30 to 40 cm, and no vegetation should overhang the fence. Maintenance involves keeping the fence in place, repairing soil erosion, covering the results of digging activities of large or burrowing animals, and removing vegetation and other debris that fall on or across the fence. On downhill slopes, the sheet flow of surface water from heavy downpours can cause a fence to collapse or buckle. Although holes in the bottom or sides of pitfalls allow for some water drainage, heavy rains or compact soils may result in occasional flooding; water should be removed from pitfalls as soon as possible after a rain (see discussion of this problem in "Straight-line Drift Fences and Pitfall Traps," technique 7).

In order to minimize trespass, baffles can be installed at the top of the fence to form a "T" shape or down-slanted eave. However, the eaves of the T should not be so large as to provide shelter to climbing species, or the overhang will have to be checked in addition to checking the pitfall. Painting the top of the fence with teflon paint has been suggested as a way to prevent or minimize trespass. Teflon paint is expensive, however, and its effectiveness has not been determined. A lip on the bucket may keep animals from climbing out of pitfalls; the lip should be removable for easy checking. Plastic lids, which fit securely on buckets, often are available. A lip can be created by cutting a circle 50 mm from the edge and removing the center. However, buckets may change shape during the course of a study, making lid positioning difficult. Research is needed on bucket lip technology and effectiveness.

In addition to (or in place of) pitfalls, various types of screen-mesh funnel traps (with single or double openings) can be placed parallel to the drift fence (see technique 7, above, for additional information). The use of various diameters of open-ended or partially closed PVC pipe placed on or near the fence can supplement funnel trapping. PVC pipes are effective in capturing certain climbing species, particularly hylids, that use the pipes as diurnal hiding places (e.g., Lohoefener and Wolfe 1984).

Traps should be checked daily, preferably in the morning, to reduce mortality from desiccation and predation. Depending on capture rate, checking traps can be time-consuming. For example, checking and processing animals from 46 pitfalls at a north Florida drift fence enclosure (230 m perimeter) took from 30 minutes to more than 8 hours per day. Some sampling regimes may require checking pitfalls two or more times daily, depending on the question being asked, researcher time, and the capture rate of organisms. If traps are not checked for two or three days, the number of animals captured will be significantly underestimated and mortality will increase (some animals may escape as numbers in the trap increase, and other animals may be removed by predators).

Pitfall traps can capture poisonous snakes and invertebrates. One should never reach under a cover or into a trap without checking it first. Long forceps can be used to stir the bottom contents so that small secretive species hiding in debris, soil, or sand will be exposed. A small reinforced aquarium net can be used to check flooded pitfalls.

Animals can be processed at the site of capture. If it is necessary to process them elsewhere, they should be placed in small plastic bags with a plastic tag or a tag of high-rag-content or waterproof paper, on which the pitfall number is written in pencil or permanent ink. As animals are processed and released, the numbers can be crossed out and the tags reused. The species, number of specimens, and pitfall location should be written in a weatherproof field data book when the trap is emptied.

Vertebrate predators—birds, small mammals (particularly raccoons in North America), and even snakes—that learn the location of pitfalls and "run" the traps (routinely visit and remove animals) can be a nuisance. Amphibian mortality from invertebrates, particularly ants, is likely to be a more serious problem. Predators should always be removed from the pitfall. Commercial mammal or ant traps (or baits) can be used to effect long-term eradication of these pests. Boards (Fig. 12) or galvanized wire mesh (see Reading 1989:fig. 2) can be positioned over pitfalls to discourage avian predators.

PERSONNEL AND MATERIALS

Installing an encircling drift fence is labor-intensive. At one study site in Florida sandhills, it took CKD and a 4-person crew two days to install a 230-m fence. Much longer periods will be required for longer fences or areas with compact substrates or complex topography. Backhoes or mechanical diggers facilitate installation. Fence maintenance (repair, vegetation trimming) for most short drift fences can be handled easily by one person as long as problems are corrected routinely. Data collection will take varying amounts of time depending on distance to study site, types and amount of data collected, numbers of animals processed, and number of pitfalls to be checked.

The materials needed to install a drift fence include fence materials, pitfall buckets, cover boards (if required), pieces of foam (for moisture and cover in the bucket, if needed), stakes, bolts or electrical ties for stakes, paint (to number pitfalls), equipment to trim weeds (weed whacker, shears, machete), and shovels or posthole diggers. Data-recording materials include rain gauge, air and water maximum-minimum thermometers, water-depth marker, marking tools, ruler (preferably clear flexible plastic), scales for weighing animals, field notebook, long forceps, sturdy aquarium net, paper tags, plastic bags, field data sheets, clipboard, and pencils. Data analysis equipment includes computer hardware and appropriate software.

DATA TREATMENT AND INTERPRETATION

The researcher should know in advance which data are to be taken and should prepare a data sheet accordingly (80-column computer data coding sheets make good data sheets). In addition to minimum data listed in Chapter 5 or emphasized for this technique, identification number (cohort or individually marked), pitfall number, method of capture (pitfall, funnel trap, by hand), capture status (first-time capture/recapture), weight, notes (e.g., reproductive condition, coloration, tail regeneration), maximum and minimum air and water temperature since last check, pond water depth (measured at same location), rainfall since last check, and weather conditions should be recorded. If available, data on barometric pressure and moon phase might be noted. The occurrence of unusual or cyclic events (weather fronts, hurricanes) also should be noted.

Data should be entered directly onto data sheets and into a computer database, if available, as soon as a sheet is completed. Computerized databases can be set up in the same format as the data sheets for easy transfer. Codes should not be used for recording original data, but can be used for convenience and to save space in the computerized database. For example, a 2-letter or 3-letter code, using the first letters of the scientific name, can be used to identify species (e.g., HC for *Hyla cinerea*), and numbers can be used to identify weather conditions described in the field (e.g., 01 for clear, 02 for partly cloudy, and so forth). Letters should be kept either in uppercase or lowercase to minimize errors (i.e., HC or hc but not Hc). Computer codes should be sufficient to allow one to distinguish among species at the site without reference to a key (e.g., codes for *Leptodactylus pentadactylus* and *Leptodactylus poecilochilus* could be LPD and LPC, but not LP and LPS). This practice keeps data cur-

rent, facilitates data proofing, and allows rapid analysis for reports and periodic project assessments. Computer databases should be matched with statistical packages to ensure compatibility. For instance, databases compiled using dBASE III+ and IV software are easily loaded into SAS programs for analysis.

The type of data analysis will depend on the question asked. Indices of species richness and diversity are reviewed in Chapter 9 in this volume and in Magurran (1988). Krebs (1989) discussed a variety of ecological tests, including those used to estimate abundance, determine sample size, and measure survival rates; a software program (Krebs 1988) for these tests also is available (see Appendix 6). Problems may occur in the analysis and interpretation of results because of uncertainties involving the magnitude and significance of trespass (Gill 1985, 1987; Dodd 1991b). Trespass undoubtedly results in the underrepresentation of some species or size classes in a sample. Also, assumptions concerning equal catchability may not be valid.

Drift fences encircling breeding sites have been used in various studies of biological diversity. Examples include studies of species assemblage structure (Cortwright and Nelson 1990), population size and dynamics (Gill 1978a,b, 1985; Semlitsch 1983; Dodd and Charest 1988), and trespass (Gill 1985; Dodd 1991b).

SPECIAL CONSIDERATIONS

The costs of installing and maintaining drift fences encircling breeding sites are high. Initial capital outlay to purchase supplies may be considerable (fencing material for a 230-m fence is approximately U.S. $575 for galvanized metal and U.S. $625 for aluminum). Personnel costs are high for installation and may be high for day-to-day operation (data collection and analysis) and maintenance (minor equipment replacement; vehicle gas and repairs). If costs can be minimized, encircling a breeding site with a drift fence is an efficient, effective technique for amphibian sampling. Drift fences typically are used with mark-recapture studies (see Appendix 2).

If a study is suspended, the fence can be opened temporarily and the pits covered. At the termination of a drift fence–pitfall trap study, the fences and traps should be removed.

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10. Quantitative Sampling of Amphibian Larvae

H. BRADLEY SHAFFER, ROSS A. ALFORD, BRUCE D. WOODWARD, STEPHEN J. RICHARDS, RONALD G. ALTIG, AND CLAUDE GASCON

There are various methods of removing amphibian larvae from water for counting and identification. These methods include seining, dipnetting, trapping, and enclosure sampling, in which larvae are captured with known quantities of water in boxes, stovepipes, or collapsible netting. These techniques provide a fast, relatively thorough, qualitative or quantitative sample with minimum personnel, material, and time. In addition, the techniques generally do not harm the animals, and so can be used to monitor rare or endangered species. The two primary goals of these procedures are to assess the species richness of larvae in a body of water and to determine larval population size.

TARGET ORGANISMS AND HABITATS

Although in this chapter we concentrate on larvae, the techniques described could be used for aquatic adult amphibians. The quantitative methods, however, depend on equal catchability of individuals and species. Because aquatic adults are often powerful swimmers, their capture with these techniques is somewhat haphazard. Thus, we recommend against using the techniques to estimate total population size and density of aquatic adults.

Each technique is most efficient in a particular type of habitat. Seining is extremely effective in shallow ponds and lakes with little vegetation. Dipnetting is frequently the simplest method for sampling vegetation-choked bodies of water, stream habitats with limited access or great structural complexity, and specialized habitats such as tree holes. Enclosure sampling is effective in shallow water habitats with relatively uniform substrates. Trapping may be the only way to sample deep-water habitats or those with complex bottoms of stones, wood, or rocks.

For large bodies of water, including large vernal pools and shallow lake habitats, seining may be the only effective way to generate sample sizes sufficient to estimate species richness and abundance of amphibian larvae, especially when samples are removed rather than marked and released for recapture. Dipnetting is most effective for estimates of abundance in small bodies of water and shallow streams (generally < 1 m deep) and with removal sampling. Its effectiveness increases as the size of the body of water decreases; in very small pools, it often is possible to count all individuals present. Enclosure sampling, which is generally used to estimate population size, is most appropriate in relatively small bodies of water as well. Traps may be effective in any habitat, except fast-moving, shallow streams, but they are generally used only in special situations where other, easier sampling efforts fail.

BACKGROUND

The primary assumptions for using any of these techniques to estimate species abundance are that all animals are equally catchable, and that sampling efforts are equal for each unit of field collecting time. The first assumption may be generally true for the same species in similar habitats but often will not hold for a diverse array of species or habitat types. When these assumptions do hold, then one may use either removal estimation procedures (see "Removal Sampling," Chapter 8) or quadrat analysis methods (see "Quadrat Sampling," technique 4, above) to estimate the total population size of each species (see "Data Treatment and Interpretation," below). Which of these approaches is most appropriate depends on the scale of the area sampled relative to the size of the habitat. In general, if a sufficiently large fraction of the habitat is sampled so that sequential samples are not independent of each other, but rather deplete the population, then removal sampling analysis is most appropriate. However, if a smaller fraction of the habitat is being sampled, and sequential samples are independent, then treating the samples as quadrat samples for analysis is most appropriate.

Some of the quantitative sampling procedures described may be applicable only in certain situations. When unequal efforts are required for different microhabitats, quantitative comparisons may become impossible. However, species richness can still be estimated qualitatively.

RESEARCH DESIGN

Sampling designs fall into two general categories. In designs used for removal estimates of population size, sampling is random without regard to the independence of the samples. In designs used for quadrat sampling, the samples must be independent. Assessing independence in aquatic habitats is not always easy, because larvae may swim many meters to escape a net or a human intruder. Our experience suggests that samples more than 5 m apart can be considered independent. Thus, if one is sampling a small pond or stream, the samples are, by definition, dependent, and quadrat sampling estimates for abundance will be inappropriate. Amphibian larvae occur in three basic habitat types: small bodies of water; ponds; and streams. We discuss sampling schemes for each.

SMALL BODIES OF WATER. Here we include tree holes, small sinkholes, puddles, and other

bodies of water less than 1 m in diameter. Such habitats are repeatedly sampled with a dipnet or small seine. The number of larvae caught is recorded for each sweep, but larvae are not returned to the water. After at least 10 sweeps fail to uncover any new larvae, it is safe to conclude that the total population, or at least most it, has been obtained. The larvae can be returned to the pool after data have been recorded.

PONDS. For temporary ponds, we suggest stratifying sampling effort by microhabitat type. The theoretical basis and techniques of stratified sampling are discussed in many statistical texts (e.g., Cochran 1963; Yates 1981) and in Chapter 4 (see "Sampling Methods"). At the simplest, we recommend using a random sampling scheme stratified by depth and shoreline location. To do this, a sampling transect is established along the pond perimeter. This transect could be a fixed length at a fixed location—for example, a 100-m transect centered on the northern shore of a pond. It could also be the entire pond perimeter. In either case the precise location of the transect can change as the pond grows and shrinks. The number of depth zones is determined separately. If enclosure sampling is used (see "Quantitative Enclosure Sampling" under "Field Methods," below), the maximum depth is equal to the height of the sampler. If that maximum depth is 50 cm, four equivalent depth zones could be used (0 to 12.5 cm, 12.5 to 25 cm, 25 to 37.5 cm, and 37.5 to 50 cm) or the zones might be divided less evenly by depth (e.g., 0 to 5 cm, 5 to 15 cm, 15 to 30 cm, 30 to 50 cm). Depth zones do not have to be of uniform or constant width.

An equal number of samples is taken each trip from each of the depth zones, which run parallel to the shoreline sampling transect, in the water. For example, 20 samples might be taken on each sampling trip. If there are 4 depth zones, 5 samples are taken in each depth along a 100-m shoreline transect, as follows. The transect is divided into five 20-m sections that extend from the shore into the water, perpendicular to the depth zones. Four random integers between 1 and 20 inclusive are used to select which 1-m segment of each section will be sampled. A sample is taken in the shallow depth zone, opposite the point indicated by the first number; in the second depth zone, opposite the point indicated by the second number; in the third depth zone, opposite the third point; and in the fourth depth zone, opposite the fourth point. This procedure is repeated for each of the transect sections. Figure 18 illustrates a sampling plan generated using this procedure. This scheme assures reasonably even coverage by depth and by shoreline location, while also eliminating bias in the selection of sampling sites. Because the sampling transect can move when the water body changes in size, problems associated with fixed sampling coordinates are eliminated.

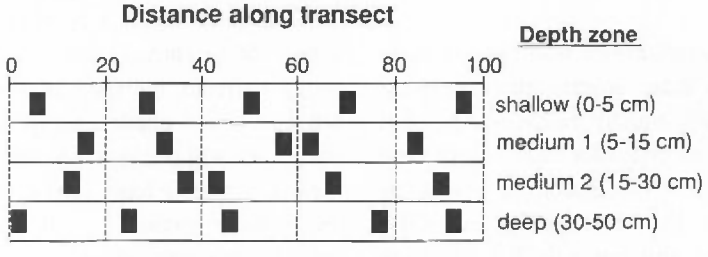

Figure 18. Diagram of a representative stratified sampling program for aquatic amphibians on a 100-m transect along the shoreline of a pond. The transect is divided into five 20-m sections along the shoreline (represented by the top horizontal line) and four depth zones. Sampling points are selected randomly, one from each depth zone in each section of the transect. Abundances are estimated separately for each zone.

A similar scheme can be used if the transect is the entire shoreline of a small pond. In this case, the total length of the transect changes as the pond expands and contracts. The sampling plan is set up in advance, and employs proportions of the length of the transect rather than absolute lengths. These proportions are converted into lengths after arrival at the sampling site.

If estimates of density within microhabitats are obtained with a stratified sampling scheme and then are used to estimate total abundances at the site, it is necessary to know the area occupied by each microhabitat. A site map that includes depth or elevation contours is constructed using simple plane-table surveying methods by establishing a benchmark for measurements of water depth on each sampling date. The area in each depth zone on each sampling date can then be reconstructed (see Harris et al. 1988 for an example of this procedure). When the surface areas occupied by each depth zone differ markedly, it may also be advisable to sample each zone in proportion to its relative area. For more details on sample allocation, consult Cochran (1963) and Yates (1981). Similarly, it is advisable to include in the site map and the stratification scheme other habitat features (e.g., substrate, areas of rooted vegetation) in the zones.

Regardless of the sampling technique used, it is useful to collect environmental data at the location of each sample. Minimum data should include water depth, water temperature, hour, substrate type, and weather conditions. Such factors as degree of illumination, dissolved oxygen concentration, water conductivity, water turbidity, and other parameters of the physical environment, as well as numbers of animals of other taxa (e.g., fish and invertebrates), may also be included.

STREAMS. Stream habitats tend to be more heterogeneous than ponds, making quantitative estimates of abundance more difficult. We therefore recommend quantification based on sampling of each habitat for a given amount of time, with larval samples averaged over all habitat types. As an example, we briefly describe a study of rain forest streams in Queensland, Australia (S. J. Richards, M. P. Trenerry, and R. A. Alford, unpubl. data). Larvae were sampled from a rain forest stream for more than 4 years. In a 500-m stretch of stream, three habitats were selected for sampling: pools (calm areas with clear water and relatively slow flow rates), runs (intermediate between pools and riffles), and riffles (rapidly flowing shallow water constantly boiling over a rocky substrate).

Riffles were sampled with five 1-minute samples. The investigator slowly moved up a riffle, turning and brushing undersides of rocks, then replacing the rocks. A dipnet placed immediately downstream caught dislodged tadpoles. The species, sizes, stages, and numbers of tadpoles collected in each period were recorded. Tadpoles were released at the upstream end of the riffle after all samples were taken. Runs were also sampled with five 1-minute periods. The substrate was a complex mixture of leaf packs, rocks, and sand, so microhabitats could not be delineated. The area was sampled with rapid sweeps of a dipnet near the substrate, with an occasional manual lifting of loose rocks. Tadpoles were released in the center of the run. Each microhabitat in pools (e.g., water over rocky substrate, sandy substrate, leaf pack) in pools was sampled with three 30-second sweeps.

Samples taken every 14 days over 3 of the 4 years of the study produced repeatable seasonal estimates of species richness and abundance with low variances. Tadpole populations at each sampling location were estimated at intervals with mark-recapture techniques to calibrate the dipnet counts. The mark-recapture and dipnetting data suggest that dipnets capture a rather constant fraction of tadpoles present, at least in the pools. The proportion captured is species-specific (*Litoria serrata,* about 0.10; *Mixophyes schevilli,* about 0.33), so the technique should be calibrated at each site.

FIELD METHODS

Several points must be kept in mind when sampling larval amphibians. First, most larvae are medium to strong swimmers that can outswim a slow-moving net. Second, larvae commonly escape by hiding on the bottom, making it essential to keep a seine or dipnet on the bottom when sampling. Third, vegetation and/or irregular bottom surfaces make any sampling difficult. Seines and rigid-frame samplers may become nearly useless in bodies of water with abundant vegetation. Sometimes an investigator can circumvent these difficulties—for example, by driving larvae from vegetation or removing bottom objects—but often will so disrupt the habitat that only species richness can be estimated. Fourth, many amphibian larvae are microhabitat specialists, so all important parts of the habitat should be identified as "strata" in a stratified sampling design.

SEINING. The seine most commonly used for amphibian larvae has a mesh size of 1.5 to 7 mm and is 1 to 1.5 m wide. For most applications, 3 to 4 m is an ideal length. A much larger seine (13–15 m long, 2 m wide, with a mesh size of 7–13 mm) is appropriate for sampling large bodies of water for large larvae; a small seine (0.5–1.0 m long, 10–30 cm wide, with a mesh size of 1.5–2.0 mm) is appropriate for small streams and pools. It is best to purchase seines pre-hung with lead weights along the lower edge and floats on the upper edge. Depending on the habitat to be sampled, some researchers attach a chain to the bottom of the seine for additional weight to ensure that no animals escape into a leaf-covered substrate. Seine poles made of 2.5-cm wood dowels are used to drag the seine. We fit the top and bottom of each pole with a 5-cm threaded eyebolt and then tie the rope of the seine to the eyes. The seine can be wrapped around the poles for storage.

For small ponds, the most effective sampling strategy is to seine directly across the entire body of water from shore to shore. It is important that the person sampling move slowly, so that the seine will remain on the bottom of the pond. However, that person must also move quickly enough to ensure that larvae do not escape. For larger ponds and lakes, it is often most productive to walk out to the depth of the seine before deploying it and then to work in toward shore in one continuous sweep. Alternatively, one can sample the mid-water without going to shore, although often many animals are lost as the seine is drawn up to the surface. For areas with dense vegetation, planting the seine in the bottom and driving larvae into the seine by walking through the vegetation is effective; the seine is then drawn through the water column as quickly as possible to collect the disturbed larvae.

A simple way to quantify seine sampling is to convert seining effort into square meters of bottom sampled; this conversion ensures that each seine haul represents about the same sampling effort. To accomplish this, one need only measure the distance traveled and multiply it by the length of the seine (measured pole to pole, which is generally about 10% less than the stretched length of the net). If the bottom is sufficiently clear of vegetation, distance traveled can be easily measured as strides; if not, short distances (up to 5 m or so) can usually be estimated fairly accurately. For large bodies of water, each seine haul should be completely independent of previous ones. This ensures that each haul gives an independent estimate of the density of animals. For smaller bodies of water, where areas seined will overlap, it may be advisable to wait a few minutes between hauls to let larvae come out of hiding.

DIPNETTING. At one extreme, a small aquarium net (about 10 cm wide) with a bendable frame is useful in capturing tadpoles from tree holes and

other small catchments. A slightly larger net serves well for general collecting situations in both stream and pond habitats. Wire-mesh sieves (kitchen strainers) with a handle work very well. The mesh is small enough to capture all but the smallest hatchlings; the net stands open out of the water for easy sample processing but is deep enough to inhibit escape of all but the largest larvae; and the frame is strong enough, if gripped close to the net, for the net to be passed through most vegetation and to be used in areas with rocky substrates. Delta nets, D-shaped nets, and flat-bottomed nets (named for the shape of the metal frame) with fine, nylon mesh and long, strong handles are appropriate in larger, deeper bodies of water that also may have deep layers of soft substrate. Net size and mesh size determine passage rates through the water; some experimentation will be needed to find the equipment optimal for the body of water and larvae to be sampled.

All microhabitats must be sampled so that species with restricted distributions will not be missed. This is especially important because we do not know the microhabitat distribution of most larvae (e.g., Alford 1986). Also, sampling should be scheduled to accommodate diel variation in larval activity and catchability (see Anderson and Graham 1967; Gascon 1991).

There are no definitive rules about the number of sweeps needed to sample a habitat adequately. It is not uncommon to cover almost all of the surface area in small aquatic sites (< 0.01 ha), whereas only a fraction of larger bodies of water are covered. Twenty to 50 sweeps can be made in an hour, depending on how much vegetation and detritus must be removed from the net and how many larvae need to be identified. A reasonable procedure is to survey each aquatic habitat for an equal period or with an equal number of sweeps. Making more sweeps in the larger habitat increases the chance of encountering rare species. Increasing the number of sweeps also increases the chance of capturing highly habitat-specific species. Because of individual differences in sampling ability, each person should collect samples from every sampled habitat. No additional species should be captured in at least the last 10% of the sampling period or sampling sweeps.

To estimate densities of larvae, some measure of water volume sampled per sweep must be obtained. This can be achieved by standardizing the length of the sweep (1 m is a comfortable sweep length) and recording the depth of water on the net frame during the sweep (i.e., ¼, ½, ¾, or full). Variation in sweep length or discrepancies between recorded and actual sweep depth will contribute to residual or unexplained variation in densities. Variations in sampling because of differences in sweep length or water depth make it more difficult to detect differences in densities between samples but should not cause densities in the areas being compared to be consistently overestimated or underestimated. Analysis of sweep sample data yields estimates of relative density. Absolute densities are best estimated with box or stovepipe sampling techniques (see "Quantitative Enclosure Sampling," below).

A growing body of literature (e.g., Wilbur and Fauth 1990; Woodward and Mitchell 1991) suggests that the nonamphibian species that coexist with amphibian larvae can strongly influence larval amphibian densities (Morin et al. 1988). Thus, recording the densities of all taxa obtained in the samples may be desirable for some studies. Other examples of studies using quantitative dipnetting include Heyer (1974, 1976, 1979), Berger (1984), and Vickery and Nudds (1991).

QUANTITATIVE ENCLOSURE SAMPLING. Enclosure sampling includes the techniques known as box sampling, quadrat sampling, and stovepipe sampling. It involves trapping animals inside an enclosure that can then be sampled, either exhaustively or until a fixed proportion of the trapped animals have been removed. Enclosure

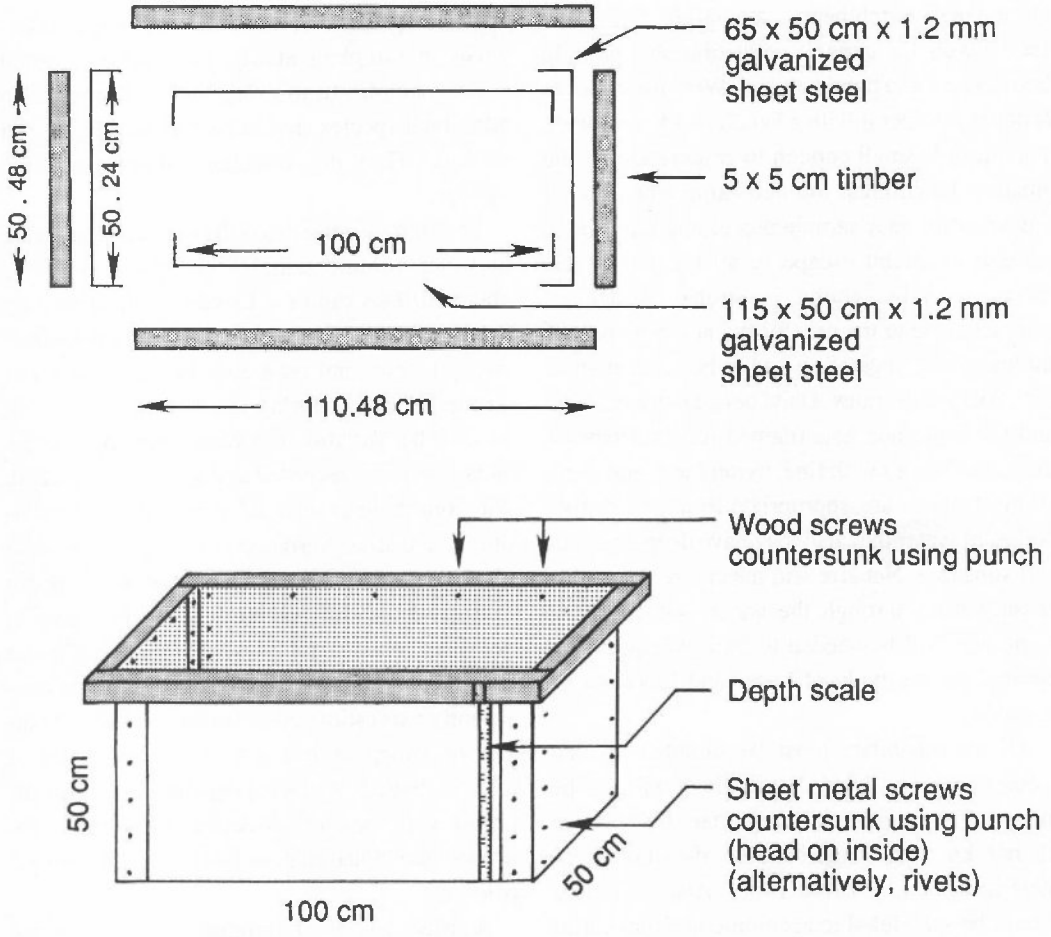

Figure 19. Construction of a 0.5 m^2 box sampler. Top view shows an exploded plan from above; lower diagram shows the assembled sampler (labels indicate inside dimensions). Depth scales should be attached to opposite corners, allowing two depth readings to be taken and averaged on uneven surfaces.

sampling is effective for habitats with shallow water and relatively uniform substrates. It has been used with some success by a number of investigators (Calef 1973; Turnipseed and Altig 1975; Morin 1983; Alford 1986; Harris et al. 1988).

The simplest enclosures are preexisting objects, such as a length of PVC (polyvinyl chloride) sewer pipe (Alford 1986). A more complex but generally more successful enclosure is the metal box sampler, 0.5 m^2 in area and 0.5 m deep (Fig. 19; also Harris et al. 1988). Depth scales should be attached to opposite corners of the box so that two readings can be taken and averaged. The sampler illustrated is relatively heavy. If a sampler is to be used by persons of limited strength or carried to a remote site, one with dimensions of 0.5 × 0.5 m (0.25 m^2) can be used.

To use a sampler like that in Figure 19, the operator moves through the water to the sampling site with a slow, shuffling gait to minimize the likelihood of scaring away animals. He or she drops the enclosure, oriented so that the sharp edge faces down, and presses it into contact with the substrate. The number of animals trapped inside can be determined in two ways.

The investigator can try to remove every larva by repeatedly dipnetting in the sampler until no new animals have been caught for some fixed period of time or number of net sweeps. Alford (1986) used a period of 5 minutes with no new captures. With the second, more efficient scheme, a dipnet is constructed that has the same cross-sectional profile as the narrower axis of the rectangular sampler and fits closely inside it. The investigator slides it into the sampler at one end, presses it against the substrate, sweeps it through the sampler, and lifts it out at the other end. Sweeps are made in alternating directions. A few net sweeps reliably remove a high and constant proportion of the animals initially captured in the enclosure.

For example, Harris et al. (1988) sampled larvae with a $1.0 \times 0.5 \times 0.5$ m sheet steel box and a 0.5×0.5 m dipnet. They swept the box from one end to the other five times for each sample and recorded the numbers of tadpoles and newts captured in each sweep. They analyzed the results and efficiency of this technique extensively. On average, 52% of the *Notophthalmus viridescens* in the sampler were removed with each sweep of the net, so that with three sweeps they captured about 90% of the total; with four sweeps, 95% of the total; and with five sweeps, 97.5% (Harris et al. 1988). Had they analyzed their data after several sampling trips, found this result, and switched to three net sweeps per sample, they could have taken more samples on each trip for a better estimate of total numbers of animals in the habitat. Investigators using this approach should conduct initial validation trials with at least five sweeps per sample. They should analyze the results of the sweeps following Harris et al. (1988) to determine the effort needed to remove 90% or 95% of animals from the sampler. This will optimize the ratio of number of samples taken to accuracy of within-sample estimates.

A few problems should be considered in planning sampling programs with fixed enclosures. First, the maximum depth that can be sampled is limited by the height of the enclosure. In addition, enclosures must be constructed of sturdy materials, such as galvanized sheet steel, to stand up to repeated use. This limits the size of sampler that can be handled by a single person, because of weight. The result is that simple box samplers (e.g., Fig. 19) are usable only in relatively shallow water. Alternatively, a two-handled sampler can be constructed for use by two people. Enclosure sampling is also limited by substrate type. To entrap animals effectively, the enclosure must make good contact with the substrate. Enclosures work well on soil or mud substrates and on fine gravel or coarse gravel interspersed with finer particles. Leaf packs and small submerged or emergent plants usually do not interfere with enclosure sampling, but rocks and large amounts of woody detritus may. If larval abundance estimates are needed for sites with deep water or unsuitable substrates, enclosure sampling must be supplemented with other devices or techniques, such as traps or mark-recapture.

If wariness or escape responses of species vary, mark-recapture or trapping may be used to calibrate the proportion of individuals caught with a rigid sampling device. Species that escape threats by flight tend to be underrepresented in enclosure samples compared with species that hide in the substrate. Disturbance by the operator when placing a box sampler may cause some tadpoles to evade capture. The degree to which this happens can be highly dependent on the skill and experience of the operator. Thus, if data collected by different researchers are to be compared, procedures for using the sampler should be as consistent as possible and should be reported in sufficient detail to allow others to employ them.

Another category of enclosure sampler is the bottom net (C. Gascon, unpubl. data). This method was designed for measuring absolute density. Bottom nets are box-shaped nets with

frames at the open end. The entire (collapsed) net is placed on the bottom of water bodies some time prior to sampling. Gascon used nets of nylon mesh screening attached to 2.5 × 1 m wooden frames. The collapsed nets may be hidden in the substrate (for example, if the substrate is mulch or leaf mold) or may simply be placed on the bottom. At sampling times the frames are lifted vertically to the surface, trapping animals in the water column above the net.

The bottom-net technique should provide results comparable to those from enclosure sampling. Bottom-net sampling has proven especially useful for sampling salamander larvae from deep (5 m) vertical-sided canals that could not otherwise be sampled (H. B. Shaffer, pers. comm.). Bottom-net sampling has two advantages over enclosure sampling: It assures that all animals in the trapped space are removed, and it allows sampling in deeper water. It also has disadvantages. First, it cannot be carried out in as wide a range of microhabitats. If the substrate has a significant amount of rooted vegetation, a bottom net cannot be seated adequately. A bottom net that is not concealed in the substrate provides a novel substrate itself, which could affect the density of larvae in the water column above it. Second, concealing a bottom net may alter the substrate in ways that change larval density. Third, because of water resistance, a bottom net is likely to rise through the water column more slowly than an enclosure sampler descends through it. Mid-water larvae thus may escape from the sample.

TRAPPING. Relative abundance can be estimated using a variety of traps designed on the funnel-trap principle. Commercially available minnow traps are cylinders, about 0.5 m long × 0.3 m diameter, with funnels extending inward at one or both ends. Animals enter through the funnels but are discouraged from leaving by the small diameters and central locations of the exit holes at the bottom of the funnels. Calef (1973) used similar traps constructed from 3.5-liter plastic jugs and funnels made of window screen to capture larval *Rana aurora*. Calef calibrated his traps in enclosures, which allowed him to use his sampling results to estimate density. He constructed enclosures in the lake, cleared them of tadpoles, placed known numbers of tadpoles in each enclosure, and trapped for fixed time intervals. This enabled him to determine the relationship between density and catch per unit effort in his traps. Calef pointed out the possibility that tadpole aggregation along the walls of enclosures may have reduced capture rates below those that would be found in an unrestrained population at the same density. He double-checked his calibration using enclosure samples and visual counts.

Eggers et al. (1982) proposed a technique for calibrating traps directly. They suggested that each trap samples animals (fishes) from a circular area with a radius that is constant for each species and habitat type, and that if the radius can be determined, trapping data can be used to estimate absolute animal densities. They placed pairs of traps at increasing distances apart, set the traps for a constant time, and examined the relationship between number of animals captured and distance between the traps. Theoretically, the number of animals trapped should increase with increasing distance between traps, rising to an asymptote at the distance equal to the sum of the capture radii of the traps. Absolute density can then be estimated as number captured per area of the circle defined by the capture radius. This technique has not been validated with amphibians and should be considered experimental at present. In general, funnel-type traps should be regarded primarily as tools for estimating species richness and relative abundance only.

PERSONNEL AND MATERIALS

Materials for each technique are detailed above. Personnel needed depends on the technique

used. In general, seining requires at least two workers, whereas the other techniques can be accomplished by one field researcher. In addition, we recommend strongly that one person be designated as the data recorder for a field trip. This practice is especially useful if several workers are dipnetting or seining; the recorder can wait on the shore and keep a running tally of each sampling team's results.

DATA TREATMENT AND INTERPRETATION

For all methods, the primary data will consist of the number of individuals of each species captured per sampling unit. This may be the number per seine haul, box sample, trap-hour, or the like. One advantage of recording data in this way is that it allows each investigator to estimate the repeatability of the sampling procedure. This is very important, because it allows for a quick estimation of the relative productivity of different microhabitats, as well as determination of the number of samples needed to estimate the mean number of individuals per sample, with a low standard deviation. If repeatability among samples is low, a stratified sampling program, different-sized samples, or use of other techniques may be warranted.

As was stressed earlier, data collected from all of these techniques can be analyzed either with techniques developed for removal sampling methods (see "Removal Sampling," Chapter 8) or with techniques developed for independent quadrat sampling methods (see "Quadrat Sampling," technique 4). For depletion methods, the critical assumption is that each sampling unit removes a constant fraction of the individuals in the habitat. Thus, if the fraction of animals removed is 50%, then 50% would be removed in the first sample, 50% of those remaining (25% of the original total) in the second sample, 50% of those remaining (12.5% of the original total) in the third, and so forth. To confirm that this assumption is being (roughly) met, the fraction of animals caught, F, should be determined for each sample.

F is calculated as follows:

$$F = \frac{\text{number caught in sample } (n+1)}{\text{number caught in sample } n}$$

If F is constant over samples, then depletion methods can be employed to estimate total population size for each species. If the sampling scheme is stratified according to depth or habitat, separate estimates can be calculated for each stratum.

When samples are independent, then quadrat sampling methods should be used. In general, the larger the number of independent samples, the greater the precision in estimates of relative or absolute density (for additional information, see Alford 1986 and Harris et al. 1988).

When independent samples are collected from a stratified sampling scheme and the area of each stratum is known, the total abundance of each species on each sampling date can be estimated as follows:

L = the number of strata sampled

n_h = the number of samples taken in stratum h (replicates)

N_h = the number of sampling units in stratum h (i.e., the maximum number of samples that could be taken without replacement in each stratum—if samples are 0.5 m^2 and the area of a stratum is 247 m^2, then N_h for that stratum is 494)

y_h = the mean count per sample in stratum h

s_h^2 = the sample variance of the count per sample in stratum h

$\hat{Y}_h = N_h y_h$ = the estimated size of the population in stratum h

$s_{\hat{Y}_h} = \sqrt{\frac{N_h(N_h - n_h)}{n_h} \cdot s_h^2}$ = the standard error of $\hat{Y}_h$

$f_h = \frac{n_h}{N_h}$ = the sampling fraction for stratum h

$\hat{Y} = \sum_{h=1}^{L} N_h y_h$ = the estimated total population size

$s_{\hat{Y}} = \sqrt{\sum_{h=1}^{L} \frac{N_h(N_h - n_h)}{n_h} \cdot s_h^2}$ = standard error of $\hat{Y}$

To apply these equations to samples taken according to the scheme illustrated in Figure 18, the four depth zones would be regarded as the strata, and the five samples taken in each stratum along the transect would be regarded as replicates. Thus, $L = 4$, $n_h = 5$, and $N_h = 20$ (assuming each sample occupies 1/20 of the transect area). Suppose the following data set was collected from the pond shown in Figure 18:

		Number of tadpoles collected				
		Sampling station				
Depth	Stratum	1	2	3	4	5
Shallow	1	1	2	4	3	2
Medium 1	2	2	4	4	6	5
Medium 2	3	5	2	7	4	3
Deep	4	0	0	1	0	2

Then:

$y_1 = 2.4$ $y_2 = 4.2$ $y_3 = 4.2$ $y_4 = 0.6$

$s_1^2 = 1.3$ $s_2^2 = 2.2$ $s_3^2 = 3.7$ $s_4^2 = 0.8$

$\hat{Y}_1 = 48$ $\hat{Y}_2 = 84$ $\hat{Y}_3 = 84$ $\hat{Y}_4 = 12$

$s_{\hat{Y}_1} = 8.8$ $s_{\hat{Y}_2} = 11.5$ $s_{\hat{Y}_3} = 14.9$ $s_{\hat{Y}_4} = 6.9$

$f_1 = f_2 = f_3 = f_4 = 0.25$

$\hat{Y}_h = 228$

$s_{\hat{Y}} = 21.9$

These data suggest that strata 2 and 3 are not significantly different, whereas strata 1 and 4 are different from each other and from 2 and 3. In addition, the confidence interval for the total population would be about 228 ± 44 (the mean ± 2 standard errors).

SPECIAL CONSIDERATIONS

The methods we describe are based on the assumption that all individuals are equally catchable. However, catchability often depends on size of the larva, details of the habitat, and even the presence of aquatic predators, a potentially serious problem that can be difficult to detect. Mark-recapture methods can be used to estimate true population size, but such techniques are exceedingly difficult to implement for most larvae (see Appendix 2). Quantitative estimates obtained using the techniques described in this chapter can be calibrated against a known population size determined with mark-recapture techniques. Alternatively and preferably, by using several different techniques, an investigator may be able to understand how a particular collection method misses certain individuals and to compensate accordingly (see Griffiths 1985).

Use of nets requires a compromise among mesh sizes. Very small mesh nets capture all larvae, but become clogged with filamentous algae and debris. They also are cumbersome and move through the water relatively slowly. Large mesh nets are much easier to use, but miss small individuals. In general, we advocate using nets of several mesh sizes to ensure that all size classes of animals are captured.

One disadvantage to seining is that it requires at least two persons to be effective. Single-person seining is far less efficient (Routman 1984). In general, the simpler the habitat is structurally, the more reliable seining is for quantifying abundances of larvae. Thus, this technique is especially suitable for surveying vernal pools, stock ponds, and other vegetation-free habitats.

Some jurisdictions require a special permit for use of seines (e.g., state of California). The legality of both seines and traps should be cleared with the local fish and game department or its equivalent.

We have purposely not recommended use of electric shocking devices for sampling aquatic amphibians. Commercially available devices are expensive, and constructing them may be dangerous because of the high voltages involved. All such devices, if not used with extreme care, can electrocute the user. Finally, in our experience, the techniques outlined in this section

work as well as, or better than, electroshocking for sampling aquatic larval amphibians.

Often, the results obtained from the sampling approaches we describe will be used to make comparisons of species density or richness across study areas. Although making such comparisons is a goal of comparative quantitative sampling, caution should be exercised to ensure that results are comparable. Of special concern are differences in how individual investigators sample. The speed with which a net is passed through the water, how well it is pressed against the substrate, how quickly it is raised, and how it is passed through vegetation can all differ among investigators, often with major impacts on sampling effectiveness. To minimize these effects, a single person or team should do all of the actual sampling, or each should contribute equally to all aspects of a survey.

In a similar manner, extrinsic factors, especially weather and human disturbance, can influence the distribution and catchability of individual animals. Again, samples taken under similar conditions of wind, time of day, and human activity levels should be comparable. To facilitate standardization, brief mention of survey conditions should be made in all published reports.

Finally, amphibian larvae are fragile creatures. Tadpoles often have delicate tail fins, and salamander larvae can have delicate fins and gills, all of which can be damaged in nets. Trauma can be minimized by keeping animals cool, uncrowded, and in the net for as little time as possible while assembling a sample for analysis. A glass tube fitted with a rubber bulb is often a useful device for drawing up individual larvae and moving them among containers. We often use commercial turkey basters (i.e., large plastic or glass syringes with rubber bulbs). In addition, larvae can be measured and staged in such devices without trauma to the larva.