A Standardized Protocol for Surveying Aquatic Amphibians

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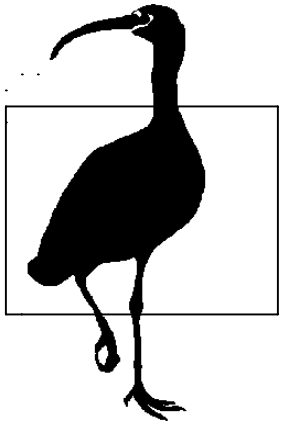

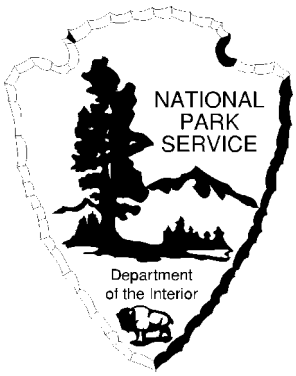

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Introduction

There is compelling evidence that amphibians in western North America and elsewhere have suffered significant declines over the last 10 - 15 years. The loss of amphibians is particularly significant because it is occurring on a world-wide basis, including our largest park and wilderness areas that are not subject to habitat loss. While many populations have declined because of a loss of habitat, other healthy, seemingly well-protected populations have disappeared for no obvious reason. The magnitude and extent of declines have been difficult to determine because of a lack of baseline data on population status or trends.

The concern about amphibians has led to surveys being conducted by an increasing number of individuals and agencies. In order to document the status of amphibian populations and provide a baseline for evaluating future changes, it is highly desirable to use a standardized survey protocol. By using such a protocol, data collected at different times, different places, and by different individuals can be compared. Without standardization, it is difficult to evaluate whether changes in amphibian populations have occurred or whether different findings reflect varying field techniques. Publication of a standardized protocol also provides a detailed reference for description of field techniques.

Though some recent publications have provided information on conducting surveys for amphibians (e.g., Heyer et al., 1993; Martin et al., 1994), the most appropriate techniques vary greatly from one region to another. Additionally, no one technique will sample all groups of amphibians equally well. This publication describes a standardized protocol for aquatic amphibian surveys that is appropriate for many species of frogs and toads, as well as some species of pond- and stream-breeding salamanders.

Applicability of Protocol

This protocol for conducting aquatic amphibian surveys has been tested and refined over the last five years at over 2,300 field sites in California. Although it was developed in California, the protocol is applicable to many other areas.

The aquatic survey protocol was designed and tested to survey for the following species of amphibians:

Western toad	*Bufo boreas*
Yosemite toad	*Bufo canorus*
Red-legged frog	*Rana aurora*
Foothill yellow-legged frog	*Rana boylii*
Cascades frog	*Rana cascadae*
Bullfrog	*Rana catesbeiana*
Mountain yellow-legged frog	*Rana muscosa*
Pacific treefrog	*Hyla* (=*Pseudacris*) *regilla*

The survey protocol also works well for some larval salamanders including newts (*Taricha* spp.). Spadefoot toads (*Scaphiopus* spp.), and larval giant salamanders (*Dicamptodon* spp.), long-toed salamanders (*Ambystoma macrodactylum*), northwestern salamanders (*Ambystoma gracile*), and tiger salamanders (*Ambystoma californiense*) are well sampled in some habitats, especially small ponds and slow portions of streams. To sample these salamanders in larger ponds, it is necessary to use a seine, a technique that falls outside the scope of this publication.

Though not the primary focus of the aquatic surveys, garter snakes (*Thamnophis* spp.) and western pond turtles (*Clemmys marmorata*) are routinely found and should always be recorded. Garter snakes are of interest because they also appear to be declining in some areas, perhaps because a portion of their prey base (amphibians) is now gone or significantly reduced. The western pond turtle is of interest primarily because it has been proposed for federal listing and is protected by state regulations in Washington, Oregon, and California. Surveys specifically for snakes and turtles need to include additional search and trapping techniques that are not described here.

Although we have not field tested this protocol outside California or with all species within the state, it is likely to work well with other, ecologically similar species such as other toads, true frogs, and treefrogs (e.g., spotted toads, leopard frogs, wood frogs, spotted frogs, California treefrogs). Certain habitat specialists such as tailed frogs (*Ascaphus truei*) and Olympic salamanders (*Rhyacotriton* spp.) should not be censused using the protocol described here. These species are found primarily in fast-flowing streams of moderate to steep gradient. Though tailed frogs or Olympic salamanders will generally be found, if they are present, they could easily be overlooked and will almost certainly be under represented using the techniques described here. This difficulty occurs primarily because these two amphibians are not readily found with either visual searches or by sweeping with a dip net (as described below). Surveys for tailed frogs and Olympic salamanders are generally most effective if 6.3 mm (1/4") mesh hardware cloth or D-shaped dip net is placed downstream as rocks are turned or removed from the stream. This technique is relatively labor intensive (Bury and Corn, 1991).

The aquatic survey protocol has not been tested with spadefoot toads (*Scaphiopus* spp.) or true toads (*Bufo* spp.) in the desert. In arid habitats, both the adult and larval amphibians are sometimes present for only a brief period (often only a few days or weeks). Additionally, there are significant year-to-year fluctuations in population size at any given site, often in response to local rainfall. Both of these factors make it more challenging to survey for amphibians in the desert. Analysis of data from these areas is also similarly difficult since it is hard to separate long-term changes in population size from normal weather-related population fluctuations. Nonetheless, the aquatic survey protocol described here would still be appropriate for these species and may well prove to be the most effective technique for surveying aquatic amphibians in arid environments.

Aquatic surveys are not used to survey or census woodland salamanders in the family Plethodontidae (e.g., Ensatina, slender salamanders, arboreal salamanders) since these species lay their eggs on land and have no aquatic phase that would cause them to congregate in or near water. Surveys for these species are generally conducted using time constrained or area constrained survey techniques (Aubry et al., 1988; Campbell and Christman, 1982; Corn and Bury 1990, 1991).

Basic Qualification for Field Biologists

The success of a survey hinges on having well qualified personnel conducting the field work. It is essential that the field crews have sufficient experience and training so that the resulting data are complete and reliable. Though aquatic surveys may seem easy to implement, they require a significant amount of expertise. The appropriate background is gained through a combination of field experience and a quality training program (see Training section).

Ideally biologists should be hired who have extensive experience conducting formal surveys for amphibians, including field work in remote areas. When a sufficient number of qualified biologists are not available, compromises must be made. In general, it is best to hire biologists with a strong background in conducting field studies of vertebrates.

It is much easier to teach field crews how to find and identify amphibians than it is to provide the experience that comes from conducting research under actual field conditions. For example, people who have spent a season searching for spotted owls or conducting

stream surveys for salmon may not be familiar with amphibians, but they have gained experience in gathering scientific data under field conditions that may be similar to what they will encounter while searching for amphibians. This type of field experience is extremely valuable, and cannot be readily taught during a training course. Rarely does a person who has just graduated from college have adequate experience to conduct aquatic amphibian surveys unless they have participated in extensive research projects as an undergraduate.

Evaluating job applicants

Applicants for field positions can be rated in four areas that are sometimes referred to as KSAs (Knowledge, Skills, and Abilities) by Federal employers:

1. Knowledge of the biology, natural history, and field identification of amphibians and reptiles.

   Field biologists must be able to find and identify amphibians correctly. Prior experience with other vertebrate groups, while not as useful as work with amphibians, still provides valuable experience in making careful observations in the field and in using field guides, keys, etc. Though this is one of the most important areas of knowledge, it is also the easiest one to teach during a training session so this point is evaluated on an equal basis with the other three.

2. Ability to implement standardized census techniques for amphibians and reptiles.

   Conducting aquatic surveys requires careful attention to detail in the field and is quite different from merely collecting animals for research or other purposes. Some people are adept at finding amphibians and reptiles, but are not disciplined enough to follow a standard protocol in the field. While it is preferable to have had this experience with amphibians and reptiles, a demonstrated ability to follow field protocols with other vertebrate groups is a strong plus.

3. Ability to take reliable, scientific notes with a high degree of accuracy, sometimes under difficult field conditions.

   The ability to take good, reliable scientific notes is very important. The written record provides lasting documentation of what was done and what was found. If the notes are poor, the survey results may be entirely useless. While it is often easy to tell field people what to record, there is a surprisingly wide range of abilities to take accurate notes in the field. Experience in recording observations in the field is quite valuable.

4. Skill at reading topographic maps. Ability to perform work in difficult terrain and under extreme weather conditions, sometimes for long hours. Ability to use good judgment and resourcefulness, and to be flexible in carrying out field work.

   This factor is intended to evaluate how much experience an applicant has had in the outdoors. It is important that field personnel know where they are, how to get back to where they started, and what to do in poor weather conditions or when things go wrong. Even well recorded scientific observations are of little use if a field crew is not sure where they were. This is also important for basic safety, especially when surveys are conducted in remote areas. It does not matter whether this experience is gained as part of general recreation (e.g., backpacking) or while conducting actual research.

Appendix A gives detailed, formal guidelines which we have used for evaluating candidates for each of the above four criteria.

Training

Aquatic surveys are not the type of work someone can simply "learn by doing." A good training course is an essential part of a successful field season. Training covers not only the identification of adult and larval amphibians, but also data collection, and both logistic and administrative matters. Appendix B provides an outline of the training course we have used for the last several years. This outline would need to be modified to accommodate local conditions, the experience of field personnel, and administrative matters appropriate for different employers.

A training course must include sufficient field time to visit a diversity of aquatic habitats (e.g., ponds, streams), see aquatic survey techniques implemented, have all participants conduct their own surveys, and spend time in the field evaluating results for accuracy and adherence to the protocol.

Since the identification of larval salamanders and anurans is an important aspect of the field work, a diversity of material must be available for examination. We not only look at live local amphibians, but also examine slides and preserved specimens. An informal slide quiz is a useful training aid at the end of the identification section. Most field guides are weak on the identification of larval amphibians. Appendix C provides some additional identification tips for selected western amphibian larvae.

Though it is beyond the scope of this protocol to expand on each point in the training course, it is worth emphasizing estimation techniques, an aspect of amphibian surveys which is a challenge even for experienced biologists. Estimating numbers of frogs, tadpoles, fish, or other wildlife is much more difficult than is generally appreciated. The technique that seems to work best for most people is to count a subset of individuals (usually 25 - 100) and then estimate how many more groups of that size would make up the total population. For example, count a group of 50 individuals and then scan the rest of the population, estimating how many sets of 50 there are.

The difficulty with any estimation technique is that there is rarely any feedback on the accuracy of the estimate. The Wildlife Counts software program (see Appendix E for supplier) is a useful training tool since it displays images of ducks on a pond, fish in a stream, birds in flight, etc., in a wide range of densities, and allows the user to estimate the number before displaying the actual count. Both new and experienced observers will find this feedback useful.

Project Supervision and Quality Assurance

The previous two sections have emphasized the importance of hiring biologists with a strong background in field work and conducting a thorough training program. Somewhat implicit in this discussion is the assumption that the project be designed, implemented, and supervised by someone with an extensive background in herpetological field work. It will be impossible to provide adequate training in amphibian biology and identification or the implementation of field techniques unless the supervisor/project leader has had sufficient field experience conducting amphibian surveys.

Additionally, the supervisor/project leader must participate in field surveys to assure that the protocol is being applied in a consistent and appropriate fashion. In general, we have followed our training course with immediate, five-day visits to field areas to work with field crews at the start of the season. These visits allow us to answer additional questions

and to assure that the protocol is fully understood and implemented. This close association with field crews is continued throughout the season and helps with both the proper implementation of survey techniques and good communication about the progress of the field work.

Establishing the Area to be Surveyed

The area to be surveyed must be defined at the start of a season so that an appropriate system of selecting sites within that area can be implemented. This section discusses how to establish the aquatic survey area and the next section considers ways of selecting the specific survey sites within a survey area. There are three main ways to define the survey area: by drainage basin, by management area, or by the habitat or range for a particular species. We will discuss each of these and offer recommendations about how to implement each type of survey.

Drainage Basin

The species of amphibians that are normally found during aquatic surveys are dependent on water for reproduction. Hence there is a biological basis for defining survey areas to include all suitable bodies of water within a naturally defined area such as a drainage basin.

A drainage basin might include one small creek, the combined drainages of several adjacent creeks, or the headwaters of an entire river system. In mountain regions, the ridges between drainage basins are often formidable barriers to amphibian dispersal and sharply define sets of populations that are likely to share dispersing individuals (metapopulations). A survey encompassing a drainage basin is clearly the best since it typically provides the most complete ecological information on the status of amphibians.

In many parts of coastal California, drainages are not topographically distinct; hence there are few barriers to amphibian dispersal. While it is preferable to define survey areas using these natural units, they do not always support genetically independent populations and it is more difficult to understand how populations in separate drainages might interact, both ecologically and genetically. Nonetheless, naturally defined areas, even when only

modestly isolated from adjacent areas, are the most ecologically meaningful areas to sample.

Management Area

Aquatic surveys are sometimes conducted to provide information about a particular management area. The area might be local (e.g., a new campground site) or extensive (e.g., an entire wilderness area). A biologist might be asked to determine what amphibians are present within the management area and what the impacts of a proposed management change might be on known or potential amphibian populations.

Conducting surveys in areas that are not clearly defined by natural barriers to amphibian dispersal can result in data that are difficult to interpret. For example, if a new 10-acre campground is proposed, how large an area should be searched? There may be no amphibian breeding areas within the proposed project, but a stream through the campground might be an important dispersal corridor. Increased human activity might also lead to the introduction of non-native animals (e.g. trout, bullfrogs) that could significantly impact local amphibians.

In general, surveys should be extended to include all populations of amphibians that might interact ecologically by occasional exchange of individuals. If this is not possible, try to establish at least a buffer zone that would be included in the survey. A minimum buffer of two kilometers is recommended, but this may not be sufficient in some areas. Any significant amphibian habitat (e.g., large meadow, series of large ponds) just beyond the buffer zone should be included as well.

Species Habitat or Range

This type of survey focuses on a particular species, typically one that is formally listed or believed to be rare. A single species survey would include all habitat for that species within either the entire geographic range or an area of interest such as a National Forest or State Park. Focusing surveys on particular species limits the habitat types that need to be surveyed. For example, when surveying for foothill yellow-legged frogs in northwestern California, we typically stop searching in the upper reaches of creeks where they become steep (high gradient), cold, and torrential. Such habitat is not suitable habitat for yellow-legged frogs. The habitat change indicates the upper boundary of the appropriate survey area for this species.

Unless one is familiar with the habitat preferences of a particular species, there is a tendency to focus on those areas. It is also important, however, to search areas that are believed to be marginal or largely unsuitable for the species of interest. Habitats that are

not used in some parts of a species' range are regularly occupied in other areas. Also, amphibians are occasionally found to occupy habitats that have been thought to be unsuitable. Hence it is always best to search farther than what would normally be considered suitable habitat.

Selection of Survey Sites

The specific sites within the survey area can be selected in a number of ways. In the following discussion, we discuss the primary techniques for selecting sites and then offer some guidance as to when each technique would be most appropriate.

Complete Survey

A complete survey includes all aquatic habitats within the survey area, e.g., the entire length of all streams and rivers, and all ponds, lakes, and meadows. Areas that are clearly unsuitable for amphibians (e.g., dry stretches of creeks, fast flowing rivers, dry portions of meadows) are not included. Surveys focusing on a particular species may also be complete within the appropriate habitat for that species (see the Species Habitat or Range section).

Complete surveys conducted in a naturally defined area, such as a drainage basin, provide the best information on the population status of amphibians. It is best if the survey area has boundaries that are barriers to amphibian dispersal. Some biologists believe that individuals routinely move from one population to another, with one or a few very reproductively successful populations providing most of the dispersing individuals for other populations. Without this dispersal, these other populations might not be self-sustaining over a long period of time. If only a few populations within a drainage are visited, the results would be difficult to interpret and the status of a particular species might be interpreted incorrectly.

If aquatic surveys are being conducted in anticipation of either a development project or significant change in management practices, complete surveys (not sample surveys, discussed later) should be conducted unless the affected area is extremely large.

Surveys based on a sampling regime are not sufficient when there are potentially serious impacts on populations of rare or declining amphibians.

Advantages - A complete survey has advantages over other techniques, both because there is no bias in selecting sites and because there is no need to extrapolate the data to sites not visited. Most other types of surveys only sample a few of the potential sites for amphibians. While a survey based on samples may be dictated for a number of reasons (e.g., cost or the size of the area to be surveyed), it is preferable to conduct a complete survey by visiting all potential amphibian habitats within the area of interest. This procedure eliminates any sampling bias and increases the likelihood that amphibians will be located if they are present.

Disadvantages - The primary disadvantages of a complete survey are the time and expense. If the survey area is small, these considerations may not be significant. In a large park or wilderness, it may not be possible to conduct a complete survey. If so, it will be necessary to select representative or random sites.

Historic Survey

It is often useful to revisit sites where certain species have been recorded in the past. This is especially true for declining amphibians that can be difficult to locate because of their patchy distribution. Historic data are often available from museums, wildlife observations kept in park files, published studies, and from communication with other biologists.

If the survey is intended to evaluate population changes over time, it is necessary to determine whether each historic site still provides suitable habitat for amphibians. Some sites may have changed such that they no longer provide suitable habitat because of obvious loss of habitat (e.g., a new housing subdivision, clear cut forest) or natural changes (e.g. success ional changes in a meadow or pond) that result in significantly different dominant vegetation. When analyzing population trends, such sites must be treated differently from sites where habitat is still suitable. Clearly this evaluation requires a fair amount of field experience and a strong understanding of amphibian natural history.

Advantages - The decline of amphibians in western North America and many other areas has been difficult to document since there were few biologists monitoring amphibian populations over the time period of interest (1970 - 1990). Historic records (largely based

on museum specimens) have provided some of the best information on the distribution of amphibians prior to the decline, especially when field notes or natural history publications accompany specimen records.

Disadvantages - While comparisons with historic sites can be valuable, historic sites are seldom random, and may not be representative of the available habitat. Many historic sites are near roads and trails, factors that may be contributing to amphibian declines.

The absence of amphibians at a historic site is not necessarily proof that the species has been lost from the area. The original identification may have been incorrect and the species never occurred there. It is important to examine the actual museum specimen, if possible. Also, the habitat may have changed. Sometimes this change can be evaluated by reading the field notes that may accompany museum collections.

Locality information for many of the older, and hence more valuable, museum specimens is often vague and sometimes refers to features that are no longer present. Some specimens collected in the early 1900s have localities that reflect where the early naturalist camped, not where the animal was found. As a result, the relationship of a collection site to the written locality is sometimes only casual. This imprecision makes it difficult to revisit a site. Fortunately, recent locality data tend to be more specific and thus more useful, but the older records are of most interest.

Sample Surveys

If the survey area is large, it may be necessary to sample some of the available sites rather than visit all potential sites. The sites to be surveyed can be selected by either a representative or random process.

Representative selection of sites

Survey sites can be selected to represent different geographic areas within 1) a species' range, 2) different habitat variables such as elevation or plant community (e.g., grassland, oak woodland), or 3) different aquatic habitats (e.g., pond, stream, lake, meadow).

For example, when conducting surveys to evaluate the distribution and population status of a widespread species, it would be appropriate to visit a large number of widely scattered sites representing different geographic parts of the range rather than conducting a more detailed, complete survey in only a few small areas.

If sites are selected on a representative basis, great care must be taken to ensure that all suitable habitats are sampled and that all variables that might affect amphibian distribution and abundance are considered. For example, if mountain yellow-legged frogs are the target species, a survey should include sites with and without trout, sites that represent a range of elevations, and sites that include a range of aquatic habitats (e.g., ponds of different depths, lakes, streams, meadows).

Advantages - The use of representative sites is a flexible approach. It allows a field crew to adjust the amount of effort according to the quality of habitat and the amphibians found. For example, foothill yellow-legged frogs are almost entirely absent from the southern Sierra Nevada foothills. When a remnant population was located in that area in 1994, additional sites were visited in the area and eventually a complete survey of the drainage basin was conducted to locate any other populations in the area.

Disadvantages - Selection of representative sites depends on a knowledge of amphibian natural history typically found only in experienced, well-trained field crews. Additionally, representative sampling will almost always result in some sampling bias; hence it is not appropriate to extrapolate these data to a larger area.

Random selection of sites

Random selection of survey sites is appealing primarily because it allows for the statistical extrapolation of data from a sample of sites to an entire area. To be statistically valid, however, the sample sites must be selected from the entire range of aquatic habitats (e.g., rivers, streams, ponds, lakes, marshes, meadows, etc.) and habitat variables (e.g., elevation, plant communities, presence of introduced fish, water quality, temperature).

This assumption is difficult to meet. The necessary procedure is to 1) define the entire set of possible sites, and 2) make a random selection from those sites. **All** appropriate habitats must be included, e.g., all suitable aquatic habitats used by the amphibians of interest. As the number of important habitat variables increases (e.g., presence of non-native fish, habitat type), the number of sample sites must increase as well.

In some cases, it might be appropriate to stratify the set of potential sites and then randomly sample within the various strata. This method is the best way to sample when there are significantly different types of animals in different habitat types or if the numbers of animals in a habitat are significantly different. If a stratified sampling regime is used, the statistical extrapolation is conducted independently for each set of samples.

Advantages - If all the assumptions for random sampling are met, data from the sites that are actually visited can be extrapolated to the entire survey area. This procedure results in more cost efficient field work and allows a large area to be included in a study. Also, if the selected sites are truly random, it reduces the bias that might be present in other sampling techniques.

Disadvantages - Random samples can be extrapolated to the entire survey area only if there are enough sample sites to ensure that the variability in the sample sites covers the range of variability for the entire area for the ecological factors of interest (e.g., population density, reproductive success, species composition). If this is not the case, the process is not statistically valid. Conclusions about the status of amphibians can be entirely erroneous if extrapolations are made from random samples that were not appropriately selected. Unfortunately, such extrapolation errors would not be detected, or perhaps even suspected, if additional surveys were not conducted.

Bias is surprisingly easy to introduce during the site selection process. For example, if one is surveying for Cascades frogs, it is not appropriate to begin all searches at sites where streams cross roads since this frog is also found in ponds and meadows, habitats that would be missed or greatly under represented with this selection technique.

Clearly random sampling is not an easy process. Often the information necessary to define all possible sites is not available. Not all amphibian habitats are shown on 7.5' USGS topographic maps or on photographs.

Recommendations for Selection of Survey Sites

Complete surveys are clearly the best if they can be accomplished. They provide the most complete information on population status and distribution, and there is little risk that an isolated, but highly significant population will not be included in the sites sampled. With the level of information gained from complete surveys, it is much easier to evaluate population dynamics, predict which are the most important populations in terms of reproductive success, and hence develop the most reliable conservation recommendations. We have used this type of survey in large regions of Yosemite, Kings Canyon, and Lassen National Parks. Complete surveys are also best when only a few populations are known, e.g., Cascades frogs at the southern extreme of their range.

Historic surveys can be valuable, but it is often difficult to make meaningful comparisons with contemporary surveys because of vague locality data (especially for the oldest records) and the problem of habitat change over time. A few studies have been

successful, however, in using historic information for evaluating amphibian population trends (Fellers and Drost, 1993; Drost and Fellers, 1994). For any study, historic data should be checked to make sure that any historic sites are identified and carefully surveyed for existing populations of amphibians.

The best technique for selecting survey sites varies with the goals of a study. Representative sampling can be quite efficient for evaluating the status and distribution of amphibians over a large area. This method is flexible and efficient, but it requires knowledgeable and experienced field personnel. This is the technique we have used successfully for much of the work at low elevations in the California foothills.

In general, studies based on the random selection of survey sites are unlikely to be successful in predicting the distribution and population status of amphibians because of the difficulty in meeting the essential assumptions of this technique. Special attention needs to be given to the study design so that there is no doubt that all appropriate areas are included in the set of sites from which random samples are selected.

Conducting Field Surveys

Aquatic surveys were designed to maximize the likelihood that 1) all aquatic-breeding amphibian species in the area will be detected and 2) their relative abundance will be accurately recorded. Aquatic surveys are most efficient at locating eggs, tadpoles, and frogs that are strongly diurnal or closely associated with water (e.g., *Bufo, Hyla*, *Rana*). Salamanders that breed in ponds and streams are also generally well sampled (e.g., *Taricha*). A few habitat specialists are not well sampled by this technique (e.g., Olympic salamanders, tailed frogs).

Time of Day and Time of Year

Aquatic surveys are conducted during the day. Eggs and tadpoles are sampled most efficiently when they can be found both visually and with a dip net. Adult frogs can also be found during the day, in some cases better than at night. During the peak of breeding, some frogs, such as the Pacific treefrogs and red-legged frogs, are more conspicuous at night. If it is important to obtain maximum counts of breeding frogs, aquatic surveys can be augmented with searches at night since adults of some species are more active at night.

Surveys are most reliable if conducted from the onset of the breeding season (generally in the early spring) to when tadpoles are just beginning to metamorphose in the summer or fall. In colder areas (e.g., higher elevations of the Sierra Nevada), adult frogs are notably less active as the weather cools in the fall (late September through October). While surveys may be able to confirm the presence of frogs during that time of the year, counts of individuals may be lower as adults become less active and the newly metamorphosed young disperse.

Aquatic surveys are usually conducted from early spring to the late summer in order to detect all life history stages of frogs: eggs, larvae, juveniles and adults. Seasonal and daily variation in activity periods will influence which life history stages are found on a given survey. For example, during a survey conducted in the late spring, eggs, larvae, and adults of one or more species may be encountered, while during the late summer only recently transformed juveniles and a few adults may be observed. Also, some species are nocturnal. Adults of some species tend to be more secretive and less active during the day; hence it is more likely that a surveyor will encounter only eggs or larvae during diurnal surveys.

For sites that are monitored from year-to-year, it is important to conduct repeated surveys at the same time each year. This does not mean on the same date, but at an ecologically similar time. For example, some species breed just as the snow disappears, but the exact date can vary by a month or more from year to year.

Equipment and Supplies

The primary pieces of equipment are a sturdy dip net, a pair of binoculars that can focus to within at least 3 m, and suitable boots or other footwear. Additional supplies include a high quality, single lens reflex camera and flash, scales, tape measure, measuring board, and data sheets. A seine may be required for sampling some ponds. Optional equipment includes supplies for taking pathology samples, genetic tissue samples, blood samples, and voucher specimens. A detailed list of equipment and suppliers is given in Appendices D and E.

Dip nets should have a sturdy wooden handle (approximately 1.25 m long by 2.5 cm thick) so the net can be pulled through dense vegetation. Mesh size should range from 2 - 4 mm. (Mesh sizes are measured when stretched to their maximum length. The functional size of the opening is typically about half the listed mesh size.) Smaller mesh sizes tend to get clogged with algae whereas larger mesh sizes allow the smallest tadpoles to pass through. Larger mesh sizes work well later in the season when tadpoles tend to be larger and the aquatic vegetation is more extensive.

When dip nets need to be packed into the backcountry, we use nets with aluminum handles. While neither the handle nor the hoop are as sturdy, there is generally not as much dense aquatic vegetation in these alpine areas.

Footwear

Footwear is very much a personal decision. We have found, however, that some combinations work particularly well. Except in the Pacific Northwest where both the streams and the weather are quite cool, we do not use chest or hip waders because they tend to be heavy and hot.

The most versatile footwear is Teva[1] (or similar) sandals worn with neoprene dive booties. Tevas provide good traction on a wide variety of substrates. Make sure the Tevas have a sole that will grip irregular surfaces, e.g., not a flat sole, but more like Vibrum soles. Dive booties provide good insulation and additional foot protection. This combination is also lightweight and easy to carry in a backpack.

When more substantial foot protection or ankle support is desirable (e.g., in boulder-strewn creeks), felt-bottomed boots with dive booties work well, but they do not generally have the traction of a good Teva sandal.

During the later part of the summer, regular hiking boots can be worn at higher elevations since the meadows are reasonably dry and we usually walk only on the bank of high mountain ponds and lakes. In this situation, leather boots with a good coat of waterproofing are preferred.

Survey Method

A description of the survey method can sound deceptively simple. It is important to emphasize that a considerable amount of training and experience are required in order to locate and properly identify amphibians.

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[1]. The use of trade names and the identification of vendors here or elsewhere in the document does not imply a U.S. Government endorsement.

Basic technique

The basic technique for conducting aquatic surveys is to 1) use binoculars to scan for basking frogs, 2) slowly walk in the water or on the adjacent bank while visually searching for eggs, larvae, and adults, and 3) use a dip net to find and capture larvae and adults. Each of these three points is discussed in more detail below, including modifications necessary to fit different habitats.

Scanning with binoculars

Begin the survey by scanning the banks, exposed rocks, floating vegetation, etc. up to 15 m ahead for basking frogs. Also, make sure to scan less conspicuous places such as under overhanging banks, in holes in the bank, and under raised logs. Some frogs are often found sitting in fairly conspicuous places (e.g., Cascades frogs, bullfrogs) while others are often in the darker, more sheltered spots (e.g., red-legged frogs).

Scanning with binoculars allows you to find frogs that may jump into the water before the surveyor is able to locate and identify them. Binoculars also help locate the more secluded animals that might not move and could otherwise be overlooked.

If you cannot see 15 m ahead because of vegetation or a bend in the creek, scan as far ahead as possible and then proceed slowly to minimize the chance of startling a frog.

Turtles are often seen with only their heads above water; in this position, they look remarkably similar to a stick. Garter snakes sometimes sit with only their heads protruding from an opening. While reptiles are not the primary focus of aquatic surveys, garter snakes are significant amphibian predators and are important to record.

Visual searches

After scanning with binoculars and tallying the individuals observed, begin walking slowly. Visually search the banks, rocks, logs, pond bottom (if water clarity permits), and the surface of floating vegetation within a few meters of your location. After walking 10 - 15 m, stop and scan ahead with binoculars before advancing further. Record the species and number of individuals seen as you proceed. Alternate binocular scanning with dip netting (see Dip netting below), and visual scanning.

Frogs can be found on the bank, on rocks, swimming, or sitting on the bottom (underwater). Tadpoles may be conspicuous, but they may also be cryptically colored or obscured by vegetation. Similarly, some eggs are fairly obvious (e.g., toads, bullfrogs) while others are difficult to see. For example, foothill yellow-legged frogs typically attach their

eggs to the downstream side of rocks, especially in the calmer portions of a creek. The egg masses tend to accumulate a covering of silt that makes them quite cryptic.

There is a tendency for people to focus their attention on a single microhabitat such as the stream bank or pond bottom. Constantly make sure that you are including all possible microhabitats in your search pattern. Also, be aware of all life history stages that might be present (e.g., eggs, larvae, subadults, adults). If you are expecting to find only adult frogs, it will be easy to miss eggs.

Dip netting

A dip net is necessary for locating and catching amphibian larvae. In general, you need to sweep a dip net through the water on a regular basis as you proceed. In clear, mountain ponds sweeping a net through the water is not critical since you can see virtually anything that you might catch. In most areas, however, dip netting is essential for an accurate survey.

Sweep the net in all types of microhabitats: plunge pools, riffles, slow moving backwaters, beneath overhanging banks, and within floating and emergent vegetation. If not blocked by dense vegetation or limited by the depth of the water, sweep the bottom with each pass of the net. Check the contents of the net after each pass through the water. Record the species, life history stage, and the number of individuals observed.

Wave the dip net over grasses and bank vegetation to flush frogs that may not have jumped as you approached. This is especially useful for mountain yellow-legged frogs, particularly on a cool day.

Attempt to capture amphibians to verify identification, collect biological data, and photograph the animal (see sections on Conducting Field Surveys and Photography). While some frogs can be caught by hand, other species are caught most easily with a net. A dip net is also useful for catching garter snakes, fish, and aquatic invertebrates for identification.

Meadow surveys

The basic survey technique is modified when surveying meadows. Meadow surveys are conducted by slowly walking along the mainstream channels and circling all potholes and pools of water. Be sure not to overlook very shallow pools and seeps (as shallow as 2 cm deep) that may be used by species such as the Yosemite toad.

Visibility is usually low in meadows, so the surveyor must rely heavily on the constant use of a dip net. Dip the net frequently within emergent and floating vegetation, and beneath overhanging banks to search for amphibian larvae, fishes and aquatic invertebrates. Make sure to sweep the bottom with each pass of the net. Check the contents of the net after each pass through the water. Also check for egg masses attached to emergent vegetation. Record the species, life history stage, and number of individuals observed.

When not following an obvious channel, it is sometimes difficult to survey the entire meadow efficiently. We have found that a zigzag path through the meadow, with 10 m wide sweeps is effective.

Where to walk

Surveys are conducted either by walking in the water or, if necessary, along the adjacent bank. Wading typically provides the best vantage from which to spot frogs, especially when there is extensive vegetation on the bank that might provide good cover for frogs. Wading can also be a quieter, more surreptitious means of moving around the circumference of a pond. Note, however, that a number of amphibian species lay their eggs primarily around the edges of lakes and pools where they would be susceptible to trampling. Be careful.

In general, you should walk on the bank if 1) the bottom is steep, muddy, or slippery; 2) the water is running too swiftly; or 3) the water is remarkably clear, as with some mountain lakes and ponds.

Personnel

Aquatic surveys are best conducted by two-person crews. For streams less than 3 m wide, one person can survey the entire width of the creek. This allows one person to search upstream while the other searches downstream. If the stream is wider than 3 m, two people can walk parallel to each other on opposite sides of the creek. When possible, walk upstream to avoid turbidity caused by walking in the creek.

Small ponds and lakes can be either surveyed by one person who walks entirely around the periphery or by two people walking in opposite directions. For larger lakes and ponds with convoluted banks, surveys can take several hours to complete. In this situation, a two-person team can complete the work nearly twice as fast. This cooperation helps maintain good morale, and results in better quality work. Similarly, small meadows can be covered by a single person, while it is more efficient to have two people in larger meadows.

Ponds that need to be seined require two people, one to work each end of the seine. Seining is an effective sampling method for amphibian larvae (Shaffer et al., 1994); however, it is impractical in ponds with dense vegetation or numerous rocks and logs.

Recording data

Record the number of eggs, larvae, and adults of each species as you conduct the survey. Do not rely on memory unless the number of animals seen is quite small.

During each survey, the total survey time is determined by running a stopwatch only during the period when you are actively searching for amphibians (see section on Duration of each Surveys below). The stopwatch is paused when recording data, moving around obstacles, photographing amphibians, and conducting other activities.

Duration of each Survey

The length of time spent at each site is variable, depending on the quality and extent of available habitat. The field crew is responsible for determining the length of time to spend surveying at each site. If the habitat becomes unsuitable (e.g., the stream dries up) or it is impractical to continue surveying (e.g., dense vegetation overgrowing a stream, physical obstacles, dangerously swift water), the survey may be discontinued at any time. If there is sufficient good habitat, the minimum time spent surveying is two person-hours. (A person-hour is calculated as one person searching for one hour or two people searching for a half hour.)

Searches of much longer than two person-hours are desirable when the habitat appears to be particularly good, if there are records of rare amphibians in the general vicinity, or if it is likely that only one visit will be made to the site. In some areas, there is a significant investment of time in getting to a site, hence it is preferable to conduct one thorough survey rather than revisit the area.

If the survey is designed to provide complete coverage for an area, surveys are continued until all appropriate habitat has been searched. This procedure may take several hours, days, or weeks, depending on the size of the area (often a watershed) that has been established as the survey area.

Weather Conditions

Rain and cold weather can interfere with effective surveys. Rain will 1) reduce visibility, making it difficult to see tadpoles and eggs through the surface of the water, 2) interfere with the use of binoculars which are helpful when scanning for basking frogs, and 3) make it harder to stay warm and dry, thus reducing the likelihood that data will be both complete and accurate. Additionally, some frogs seek more protected areas while it is raining. In general, surveys should not be conducted in the rain. In parts of the Pacific Northwest, however, it often rains and it might not be practical to exclude rainy days.

Cold weather reduces the activity of frogs and toads, especially at high elevations. Mountain yellow-legged frogs are notably more difficult to locate in cold conditions. The tadpoles tend to spend the cooler parts of the day in the deeper parts of a pond or lake. They are most conspicuous from late morning to late afternoon. As the weather gets cooler in the late summer (September), adult frogs are less conspicuous. It is best to conduct surveys from mid-morning to late afternoon. When searching for adults in cooler conditions, turn over rocks at the edge of the water, both on land and in shallow water.

Nocturnal Surveys

Aquatic surveys are most efficient at locating eggs, tadpoles, and frogs that are strongly diurnal or closely associated with water. However, some adult frogs and toads are found in greater numbers at night, e.g., Pacific treefrogs and red-legged frogs during the height of their breeding season. If it is important to maximize the number of adults counted, diurnal aquatic surveys can be augmented with searches at night. The same basic technique can be used, but the search typically concentrates on adults, not eggs or larvae.

Nocturnal surveys are conducted by using a bright light to look for eye shine of adult amphibians. Since the angle of reflection is small, hold the light near your own eyes so you sight down the beam of light and watch carefully for a bright reflection from either one or both eyes, but with practice, this technique can be carried out most effectively with a pair of binoculars. With some lights, it is convenient to rest the binoculars on the light so that they move in tandem. Surprisingly, the eyeshine from some spiders is nearly as bright as that of frogs. With practice, reflections from spiders, raindrops, etc. can be distinguished from frog eyeshine.

While headlamps with 2 or 4 AA batteries are useful for finding your way around, they are generally inefficient for locating frogs. A light with at least a 6-volt lantern battery or a separate gel cell works best. For searches lasting only 30 - 40 min, there are some

250,000-candle power rechargeable lights that work well. Appendices D and E provide a list of equipment and suppliers.

Monitoring Amphibian Populations

Limitations of time and budgets often dictate that aquatic surveys can be conducted only once at any given site. If the field work is carried out by experienced and well-trained biologists, a single survey can evaluate the presence and abundance of species at a site, particularly if the survey is timed to coincide with the peak abundance of larvae or breeding adults.

Monitoring a particular site can provide valuable additional data that would not be available with a single survey. Visiting a site several times throughout a season can provide information on both reproductive effort (i.e. number of eggs) and reproductive success (i.e. number of tadpoles that survive to metamorphosis). Reproductive effort and reproductive success are both important indications of the health of a population. For example, visiting a site late in the season and finding only a few metamorphosing tadpoles would clearly indicate a low reproductive success. This failure could be caused by few adults laying eggs or by poor survival of either the eggs or tadpoles. By visiting a site several times throughout the season, a researcher would be able to evaluate these possibilities and thus have a far better opportunity to determine the cause of the low reproductive success.

If possible, a formal program should be established to monitor the status of amphibian populations. We offer some guidelines on selecting sites and implementing such a program.

Monitoring Considerations

Selection of sites

A few amphibian species have declined to the point where only a few populations remain. In such a case, all populations should be included in a monitoring program.

For many species, there is a substantial number of populations with a wide range of population size and reproductive success. A good monitoring program should include sites that represent both the range of environmental conditions (e.g., habitat type, elevation) and population status. For example, a monitoring program should not include only the largest population for a particular species, nor should it include only the smallest, most endangered populations.

Consideration should be given to the long-term stability of a monitoring site. Stable conditions will make it easier to evaluate factors that may cause population fluctuations. It is also useful to have some sites where the effects of fire, logging, construction, etc. can be evaluated. Hence, it is usually best to select sites that represent a wide range of conditions.

Frequency of monitoring

Monitoring programs can be implemented at several levels of effort. It is best if the selected sites are visited at the beginning of the breeding season (to evaluate initial breeding effort) and again when the larvae begin to metamorphose (to evaluate reproductive success for the season). Since it is not always possible to judge when the peak of the breeding season will occur or when the larvae will metamorphose, it may be necessary to make several short visits at both the beginning and end of the season.

Monitoring can also be conducted annually. Clearly annual visits will not provide as much information on the status of a population as more frequent visits. Over a number of years, however, population trends can be tracked, but there will be less information on any changes in population size.

If a population is visited only once a year, the survey should be made at the peak of breeding so a maximum number of adults can be counted. Counts of adults made at other times of year may not reflect the number of adults in the population and counts of larval amphibians represent that year's reproductive output, not the size of the adult breeding population.

Monitoring of sites at intervals of more than a year can determine whether a population is still present, but without information on yearly fluctuations in population size, only very general conclusions can be made.

Priorities for Data Collection

This section provides a priority order for the various procedures that should be performed with the amphibians found during aquatic surveys. The procedures are listed in order with the most important information being collected first.

All amphibians captured should be processed at least to the point of a visual evaluation of their health. A sample of amphibians at each site should be weighed, measured, sexed, and photographed.

Additional data on pathology, blood, and DNA samples may also be needed. The pathology and DNA samples are easy to collect and require relatively little time or expense. They should be included as part of any aquatic survey, though they do require the acquisition of some extra supplies and the cooperation of other researchers qualified to analyze the samples. Collecting blood for toxicologic analysis (e.g., pesticide effects) is rather specialized and should only be undertaken as part of a formal research program designed to evaluate pesticides.

Capture - Try to capture at least the first 12 amphibians of each species at a site. At some sites, such as the higher elevations of the Sierra Nevada, only one species occurs for each of the three local genera, so it is not necessary for experienced field workers to catch them to confirm identification. It is, however, important to capture the animals to document their presence (at least with photographs) and gather data on their health, size, weight, and sex ratio.

Count and identify - The most basic information to gather is the number and identity of each species found at a site. Standard field guides work well for identifying adults (Nussbaum et al., 1983; Stebbins, 1985; Leonard et al., 1993). Appendix C supplements information in the field guides for the identification of selected larval amphibians.

Health - A visual assessment of the health of all amphibians (e.g., eggs, larvae, adults) is important. Sick or diseased amphibians do not survive long in the wild and may provide valuable data on potential causes of population declines. If an animal obviously is unhealthy, it is best to collect it. Follow the procedures in the Pathology Specimen Collection section.

Larval or adult amphibians might have skin abnormalities (abnormal reddening, ulcers, tumors, cottony fungal growths, etc.), or external parasites (leeches). While most amphibians have difficulty moving at cold temperatures, sick adults may be characterized

by not having a righting reflex, loss of use or coordination of one or more limbs, abnormal positioning of one or more limbs while at rest, or by having spastic or epileptic (convulsive) movements.

When examining eggs, the only disease likely to be noticed is a fungal infection. Fungus will appear as a white, cotton-like growth on the egg mass or within the jelly of individual eggs.

Sex - The sex of many adult amphibians can readily be determined, especially for frogs. Standard field guides describe the distinguishing characteristics such as swollen thumbs or dark throats of male frogs.

Weight - A sample of 12 adult or subadult frogs should be weighed and measured at each site to assess the general health of the population since healthier animals are likely to weigh more for their size. Most amphibians are easily weighed in a small plastic bag using a spring scale (e.g., Pesola scale). Use the data sheet to record the total weight and subtract the weight of the bag. The bag is more stable if the animal is placed in a corner and then the bag is rolled into a cylindrical shape. This reduces the area of the bag that is exposed to a wind and also keeps the animal from moving as much. Use your body as windbreak if necessary.

Length - The length of a frog is measured most easily using a ruler with a stop at one end. Such rulers are not commercially available, but can easily be made. The most commonly used size is constructed of a wooden base, approximately 20 x 5 cm. A 5 x 5 cm stop is nailed and glued to the end. A flat, plastic 15 cm (6") ruler is glued to the center of the wooden base with the metric scale beginning at the stop. Use a good, waterproof glue to attach the ruler.

When measuring a frog, hold its head up against the stop and then gently press down on the vertebral column and pelvic girdle so that the frog's back is parallel with the ruler. Measure the distance from the tip of the nose to the vent (base of the hind legs).

Small- or medium-sized salamanders are measured in a Ziploc bag. Place the salamander in the bottom of the bag and hold the ruler along side the body. Measure from the tip of the nose to the posterior end of the vent.

Photography - Photographs can be used to document the presence of amphibians at sites where voucher specimens are not collected. To ensure that an animal does not escape

while collecting additional data, photographs should be taken as soon as the basic information (described above) is recorded. See the Photography section for details.

Pathology - In most wildlife species, signs of illness are usually well hidden (D. Earl Green, pers. comm.). Amphibians that appear to be unhealthy are probably very sick and are unlikely to live much longer.

Even if an amphibian is not clearly sick or showing signs of abnormalities, it is valuable to collect at least external samples and blood samples to determine normal, baseline levels for diseases, parasites, and blood characteristics. External samples may include skin or cloacal swabs for bacteria and fungus cultures, removal of external parasites, or collection of voided body wastes (excrements). See the Pathology Specimen Collection section for details.

Make sure to wash your hands after handling any sick amphibians so as to reduce the likelihood of spreading any disease or infection to other individuals.

Blood - Blood can be collected as part of a baseline evaluation of an individual, a population, or a species. Blood samples range from simple blood smears on glass microscope slides (for examining white blood cells or blood cell protozoan parasites) to whole blood collection which can be used for a wide range of analyses (e.g., serologic, serum chemical, toxicologic, hormone, DNA/RNA), but which may require a centrifuge and liquid nitrogen. The preparation of blood smears is described in the Pathology Specimen Collection section while the more specialized procedures for collecting whole blood samples are described under Blood Collection via Heart Puncture.

DNA - Collection of tissue for mitochondrial DNA analysis is straightforward, especially if only small- or medium-sized larvae are involved. Samples can be stored in vials of 95% ethanol or in liquid nitrogen. See the Tissue Collection for DNA Analysis section for details.

Voucher Specimen - While we do not recommend the routine collection of formalin-preserved museum specimens, it may be appropriate to do so in some cases. If so, make sure to obtain all possible data prior to sacrificing the animal. See the section on Voucher Specimens for guidelines on collection of vouchers.

Aquatic Survey Data Sheet

An Aquatic Survey Data Sheet has been developed which is used to record all data from the aquatic surveys. This form should be completed even if the survey is conducted at night, includes seining, or includes time-constrained surveys. By using one basic data sheet, consistent information on the location and species found is recorded in a standard fashion. A sample aquatic survey data sheet is shown in Appendix G.

Overview

Fill out a survey sheet for each locality. If there are two or more habitats at a site, use a separate data sheet for each distinct habitat. For example, if there is a lake with a stream flowing from it, fill out one data sheet for the lake and another for the stream. Meadows may be problematical since a meadow might have a small stream, marshy areas, and perhaps a shallow pool. In general, you should include all these as part of the meadow unless the different areas are decidedly discrete and would clearly support a different frog fauna. This decision is simply a judgment call on your part.

Side 1

Site: This is an important number since it will be used to identify all tissue, blood, and pathology samples. Number all sites consecutively beginning at one. Prefix the number with the first letter of the name of the park or other area where you are working. For example, a sample from Redwood NP might be numbered R-59 while one from Yosemite NP could be Y-35. Sites that are immediately adjacent, but distinct (e.g., a lake with a stream flowing into it) are distinguished with suffix letters such as Y-132A and Y-132B.

Date: Write the date as month, day, year (e.g., Aug 11, 1993). The three letter abbreviation for the month is less ambiguous and more readily recognized than 8-11-93.

Begin Time: Record the time when field work began, not time you arrived at the locality.

Total Time: This is the total time (in minutes) spent searching for amphibians. It is the time spent walking along a creek or around a lake while looking for amphibians. If there are significant obstacles that must be avoided (e.g., major log jam, cliff face around part of a lake), do not include time spent avoiding these hazards. Also, do not include time spent processing specimens or recording notes.

If two or more people spend differing amounts of time searching, enter the average total time. For example, if one person spent an hour and another person spent 50

min, the total time should be 55 min. This time will be multiplied by the number of qualified observers circled in the next block.

Observers: Enter the names of the observers beginning with the name of the person filling out the data form. Circle the number of active, qualified observers who contributed to the survey.

Locality: This needs to be a description that would allow someone not familiar with the area to find the exact spot again. For example,

> Highway 1, 4.6 mi S of junction with Sir Francis Drake.
>
> Dry Creek, 0.3 mi upstream from junction with Hwy 216.
>
> Rice Creek between Crumbaugh Lake and Cold Boiling Lake.

Use mapped landmarks that are not likely to change. A distance from a large town or city is not particularly useful since it is difficult to tell from what point the distance was measured.

Mileages are assumed to be via road unless indicated otherwise. Sites reached by dirt roads that would be hard to find on a map will require careful descriptions. Use Forest Service road names if available. You might also need to give distance to a paved road junction. Backcountry localities can be given as the name of a mapped lake or as an airline distance from such a lake. When using airline distances, make sure to indicate a compass direction at least as detailed as NNW or ENE. Some localities are difficult to describe and the UTM coordinates will be the most descriptive, but not everyone has GPS equipment for relocating the site.

Try not to describe a locality as if it was a treasure hunt. For example, do **not** report a locality as:

> Take Redwood Ck Rd, go 0.5 mi to Bald Hill Road, follow trail to bridge where a large tree has fallen.

Owner: Circle the appropriate owner. The abbreviations refer to Unknown, National Park Service, Forest Service, Bureau of Land Management, State, Private, Other.

Elevation: This will need to be taken from a 7.5' topographic map. The elevations given by GPS most units are not sufficiently accurate. You might write the elevation down in pencil in the field, but it would need to be confirmed later by plotting the UTM coordinates on a map. Make sure to circle the units used. These units will normally

be feet if the elevation is taken from a topographic map. Since a few of the newer USGS maps have elevation in meters, it is necessary to indicate whether elevation is recorded in feet or meters.

Distance to Public Paved Road, Public Dirt Road, and Mapped Trail: Some evidence indicates that proximity to roads and trails relates to the presence or density of frogs. By recording these distances, it will be possible to examine the data for this relationship.

Public roads are roads that can be driven by the general public. Public roads do not include private logging roads, private driveways, or roads for "official use only." If a road has a gate, it is not generally accessible and hence not public. Public access does include roads and trails that are seasonally closed for reasons such as snow.

Always include the distance to a paved road. If there is a dirt road that is closer, include the distance to it as well. If access is by trail, include distance to the trail. For example, if a paved public road skirts a meadow site, include only the distance to the paved road. If you travel on a paved road which then turns to dirt and ends at a trailhead, include the shortest distance to the nearest trail, dirt road, and paved road. If a paved road leads to a trailhead, include only those two distances. Determine the distance to the closest point along a road or trail. In other words, if a trail ends at a lake, the distance from the lake to the trail is 0.0 km. In some cases, you will need to use a map to determine these distances.

Topographic Map: This is the name of the USGS topographic map as indicated on the corner of the map. Circle either 7.5' or 15' for the scale of the map.

UTM: This is a pair of numbers that are basically x and y coordinates. In our area, they are North and East. The most accurate coordinates are obtained by using the GPS and later confirming them with a 7.5' topographic map. Always record the UTM coordinates in the field if it is possible to lock in on at least four satellites. If fewer than four satellites are available, record the UTMs from a topographic map.

Circle whether the UTM coordinates were obtained from the GPS or from a map. If you used both, circle both. Also, circle the number of satellites that were used for any GPS readings as well as the zone (10 or 11).

If you walk a length of creek, you should enter the UTM coordinates for both the beginning and ending location. For lakes and ponds, enter data for only one site near the edge of the water.

Weather: Circle the most appropriate choice for cloud cover and rain/snow.

Wind: Circle the most appropriate choice. Light wind is < 5 mph, moderate 5 - 20 mph, and strong > 20 mph.

Air Temp: Measure the air temperature in the shade at approximately 1 meter off the ground. Circle F for Fahrenheit or C for Centigrade.

Water Temp: Measure the water temperature at approximately 15 cm deep either in the vicinity of any amphibians that are located or approximately 0.5 meter out from the edge of the water. Since water temperature can be quite variable in standing water, try to select a location that is representative of the site. Circle F for Fahrenheit or C for centigrade.

Habitat Condition: Circle the number that best represents how natural or altered the habitat is. A 1 would represent relatively pristine habitat such as old growth forest or an undisturbed mountain meadow with no obvious sign of human disturbance. A 3 would represent a second growth forest or a lake with a few picnic tables and perhaps a small parking lot at one end. A 5 would represent a clear-cut forest with little regeneration or a channelized stream in an urban area.

Site Description: Indicate the general habitat type that best describes the area surveyed. If the survey includes significant portions of two major habitat types (e.g., meadow and lake), use a separate data sheet for each site. See discussion under Site above for guidelines on designating site numbers.

Drainage: Consult a topographic map to determine whether the body of water is permanent or seasonal (e.g., dries up by the end of the summer). If the map designation seems in error, note this in the comments, but record the drainage as indicated on the map.

Site Length: This the length of a lake, meadow, or section of stream searched.

Aver. Width: This is the average width of aquatic habitat surveyed. If a significant amount of adjacent habitat is also searched, note this in the comments section, but do not include the dimensions here.

Max. Depth: Estimate the maximum depth if it is $\leq$ 3 m, otherwise, enter 3 m+ since it is generally not possible to estimate depths greater than this. For a stream or river, this measurement would be the depth of the deepest pools.

Aver. Depth: This is the average depth out to a distance of 3 m from the edge. For a stream, it would include 3 m from both banks. For example, if the bottom sloped evenly to a depth of 1 m at the 3 m distance out, the average depth would be 0.5 m.

Water Flow: All lakes, ponds, and meadows are recorded as still. Streams and some pools in streams have a measurable flow rate. Estimate water flow by putting a small stick, piece of bark, or wood chunk in the water and time how long it takes to go 10 feet. Slow is $\geq$ 11 sec, Med = 7 - 11 sec and fast $\leq$ 7 sec.

Water Turbidity: Clear is like tap water. Many mountain areas will have mostly clear water. Turbid water has so much suspended sediment that you cannot see through it more than 1 - 2 cm. You might find this condition either after a heavy rain or where there has been disturbance from cattle wading in the water or from road construction.

Mid-day Shade: This may be difficult to estimate, but is of most interest for streams where shading can be important in affecting water temperature. Estimate the percent cover for shade at mid-day with any trees or shrubs completely leafed out.

Emergent Vegetation: This represents the percentage of water surface that is covered by vegetation that is rooted in the substrate. Such vegetation would include rushes, cattails, grasses, etc.

Floating Vegetation: This is the percent cover for plants such as duckweed that float on the water.

Watershed: This can be a difficult and subjective category. The watershed of interest is the area that drains into the site surveyed. It does not include areas downstream or nearby streams that do not drain into the site visited. In some cases, the watershed may begin only a short distance from the site surveyed. Other times it may extend for many kilometers.

Indicate the predominant land uses influencing the watershed with a "P" for predominant. Note any other land uses with a check mark.

Substrate: Indicate the predominant type of substrate with a "P" for predominant. Note which other substrate classes are present with a check mark. Silt is a fine, mud-like sediment. The others are various diameters of broken rock gravel, and sand.

Predominant Vegetation: This is the dominant plant species in the area being surveyed, e.g., Douglas fir forest, redwood forest, oak savannah. In other areas, shrubs such

as *Ceanothus* or perhaps grassland will be the dominant vegetation. List plant species in order of dominance when possible.

Fishing Tackle: The presence of lost fishing tackle indicates that someone believed fish occurred in the area surveyed. If fish were not seen, the presence of tackle would suggest that fish might be present.

Fish Present: Circle the appropriate answer.

Species and Approx. Number: Try to identify any fish seen at least to the family level. You may need to capture fish and even preserve a specimen for later identification. If you are not able to catch the fish, give as good of a description as possible, e.g., minnow, mosquitofish, trout, sunfish.

Estimate the number of fish seen within the area surveyed. Do not extrapolate fish seen to the total number you think might be present. If you believe the number you have observed is much less than the number present, note that in the comments. In most cases, it is sufficient to know whether or not fish are present, but it might be possible to rank sites with fish using estimates of abundance.

Species Found: List all species of amphibians seen. It is preferable to use scientific names. You can abbreviate the names with the first two letters of the genus and the first two of the species. For example, the abbreviation for the Yosemite toads (*Bufo canorus*) would be BUCA and the western toad (*Bufo boreas*) would be BUBO. Note that some four letter combinations are not unique (RACA could be either *Rana cascadae* or *Rana catesbeiana*) so five letters would be required. If in doubt about the scientific name, use common names.

Garter Snakes: Amphibians make up a significant portion of the diet of garter snakes in some areas and declining amphibian populations may subsequently lead to declining populations of snakes. Hence it is valuable to document all garter snakes observed during amphibian surveys.

Garter snakes will be identified, weighed, and measured (snout-vent length and tail length). Record all garter snakes in the Species column on the front side of the data sheet and any measurements on the back. Use the comments section if necessary. If the snake has an obvious food bolus, this should be noted as a comment since it could affect the weight. If you cause the snake to regurgitate a food item, try to identify it and make a note in the comments. Weigh the snake before attempting to obtain a food sample.

Pond Turtles: Western pond turtles have apparently declined in some areas and are a formal candidate for federal listing. Record all pond turtles seen and attempt to catch them whenever possible. Measure the length of a turtle as a straight line from the anterior-most point on the shell to the posterior most point. Males have a concave plastron (ventral shell) while females have a flat plastron (Stebbins, 1985). Record the number of rings on the scutes. Normally these are easily distinguished up to at least eight.

Adults/Juv.: Record the number of adult and subadult amphibians actually seen. You may need to estimate this number for large populations. If so, note that it is an estimate. If you catch some of the amphibians (or can otherwise tell sex), indicate numbers of males, females, and unknown, i.e. 7 M, 4 F, 22 U.

Larvae: Record the number of tadpoles and larval salamanders. For some frogs, larvae do not metamorphose for 2-3 years. If you can clearly distinguish size classes, give a count for each class. Indicate whether the numbers are an estimate or actual count. Do not estimate numbers of individuals at the site, but rather the number you actually saw.

Eggs: Note the number of egg masses seen and estimate the number of eggs per mass. If the eggs do not appear healthy, note that in the comment section. Unhealthy eggs might be discolored or show signs of a fungal infection.

DNA #: Number tissue samples consecutively from 1 - 25. Indicate which numbers were stored in ethanol versus nitrogen. For example, if you collect 6 tissue samples of *Bufo boreas* in nitrogen and another 5 in ethanol, indicate "1-6 N, 7-11 A" on the line for BUBO.

If you also collected 12 samples in ethanol for *Hyla regilla*, indicate "1-12 A" on the line for HYRE. See also Tissue on the back of the form. A description of how to collect tissue samples is given below in the Tissue Collection for DNA Analysis section.

Survey Method(s): Indicate how you detected or searched for amphibians. Circle all techniques that were used. For example, you might walk around a pond with a dip net sweeping for tadpoles and note a calling treefrog at the same time.

Other: Circle the appropriate word if a voucher specimen, pathology sample, or a photograph was taken.

Side 2

Site: It is important to record the site and date on the back of each form if the forms are to be photocopied. That way, there is a way to confirm that the front matches the back of the data form.

Date: Enter the date.

The top two boxes are for recording data on frogs, garter snakes, and turtles that are sexed, weighed, and/or measured.

Species: Record the species name for each individual processed.

Tiss.: If you weigh and measure an individual from which a DNA tissue sample was collected, indicate its sample number here. These numbers can range from 1 - 25. If you collected any pathology samples, put a "P" in the box. It is possible to have both notations in the same box, e.g., "2 P"

Sex: Record either M, F, I, or -. Do not use male and female symbols. M = Male, F = Female, I = Immature, and U = unknown. The U might be used for a snake or frog that is clearly adult, but you are not sure of the sex. Subadults may also be difficult to sex.

Weight: Enter the weight of each amphibian weighed. Western pond turtles are weighed only if sufficiently large scales are available. It is sometimes possible to record the total, tare, and net weights in the box. Otherwise, use the comment section to do the calculations.

Length: This is the SVL (snout-vent length) for frogs. Turtles are measured as the straight-line distance from the anterior part of the carapace to the posterior. For snakes, record SVL as well as tail length. The two measurements should sum to equal the total length of the snake.

Comments: Write any comments here. In addition to the things noted above, you should comment on parasites, disease, unusual densities of aquatic predators, and deformities. Parasites might include leeches or similar invertebrates. Describe all pathology samples taken in the comments section.

Universal Transverse Mercator Coordinates (UTMs)

It is useful to describe localities with both a written description as well as an independent system of coordinates that can be found on readily available maps such as USGS topographic maps. The Universal Transverse Mercator (UTM) system of coordinates provides one of the best means of defining localities. UTMs are marked on most topographic maps and are much more convenient and intuitive to use than either latitude / longitude or legal descriptions based on range, township, and section. The main disadvantage of UTMs is that they are not as well known as the other systems, but that is rapidly changing since UTMs are an integral part of most Geographic Information Systems (GIS).

UTM coordinates consist of a pair of numbers (e.g., 4471943 N, 617554 E) that are essentially x and y coordinates. On USGS topographic maps, UTMs are usually shown along the map margins as blue tick marks at 1-kilometer intervals. While some of the more recent topographic maps already have the marks connected to form a 1 km grid, this is easily done on the other maps with a long ruler and a sharp pencil. Care must be taken to connect the correct tick marks since the UTM grid may not be exactly parallel with the printed edge of the map.

It is easiest to understand UTMs by examining a topographic map. We will offer one example here. On the 7.5' Lassen Peak, CA map the numbers 4472, 4473, 4474, etc. go up the left side of the map and 618, 619, 620, etc. are marked across the bottom. A point near the lower left corner of the map is designated as 4471 and 617. This position on the map represents a point 4,471 km north and 617 km E of some reference point (off the map). Note that 4,471 km is the same as 4,471,000 meters (1 km = 1000 m) and 617 km = 617,000 m. Since most Global Positioning System (GPS) units display UTM coordinates to the nearest meter, we record UTMs in meters just to maintain a consistent number of digits in the UTM coordinates. Clearly the last two digits are not significant since we do not really know our position to the nearest 10 meters. Hence the point on the Lassen Peak map mentioned above would be 4471000 N, 617000 E.

Since localities are recorded with greater than the 1 km precision marked on topographic maps, we use a transparent grid that is subdivided at 0.1 km (100 m) intervals to determine the UTM coordinates. The transparent grid is placed over the location of interest on the map and the edges of the grid are aligned with the 1 km grid that is either printed or drawn on the map. With the transparent grid in place it is easy to read locations to the nearest 100 m and merely estimate the next two digits so the coordinates are always

in meters. Locations are presumed accurate to within 0.1 km; hence the last two digits are merely used to maintain a consistent number of digits with the GPS readouts.

One reason that UTMs are convenient is that distance between sites can be readily calculated. For example, if site A is at 4471943 N, 617554 E and site B is at 4471393 N, 617631 E you can apply a bit of basic geometry to determine the distance from one to the other. Subtract one N coordinate from the other, and do the same with the E coordinates to arrive at 550 N and 77 E. The distance is the square root of ($550^2 + 77^2$) or 555 meters. These are pretty quick and easy calculations using a calculator as shown below:

4471943	617631
-4471393	-617554
550	77
550 x 550 = 302,500	77 x 77 = 5,929
302,500 + 5,929 = 308,429	Square root of 308,429 = 555 meters

UTM coordinates are referenced to a particular zone. The zone is noted in the text in the lower left corner of USGS topographic maps. Fortunately, unless research is being conducted across the boundary of two zones, UTM zones are not of particular concern, though it is necessary to record them as part of the locality description. Unfortunately, the boundary between zone 10 and zone 11 runs north-south along the eastern border of California from the NE corner of the state south through Lake Tahoe and continues straight south through the Central Valley. Hence UTM coordinates from Lassen Volcanic National Park are in zone 10 and those from Yosemite National Park are in zone 11. If you are not interested in calculating distances between two points, it is sufficient to note the appropriate UTM zone. Calculations of distance across zones requires that correction factors be added to UTM coordinates in one of the zones. It is beyond the scope of this protocol to describe cross-zone corrections, though they can be done relatively easily using a GIS.

Appendix F provides a template for making transparent UTM overlays. Photocopy the page onto transparencies and cut out the grid. It is useful to have several 7.5' and 15' overlays since they are extremely useful, but are easily lost.

Global Positioning System (GPS)

The Global Positioning System (GPS) is a relatively new technology for determining the UTM coordinates or latitude / longitude anywhere in the world. GPS is extremely accurate, relatively quick, and increasingly affordable. With sophisticated equipment, it is possible to determine a location to within less than 1 cm. Even small, hand-held units such as the Trimble Scout can achieve accuracies of about 100 m, quite enough for most amphibian surveys.

The global positioning system consists of a constellation of satellites (launched by the U.S. military) and a mobile GPS unit, which receives radio signals transmitted by the satellites. There are 24 GPS satellites in orbit around the earth. They are not geostationary, so their positions constantly change.

The GPS units function by locking on to the signals from several satellites. The more satellites the unit can lock on to, the more accurate the resulting location will be. It helps to have a clear view of the sky. Mountains, dense forests, canyons, buildings, and human bodies interfere with signal reception. Readings are difficult to obtain from inside a car or building. If too few satellites are being received, it is often helpful to move a short distance. In some situations, moving only 5 - 25 m can make a significant difference in the number of satellites the receiver can pick up and hence the accuracy of the positional data.

When working in areas without distinctive landmarks such as the backcountry of the Sierra Nevada, it is strongly recommended that a good quality GPS be utilized to help confirm locations.

Photography

Good photographs of both the habitat and the amphibians found are essential. Photographs can also be useful to document how the field work is conducted. This section provides basic information on what to photograph and some basic techniques useful for photographing amphibians.

What to Photograph

Habitat photos are intended to provide not only a visual corroboration of the written field notes, but also to document the status of the habitat at the time of the survey. Habitat photographs should be archived along with photographs of specimens and field work.

Photographs of amphibians can be used 1) as vouchers to confirm the identity of animals and 2) to augment a traditional museum voucher specimen. The photograph must be of good quality and the critical identification traits must be clearly shown.

Photographs of the first three adults/subadults should be taken for all but the most common species (e.g., Pacific treefrogs) at any site where a voucher specimen would otherwise be collected. Photographs should be taken of the dorsal view, and, with some ranid frogs (e.g., yellow-legged, red-legged), a ventral view of the extended legs.

Tadpoles can be photographed as well, but it is much more difficult to confirm the identification of tadpoles based on photographs.

Labeling Photographs

Photographs are only of use if they are adequately labeled. The following information must be written on the slide or on the back of a print: date taken, locality description, site number, county, state, species photographed, and the name of the photographer. Use a pen with indelible ink. Note that it is increasingly common for slides to be mounted in plastic mounts that are very difficult to write on without a special pen (sold in some photo stores). Try to find a company that still uses the traditional cardboard mounts.

The locality must include a clear description of the site so that another person could return to the same place. For example, "Desolation Wilderness, El Dorado National Forest" is much too vague. It is also necessary to avoid unclear or confusing place names. There are numerous "Frog Lakes" and "Mill Creeks" so these names will not be sufficiently specific. Including the county on the label will help, but even that may not be enough. In general, use place names on 7.5' or 15' topographic maps as reference points. Some places will be unnamed, in which case it will be necessary to describe the site either with township, range, section, quarter section; with UTM coordinates; or with a detailed description that references a mapped site (e.g., small pond, 1.2 mi. WSW of Tioga Lake, Yosemite National Park).

While it is possible to label photographs with only the date and site number, it is not always convenient or possible to look up these numbers in the appropriate database.

See the section on Locality under Aquatic Survey Data Sheet for additional discussion of recording localities.

Basic Camera Equipment

A high quality, point-and-shoot camera is fine for habitat photos, but is of little value for photographing amphibian specimens. A good single-lens reflex camera with a macro lens and flash is necessary to take adequate photographs of amphibians. The camera must allow the aperture (f-stop) to be set. Sometimes these cameras are referred to as aperture priority. Shutter priority cameras allow the user to select the shutter speed, but the camera sets the f-stop. Since depth of field is directly related to the f-stop, the user must be able to be able to control this variable when photographing amphibians.

Though there are many combinations that work well, we have obtained high quality photographs with an Olympus OM-4T camera, a 50 mm f-3.5 Olympus macro lens, and an Olympus T32 flash. As described below, the flash is a necessary accessory.

Appendices D and E provide a list of equipment and suppliers for photography.

Basics of Photography

A fundamental understanding of photography is needed to take adequate photographs of amphibians and their habitats. While a number of books are available on field photography (e.g., Blaker, 1976), the most important points will be discussed below. The key point to understand is the relationship between depth of field, film speed, and light.

Film speed, lighting, and depth of field

Depth of field refers to the depth of the photograph that is in focus. If you take a photograph while sighting down a picket fence, a photograph with a shallow depth of field might have only a few pickets in focus while a photograph with a large depth of field might have 30-40. When photographing small animals with a macro lens, the depth of field can range from less than a centimeter to several centimeters. Clearly, a large depth of field is

desirable to ensure that the entire animal is in focus. Make sure that the selected film speed is appropriate for synchronization with the flash. This is typically 1/60 sec.

The depth of field is determined by the f-stop, with f-16 having a good depth and f-2 having very little. Unfortunately, f-16 lets in much less light than f-2 and hence a picture taken with natural light would require a much longer exposure at f-16 than it would at f-2. Without a flash, there may simply not be enough light to use a higher f-stop and to obtain adequate depth of field. A small flash can provide the necessary light as well as add light to areas that might otherwise be in a shadow. Thus the flash can provide more even lighting and also allow more of an animal to be in focus.

Film speed also plays a role in the interaction between light and depth of field. Some films require less light and are referred to as being faster. Films with a higher ISO/ASA number react more quickly to light, facilitate the use of higher f-stops, and thereby allow greater depth of field. The disadvantage of faster films is that they tend to have less detail in the final photograph. In general, films with an ISO/ASA rating of 64 - 100 work well in most situations, but it may be necessary to use 200 - 400 films in dark areas such as deep forests.

We generally use Kodachrome 64 slide film with a flash with a guide number of 32 (ISO/ASA 100, meters).

Film handling and storage

Undeveloped film is sensitive to light, chemicals, and heat. When changing film, keep the camera in the shade. Shading the camera from the sun with your body is usually sufficient to prevent problems.

Both exposed and unexposed film is highly sensitive to formalin, both the liquid and the gas fumes. Keep both the camera and any film well away from formalin and make sure to wash your hands if they have been exposed. Heat can cause film to fog, especially once the film has been exposed. Do not store film in a glove compartment or the outside pocket of a pack.

Store film in the plastic containers in which it is normally sold. This container provides some temperature protection as well as good protection from light and chemicals.

Photo Board

A standardized photo board is quite helpful when documenting amphibians because it 1) provides a grid (usually 1 cm squares) that can be used to estimate body size, 2) allows data to be written directly on the board (with a dry erase pen) so all the critical data become part of the photograph, and 3) provides a consistent, neutral background that contrasts with the animal.

The sample photo board shown in Appendix H was adapted from one designed by Martin (1994). The actual board can be made by photocopying the sample on dark gray or light charcoal paper that is then cut to size and laminated with heavy plastic. Heavy lamination works best since it provides both the stiffness and waterproofing that allow the board to be placed behind egg masses, even underwater.

The color of the paper used in making a photo board is important since automatic cameras adjust the exposure to average the dark and light features in a photograph. Pictures taken on a white background will result in specimens that are so dark as to be unidentifiable in many cases.

Specimens should be placed at the top of the gridded area so the photograph includes only the essential parts of the board, not the whole thing.

When using a flash, do not take photographs looking straight down at the board or through the water since the light will reflect and make it difficult or impossible to see what is in the picture. Instead, shoot the picture from a slight angle to alleviate reflection problems.

Tadpoles are often photographed best when placed in a small, shallow container, such as a jar lid, which is then set on the board.

Though it is important not to let amphibians dry out, an individual that is slightly dry on the underside will tend to stick to the board and allow the photographer to take the picture before the animal moves off (David Martin, pers. comm). A second person can also be used to corral the animal and keep it suitably positioned for a photograph.

Photographic Esthetics

Amphibian photographs taken in a natural setting are less important than those on boards, but are nice to have. When taken with a good macro lens and flash, they can even be more diagnostic than the photographs on a photo board because there are fewer

problems with light and reflection. To improve natural pictures, use the following suggestions.

Make sure the animal's eye is in focus. A photograph with the eye or significant part of the head out of focus is not very appealing. Eyes are very important in how people perceive animals as well as other people.

Get down at the animal's level, e.g., lie on the ground. This angle gives the photograph a more intimate feeling.

Fill the frame with the specimen, or better yet, don't include quite the whole animal. (This is not the case with pictures taken for documentation purposes.)

Make sure that no distracting pieces of debris are stuck to the animal. This is best accomplished by having a bottle of water that can be frequently poured over the animal. The water has the added benefit of keeping the animal moist and in good condition.

It sometimes helps to pour water on the spot where the animal is to be placed. This practice provides some moisture for the specimen, and also makes a more even background for the photo, especially when the background is a rock or dry log.

Pathology Specimen Collection

Little information exists on the diseases and parasites of wild amphibians. Significant contributions to our understanding of amphibian population dynamics can be made by collecting samples from healthy, as well as sick, individuals. While the benefit of determining the cause of illness for sick or dead individuals is obvious, it is important to emphasize that baseline information on healthy amphibians is largely lacking for most species.

The collection of specimens for pathological evaluation is greatly facilitated if field biologists anticipate the opportunity to collect specimens by making arrangements for their evaluation and by carrying some basic sampling supplies when in the field. This section outlines the appropriate procedures.

Appendices D and E provide a list of equipment and suppliers for the collection of pathology/disease samples.

Selecting a Pathology/Disease Laboratory

If you expect to gather pathology specimens, it would be appropriate to contact someone at a qualified pathology/disease laboratory at the beginning of the field season. This contact will allow you to confirm their interest in the program and to determine the best procedure for shipping samples. While there are a number of laboratories that might be capable of analyzing samples, it is best to deal with a laboratory that has had extensive experience with amphibians.

Dr. D. Earl Green has developed considerable expertise in analyzing pathological samples from amphibians. He can be contacted at:

Dr. D. Earl Green
Animal Health/Diagnostic Laboratory
8077 Greenmead Drive
College Park, Maryland 20740

(301) 935-6074 - voice
(301) 935-6072 - fax

ab15@umail.umd.edu - e-mail

Selecting Amphibians to Sample

Sick, living amphibians

The most valuable specimen is a sick, but still living amphibian. Sick or diseased amphibians do not survive long in the wild and may provide valuable data on potential causes of population declines. If an animal obviously is not healthy, it is best to collect it for analysis. This is also true for egg masses or tadpoles that do not appear healthy.

Send sick, living amphibians (adults, tadpoles, or eggs) directly to a pathology laboratory. It is important that sick amphibians be sent as soon as possible. See the Packing, Storage, and Shipping of Animals and Samples section for specific instructions.

Make sure to wash your hands after handling any sick amphibians so as to reduce the likelihood of spreading any disease or infection to other individuals.

Healthy, living amphibians

There are three increasingly invasive ways to sample healthy amphibians. Each of these techniques is described in more detail below.

1. Capture, sampling, and release of animal unharmed.

   Swabs and body excrements can be taken from skin surface, cloaca, and feces or urine. These swabs are cultured to provide information on the presence of bacteria and fungi. Swabs from egg masses may be collected without harming them.

2. Collection of blood from live animals that are then released.

   Collection of blood samples from live amphibians is a relatively simple process, yet it is only rarely done. Blood samples typically consist of one or two drops of blood. Blood smear slides can be examined for the number and types of leucocytes (white blood cells), bacteria, blood parasites, and at least one type of virus. Somewhat larger quantities of blood (a few milliliters) can be used for a wide variety of analytical tests. In most situations, amphibians may be released alive after obtaining a blood sample.

3. Collection of entire animal.

   Whole, live eggs, larvae, or adults can be sent to a pathology laboratory for a complete diagnostic analysis.

Collecting samples from fresh, dead amphibians

Fresh, dead amphibians are nearly as useful as sick individuals if they are processed promptly. A specimen is considered fresh if it appears to have died within minutes (warm conditions) or hours (e.g., cold mountain lakes or streams). In general, if you can obtain an unclotted blood sample and the carcass is free of blowfly eggs around the mouth and eyes, the animal is probably sufficiently fresh.

Collecting samples from long dead amphibians

Amphibians that are not recently dead are usually not pathologically interesting. Amphibians tend to decay quite rapidly, and it is rarely possible to distinguish between microorganisms that contributed to the death versus those that were present in the environment and flourished after the animal died. It is not recommended that samples be

collected from anything but live or freshly dead animals. Long dead amphibians may be valuable for some poison tests or for DNA analysis, in which case carcasses should be placed in a plastic bag or vial and then frozen.

Basic Collection Procedures

Process each animal in the priority order listed in the section on Collection of External Samples and Collection of Internal Samples. The order is important since it places the most important samples first and also reduces the likelihood of contaminating subsequent samples.

Time is of the essence, at least until the blood sample has been collected.

Contamination of samples

Care must be taken to avoid contamination of pathology samples. Bacteria and other microorganism on the human skin can easily contaminate samples and either confound or completely invalidate the results.

At a minimum, field workers must carefully wash their hands both before and after handling amphibians for pathological analysis. This not only reduces human contamination of the sample, but also adds a level of protection from those few amphibian diseases that are transmittable to humans (e.g., various species of *Leptospira*, *Salmonella*, and *Yersinia*). Though it is probably not really necessary, some field workers have opted to wear surgical gloves when collecting samples.

Labeling containers

Label all specimens, vials, microscope slides, culturettes, etc. with the date, site number, and four-letter code for species (first two letters of the genus and first two letters of the species name). When collecting samples from more than one individual, give each individual a unique number that is then recorded on each sample. This will allow the lab to determine which samples came from each individual. For example, if both blood smears and swab samples are collected from each of four frogs, a unique number will allow the lab to determine which swab and blood samples came from the same frog.

For samples from specific organs, indicate the organ type on the label. Use a fine point, waterproof pen such as a Sharpie, but note that Sharpie ink dissolves in ethanol.

Photographs

If the cause of death or disease is conspicuous, take documentary photographs (see the Photography section).

Collection of External Samples

External samples are useful for detecting the presence of bacterial and fungal diseases.

Swab samples

Swab eggs, larvae, or adults with a Mini-tip Culturette. Pull the cotton swab out of the protective container, take the samples as described below, replace the end over the cotton swab, and crush the end of the cap to activate the preservative. Do not touch the swab as this could contaminate the sample with human bacteria. Take 2-3 swab samples.

If the sample is a cluster of eggs, gently push the swab into the egg mass. If eggs are in a linear strand (e.g., toad eggs), swab at least the outside of the strand of eggs.

For adults and larvae, swab the ventral surface and thighs (if present). If there is evidence of disease or a lesion, collect two swabs of the lesion and two swabs from normal-appearing skin.

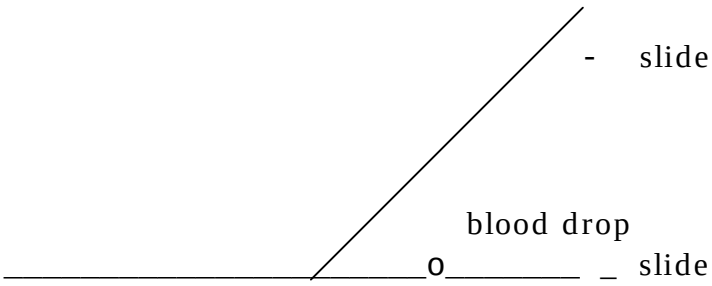
Making a blood smear

Obtain a drop of blood by clipping a toe, a tail tip, or (for larval salamanders) the tip of a gill. Blood can also be obtained by drawing a sample directly from the heart using a syringe with a small gauge needle (e.g., 28 g insulin syringe). The heart lies directly under the sternum and can usually be pierced without dissection. If this is not successful, complete the other external samples and then promptly collect a blood sample from the dissected heart.

If the frog is recently dead and the blood does not flow freely, it is sometimes helpful to use a capillary tube to collect a sample from one or several toes. Clip the end of a toe and place the tube against the exposed tissue. If necessary, massage the foot from the wrist outward to force the blood out. Small samples from several toes can be combined in the capillary tube prior to making the blood smear.

Place a drop of blood near the frosted end of a microscope slide. If the sample was collected in a capillary tube, gently blow on the other end of the capillary tube to get the blood out. You can also remove the blood by aspirating it out with a new (non-heparinized) syringe.

Take a second slide, rest it near the middle of the first slide, and draw the second slide back until it contacts the blood drop. Allow the blood to spread along the base of the second slide, and then quickly slide it away from blood drop to produce a thin smear of blood. The orientation of the two glass slides is illustrated below.



-> draw slide back to blood drop, then

<- rapidly push toward other end

Regardless of how well this works, you only get one chance since the blood dries fast. Let the sample air dry 2-3 min while you label it. If possible, collect 2-3 blood samples, each on a separate slide. As soon as the blood dries, the slides must be protected from ants and flies that will eat the blood. Slides may be stacked directly on top of each other if necessary, but it is better to place slides in a slide box.

Collection of blood serum

If serum separator tubes and a centrifuge are available, collect whole blood using a syringe with a small gauge needle (e.g., 24 - 28 g). Empty the blood into a serum separator blood collection tube. Allow the blood to clot in a cool area for 2 - 12 hours and then spin for 2 min in a centrifuge. Do not centrifuge the blood sooner than 2 hours after the sample was collected.

If a centrifuge is not immediately available, the blood may be left in the serum separators in a cool area for 2 - 4 hours and then refrigerated for up to three days before being centrifuged. The tubes of blood should not be shaken during this holding time, however.

Ship the serum separator in an ice chest (with plenty of ice) to the pathology lab.

Collection of Internal Samples

It is assumed that living, sick or diseased amphibians will be shipped directly to a pathology laboratory as described above. Hence this procedure is used only on animals that are already dead. If the animal is still alive, it will be necessary to euthanize it prior to collection of internal samples. See the Anesthetizing and Euthanizing Amphibians section for procedures.

After collecting external samples as described above and prior to dissecting a specimen, it is necessary to prevent contamination between samples by sterilizing all dissecting instruments in 70% ethanol and briefly immersing the frog, salamander, or larva in 70% ethanol to sterilize the skin.

Collecting blood samples

Place the amphibian on its back and make one long incision on the ventral midline from the throat to almost the vent (anus). This is easiest to perform if the initial incision cuts only the skin and a second incision cuts through the muscles of the abdominal wall. If you have not obtained a blood sample, proceed immediately to the heart. Use a syringe with a small gauge needle (e.g., 28 g insulin syringe) to draw blood directly from the heart. If this is not successful, make an incision in the heart and use a capillary tube to collect any pooled blood. Make a blood smear as described above.

Even if you cannot collect blood with a syringe or capillary tube, try to make a smear with a clot or a section of the heart by smearing it across a slide.

Label the slide with waterproof ink and allow the blood smear to dry 2-3 min.

Swab and frozen samples

Remember to rinse all dissecting instruments in 70% ethanol after the initial incision and prior to dissecting **each** organ. This is really important.

At a minimum, take a swab sample from the heart, liver, and intestine. If possible, also collect samples from the body cavity, gonads, kidney, lungs, and any lesions, lumps, abscesses, discolored organs, or other abnormalities. The intestines must be sampled last

since there is a significant chance of bacteria spilling from the intestine and contaminating other organs that do not normally have bacteria.

Swabs are collected by gently cutting into the surface of the organ and then swabbing the interior of the organ with a Mini-tip Culturette. Clean the scalpel blade or scissors with 70% ethanol between incisions of each organ and use a different swab for each organ.

When labeling the culturettes, make sure to note the organ that was sampled. Crush the end of the swab to activate the preservative. Culturettes should be kept cool and mailed so that they reach the pathology/disease laboratory within three days. Culturettes may be less useful after three days, but they should still be sent to a lab even if shipping is delayed.

If you have access to liquid nitrogen, place samples of each organ in individual cryovials, label with the name of the organ, locality, and species, and then put the cryovial directly into nitrogen.

Preserve the remaining portion of the amphibian in 10% formalin. Formalin preserved specimens can be transferred to 70% ethanol after 7 - 10 days. If formalin is not available, preserve the animal in 70% ethanol.

Make sure to wash your hands after handling any sick amphibians so as to reduce the likelihood of spreading any disease or infection to other individuals.

Packing, Storage, and Shipping of Animals and Samples

Packing amphibians for shipping

If a live adult frog is to be sent to a laboratory, place it in a plastic Ziploc bag with a damp (but not soaking wet) paper towel. The bag should be reasonably full of air, both to provide the limited oxygen needed by the animal, and also to offer some protection from being crushed.

Eggs and larvae can be put in Nalgene bottles or Ziploc bags for shipping. If Ziploc bags are used, the heavier freezer quality bags are preferred for eggs and larvae since a bag of water is fairly heavy. Make sure air comprises half the volume of the shipping container.

Storage of amphibians

If either sick or healthy amphibians need to be kept overnight or over a weekend, pack them as described above, and then place them in a refrigerator. The lower temperatures will help slow the development of some diseases and keeps the metabolic rate of the amphibians low.

Though amphibians would normally be kept only for short periods of time, they can be maintained (without feeding) for several weeks or more in a refrigerator. If it becomes necessary to hold animals for more than a day or two, make sure to change the paper towels or water every few days, especially during the first few days after capture. Food that was eaten just prior to capture will pass through the digestive system, even at lower temperatures. If the container is cleaned every day or two, this waste material is removed and the opportunity for bacteria or fungus to grow (in a nutrient-rich environment) is greatly diminished and hence the survival of the amphibian increased.

Shipping containers

Place the Ziploc bag in an ice chest (the small, plastic, one quart size is best). During most of the year, it is important to add a couple of packs of "blue ice" to keep the amphibian sufficiently cool. Make sure that the ice will not shift and crush the specimens.

Arrangements can generally be made with the pathology laboratory to return empty ice chests.

Shipping

All refrigerated and frozen samples should be mailed to the pathology laboratory via overnight, express mail. Some carriers prefer not to handle live animals. Labeling the package as "Amphibian Pathology Specimens" is usually sufficient. Check with individual carriers regarding their regulations for shipping live animals and/or specimens.

Temporary storage

If the swab samples or a live amphibian cannot be mailed immediately, keep them cool, preferably in a refrigerator. In the backcountry, it might be possible to use snow or cool stream water to refrigerate specimens.

Anesthetizing and Euthanizing Amphibians

Some procedures, such as the collection of blood samples, are easiest to conduct if an animal is anesthetized. We have also anesthetized mountain yellow-legged frogs during implantation of PIT tags, used for the recognition of individual frogs. Less frequently, it is necessary to euthanize amphibians. This might be the case if tadpoles are being collected for tissue samples and they are too large to fit into the collection vials.

We describe here the techniques that we have found to be the most efficient and humane. These procedures have been used on several species of frogs (*Hyla regilla*, *Rana muscosa*, *R. aurora*, *R. catesbeiana*) and may well work well with salamanders. We have noted, however, that there are some species-specific differences in reactions to the anesthesia. Hence it would be appropriate to closely monitor the anesthetic procedure for each new species that you work with. It may well turn out that the solution needs to be a bit weaker or stronger to work suitably.

Anesthetizing Amphibians

Prepare a saturated solution of benzocaine by dissolving the benzocaine crystals in a small container (e.g., 4 oz bottle) of 70% ethanol until the solution becomes saturated. This is indicated by the presence of undissolved solid (crystals). Shake the container periodically to assure that the solution is well mixed.

Make a 0.02% solution of benzocaine anesthetic solution by adding 0.2 cc of saturated benzocaine to 1 liter of fresh water. Mix the solution by shaking it gently.

Put each frog in the anesthetic (0.02% benzocaine). Make sure to place a lid over the bottle so the frog does not jump out before it is anesthetized. Allow the frog to swim in the solution for 1 - 2 minutes, or until its movement slows down significantly (Tyrone Hayes, pers. comm.).

The time it takes to anesthetize a frog varies, depending on the individual frog as well as the age and strength of the anesthetic solution. Monitor each frog closely when it is in the anesthetic solution. The transition from a very active, swimming frog to a still, sinking, anesthetized frog is often quite rapid. It is better to remove the frog from the anesthetic a little early, than to wait too long have an over-anesthetized frog that takes several hours to recover or perhaps even dies.

When the frog appears to be suitably anesthetized, remove it from the anesthetic and evaluate how well anesthetized it is. The frog is ready for blood collection when you pinch a hind toe and the frog does not retract its foot. If the frog is still responsive, return it to the anesthetic solution until the appropriate level of anesthesia is reached.

Recovery from Anesthesia

Rinse the frog in fresh water to remove any residual anesthetic. Then place the frog in a cool, moist environment for recovery. We generally use an ice chest lined with damp paper towels.

Most frogs will become somewhat alert within 1/2 hour, and fully alert within an hour after removal from the anesthesia. If a frog is not obviously recovering within 1/2 hour, check for a heartbeat and rinse again in fresh water.

Even though survival rates for anesthetized amphibians approach 100%, individuals occasionally die. Any animal which dies should be used for a thorough pathology evaluation (see Pathology Specimen Collection), have tissue collected for DNA analysis, and then be preserved as a museum voucher specimen. The pathology evaluation is of particular value since the frog was presumably healthy at the start of the procedure and can thus provide useful baseline information on parasite loads and diseases for apparently healthy frogs.

Euthanizing Amphibians

Amphibians can be euthanized with a solution of chloretone. Add chloretone crystals to a small container (e.g., 4 oz bottle) of water until the solution becomes saturated. This is indicated by the presence of undissolved solid (crystals) after a few hours or overnight. Shake the container periodically to assure that the solution is well mixed. Since the chloretone dissolves rather slowly, it is a good idea to begin preparation of the concentrated solution at least the day before it is needed.

Add 4 cc of the saturated chloretone to a container with one liter of water. Use the dilute chloretone solution for euthanizing amphibians. Place the live amphibian in the solution and wait until all movement has stopped for at least 5 min. The dilute chloretone can be used repeatedly, but it gradually becomes less effective and will take longer for animals to die. When this happens, add an additional 1 - 2 cc of the concentrated (saturated) chloretone solution. (Heyer et al., 1993)

Blood Collection via Heart Puncture

Occasionally it is necessary to obtain a blood sample from a live frog. Blood samples can be examined for protozoan parasites, viruses, hormone levels, toxic chemicals (e.g., lead, mercury, pesticides, industrial pollutants), antibodies (titers) to infectious diseases, serum chemicals (e.g., glucose, sodium, enzymes), and unusual levels of proteins that might reflect exposure to herbicides and pesticides. Blood can also be used to analyze DNA or RNA.

The method described here has worked well for frogs as small as Pacific treefrogs and would be fairly easy to implement with larger species. Survival rates for Pacific treefrogs (from which blood has been drawn via a heart puncture) are generally about 95%.

Note that time is of the essence for several aspects of this work. It is necessary to have all supplies readily available and be prepared to move directly from one step of the procedure to the next.

Appendices D and E provide a list of equipment and suppliers for the collection of blood samples.

Note that techniques for collecting blood for blood smears or as part of a necropsy are discussed in the Pathology Specimen Collections section.

Anesthetizing frogs

Though anesthetizing amphibians requires additional time and preparation, it is an appropriate step because it makes blood collection easier and it is more humane for the frogs. The appropriate procedure is described in Anesthetizing and Euthanizing Amphibians section.

Drawing Blood

Syringe preparation

Draw 0.1 cc of heparinized saline into a 28 g insulin syringe. After obtaining the heparinized saline, continue pulling the plunger back a few centimeters to coat the inside of the syringe with the heparinized saline. Once this is completed, expel saline from syringe by firmly pressing the plunger all the way in. It is important to make sure that there is not

an excess of heparinized saline remaining in the syringe or it will dilute the blood. While this is not a serious problem, it should be avoided.

Blood collection

Use the heparinized syringe to collect blood directly from the heart. Place the frog on its back and visually locate its heart by observing the heartbeat. Aiming for the heart, insert the needle into the chest of the frog. This procedure generally works best if the insertion point is about 5 - 10 mm posterior to the heart and the bevel edge of the needle is facing upwards.

Once the needle has entered the chest and reached the vicinity of the heart, draw the plunger of the syringe back slightly, up to 5 mm. If you see blood in the base of the syringe, your needle should be within the heart and the syringe will gradually fill with blood. You may need to pull the plunger back further either to maintain the negative pressure or to obtain sufficient space for the blood. For estrogenic studies, collect 0.02 cc (20 microliters) of blood for species the size of Pacific treefrogs (2 - 4 g). Larger amphibians can supply proportionately larger amounts.

If you draw back on the plunger and see air or a clear, serous fluid in the base of the syringe, you have probably not entered the heart. You should rotate the syringe slightly since the needle bevel may simply be up against the wall of heart. If this is not successful, either insert the needle further (since it may not have reached the heart) or withdraw the needle slightly and approach the heart at a slightly different angle. You usually do not need to withdraw the needle from chest, merely pull it back and redirect the angle. Placing a thumb and forefinger on each side of the heart sometimes helps to keep it from sliding around as you work.

Try not to get discouraged if you don't hit the heart immediately- it takes some practice to draw blood, especially with small frogs.

For larval salamanders, you can clip the tip of a gill and readily obtain an adequate blood sample (Brad Shaffer, pers. comm.)

Transfer of blood

If blood is being drawn as part of a pathological analysis, use the blood to make a blood smear on a glass microscope slide. This procedure is described in detail in the section on Pathology Specimen Collection. If blood was collected as part of an estrogenic study, proceed with the procedure described below.

Immediately transfer the blood from the syringe to one or two hematocrit capillary tubes. This can be done with one of two methods.

1. Gently press in on the syringe plunger until a small drop of blood forms at tip of the needle. Touch the drop with a capillary tube and the tube will fill via capillary action. Repeat this process until all of the blood has been removed from the syringe. This technique requires very steady hands and may not work well for everyone. It has the advantage of accumulating the blood in one continuous portion of the capillary tube without intervening air pockets.

2. Place the tip of needle inside a capillary tube and gently push the plunger in to fill capillary tube. Try not to get air bubbles in the tube. If possible, use a single capillary tube for the blood sample.

You might be able to remove some of the air in the syringe by pointing the needle up and slowly pressing the plunger in until blood appears at the tip. You might also lose some blood doing this, so be ready with a capillary tube.

Centrifuging Blood and Freezing Plasma

Centrifuge the capillary tubes **immediately** after filling them with blood. If the blood sits longer than two minutes before it is centrifuged, it will no longer be useful.

Prepare the capillary tube for centrifuging by covering one end of the tube with your index finger pressing the other end into Critoseal sealant. This should fill 1 - 2 mm of the tube with sealant and keep the blood in the tube while centrifuging. Place the capillary tube in the centrifuge with the sealed end toward the outside. The blood sample should be centrifuged for 60 seconds at 6,000 RPMs.

Immediately after the sample is centrifuged, separate the clear or light red plasma from the red blood cells (which are clumped in the lower 1/2 of the tube next to the sealant). Do this by breaking the capillary tube just above the level of the red blood cells. The capillary tube can be scored with a file or carefully broken with your fingernails. Expel the plasma into a labeled cryovial by gently blowing on the non-broken edge of the capillary tube to force the plasma into the cryovial. You can also remove the plasma by aspirating it out with a new (non-heparinized) syringe.

Freeze the plasma sample in liquid nitrogen **immediately** after it is placed in a cryovial.

The red blood cells can be removed from the capillary tube and saved for mitochondrial DNA analysis, but only if the sample was collected without the use of heparin. See Tissue Collection for a discussion on other collection procedures for DNA samples.

Amphibian Measurements

Though it may not be critical for some studies, we recommend that you collect a snout-vent length and weight for each frog. Weights and measures of anesthetized animals are generally more accurate than those obtained from live animals.

Tissue Collection for DNA Analysis

Increasingly sophisticated techniques are available for the analysis of genetic variability within and between populations of amphibians. This type of information is valuable not only for our basic understanding of amphibian biology, but also for very practical management decisions affecting the future of amphibian populations. For example, information on genetic diversity is being used by managers to determine which amphibian populations are most important to protect and which frog populations should be used as donors for reestablishing frogs in regions where they have been lost.

The collection of tissue samples for DNA analysis can be quite simple, especially if tissue is stored in vials with 95% ethanol. If funding is not available for immediate analysis of tissue samples, they can easily be archived for later use. Since many amphibian populations are declining at a rate that may lead to local extinctions in the foreseeable future, the collection of tissue for DNA analysis is of significant value considering the minor impact of removing a relatively small number of tadpoles from the population.

Appendices D and E provide a list of equipment and suppliers for the collection of tissue samples.

Geographic Distribution of Tissue Samples

Tissue samples generally do not need to be taken from populations separated by less than two kilometers (approximately one mile) unless one is specifically trying to evaluate genetic variation on a very local level. Exceptions to this recommendation would be populations separated by a significant barrier to amphibian dispersal which may have been in place long enough to lead to genetic differentiation.

Populations from different drainages should be sampled whenever possible if there is at least a modest barrier to dispersal and the distance between populations along connecting streams and rivers is more than two kilometers (approximately one mile).

Number of Tissue Samples

Collect up to 25 tissue samples, depending on the number of individuals present in each local population. Individuals refers to the total number of tadpoles, transformlings, subadults, and adults of a given species at a single site. Apply these guidelines independently to each species at each site.

Number of Individuals in the Population	Number of Tissue Samples
< 10	0% of population
10 - 15	10%
15 - 25	20%
$\geq$ 25	10%, up to a maximum of 25

Note that if there are 10 or fewer individuals, no tissue samples should be collected. Take photographs, but release all animals at the point of capture. For a small population, it may be possible to return after eggs have hatched in order to collect tadpoles.

In priority order, collect entire tadpoles, subadult toes, and adult toes. Do not collect portions of tadpoles (e.g., tail tips). Since tadpoles can be difficult to identify, it is important to have the tadpole as a voucher specimen. Also, a tadpole with a missing tail tip might be more vulnerable to a disease or parasite that could become epidemic in the population. While the same might be true for adults, they may be better able to cope with disease. Also, the potential contribution to the local population is far greater for adults than tadpoles.

Collection Procedures

Labeling vials

Vials are easiest to label and fill if using a vial rack. Label each vial both inside and out. The outside label is the more important since it can be read more easily. The inside serves as a backup in case the other label becomes unreadable. Use small waterproof tags for inside labels. Prepare both labels with permanent ink (e.g., fine point Sharpie, but note that Sharpie ink dissolves in ethanol), though pencil can be substituted if needed. Do not use regular ballpoint or standard felt tip pens since these are not waterproof and the ink will simply wash off. Inside labels should be made from a sturdy paper, preferably 100% rag, 28 lb. (see Appendices D and E).

Labels need to include both a number and species code. Typical labels would read:

Y-22-5	S-325-4
BUBO	RABO

The first letter is the code for the area where you are working. The first number is the site number from the upper right hand corner of the data sheet. These are consecutive numbers for all sites surveyed during the year. The third number is the tissue sample number. These numbers range from 1-25 for each species at each site.

The first example above indicates that the tissue was collected by the Yosemite crew at the 22nd site they surveyed. The vial represents the 5th sample of tissue from a *Bufo boreas* at that site.

The second example indicates that tissue from a *Rana boylii* was also collected by the Sequoia crew at site 325. The vial represents the 4th sample of tissue from *Rana boylii* at that site.

Preservation of tissue samples

Put tissue samples from each individual in separate vials. Do not put tissue from two individuals in one vial. It is best to collect the first five samples in liquid nitrogen, if available. Otherwise put tissue in 95% ethanol (not 70%). While it is not necessary to preserve samples in liquid nitrogen for DNA analysis, liquid nitrogen preserves proteins and allows for additional analysis with electrophoretic techniques.

Use cryovials with screw caps for nitrogen samples and microcentrifuge (=Eppendorf) tubes with flip-tops for ethanol. If you run short of the microcentrifuge tubes, you may use cryovials for both nitrogen and ethanol, but they are more expensive. **Do not** put microcentrifuge tubes in nitrogen since they can pop open.

The ideal sample is a tadpole that just fits in the vial. Larger tadpoles will sometimes fit with only a little effort. Smaller tadpoles are also acceptable, but are a little harder to work with in the lab. Toes fit with ease, but collect toes only if tadpoles are not available and if the population is large enough. Clip the tip of the outer toe on the front foot using fingernail clippers. Small scissors can be used, but the use of scissors tends to result in more bleeding that fingernail clippers. Clippers should be cleaned with 70% ethanol between use on animals to prevent cross-contamination of samples and to prevent spread of disease between individuals.

Tadpoles that are too large to fit in the container will need to be euthanized (see Anesthetizing and Euthanizing Amphibians). Once dead, place all or part of the liver and tail in a cryovial and freeze in liquid nitrogen. Preserve the remainder of the animal in 10% formalin as a voucher specimen. Make sure to label the voucher specimen with the same number that was used for the frozen tissue sample.

Preserving tissue in ethanol

Make sure to use 95% ethanol, not the 70% used for preservation of museum specimens. Also, note that ethanol used for preservation of specimens is denatured and is **not safe** to drink.

Filling vials is easiest if the vials are placed in a rack and then filled using a 10 cc syringe (without a needle). Fill each vial about 3/4 full of ethanol. Filling the vials much more is not necessary since the tissue will displace some liquid anyway. It is convenient to fill 50 - 75 vials at a time and keep them in a Ziploc bag for use in the field.

The tissue to liquid ratio should not exceed 1 part tissue to 2 parts ethanol. A 1:9 ratio of tissue to fluid is ideal. In other words, do not cram a large tadpole in a vial with only a small amount of ethanol because the tissue will not be preserved properly and the sample will be useless.

To make sure the sample is well preserved, open the abdominal wall with a pair of scissors so that the ethanol can penetrate. The tail also can be cut into several pieces.

If the tissue sample is a toe tip, no special preparation is necessary. Toes are small enough that the preservative will easily penetrate and the liquid to tissue ratio is usually much better than 1:2.

Preserving tissue in liquid nitrogen

Use only the cryovials with screw tops when storing samples in liquid nitrogen since other vials may crack or pop open. After labeling, drop the vial directly into the nitrogen tank. **Do not** look into the nitrogen tank as you drop the vial since there might be a splash. Safety glasses are recommended when using liquid nitrogen.

Tadpoles will die very rapidly when frozen and hence there is no need to euthanize them prior to storing them in liquid nitrogen.

If you are not near a source of nitrogen, you can keep live tadpoles and/or frogs in Nalgene bottles or Ziploc bags. If an animal dies in transport, still use it for a tissue sample, but process it as quickly as possible because amphibians deteriorate rapidly, especially in warm weather. Also note the amount of time the animal was dead before being processed.

Voucher Specimens

The collection of voucher specimens is somewhat controversial. Recent surveys for amphibians have benefited from museum collections that provide data on when and where a particular species formerly occurred. Such specimens were, for the most part, collected at a time when amphibian populations were believed to be doing well and the loss of even large numbers of animals was not likely to cause a long-term change in local populations. Today, many amphibian populations have declined significantly or have been lost entirely. Collection of only a few individuals (especially adults) may cause serious harm to some smaller populations.

Clearly it is appropriate to consider carefully if and when specimens should be collected and to consider the alternatives since any collection will remove individuals from a population. This loss is most important for reproductive adults, and less so for amphibian larvae, especially for species which produce large numbers of eggs (e.g., some toads).

Appendices D and E provide a list of equipment and suppliers for the collection of vouchers.

Traditional Voucher Specimens

Most specimens in major museums have been preserved initially in formalin and later transferred to ethanol. Formalin is a good preservative that both fixes the specimen in a rigid position and also penetrates the tissue well. Amphibians preserved in formalin can typically be used in taxonomic studies based on morphometrics (measurement of body parts) as well as studies of food habits, parasite loads, reproductive condition, etc. Unfortunately, formalin destroys most or all of the DNA that is increasingly being used for analysis of genetic variation in amphibian populations.

Voucher specimens are particularly useful for species groups that are not taxonomically well understood (e.g., *Batrachoseps*). In some amphibians, genetic differentiation is not always reflected phenotypically and it is not possible to recognize different species visually. In such cases, it would be appropriate to collect at least a few representative individuals.

Some professional publications require a museum specimen to document the distribution or occurrence of a species. This specimen does not necessarily need to be a mature adult, but photographs or written reports of animals are not always acceptable.

The impact of collecting can be reduced by collecting early life stages, notably medium-sized tadpoles or salamander larvae. For many amphibians, these can be recognized to species and will function to document the occurrence of a species at a site. Larval amphibians will not, however, be useful in many morphometric taxonomic studies since this type of work typically focuses on adults, which constitute most of the specimens in museum collections today.

If voucher specimens are collected, some tissue should be preserved separately as described in the next section. Voucher specimens should be stored in a major museum collection. Privately held specimens are not generally available to other researchers and hence cannot be used in most research studies. This lack of availability may lead to the unnecessary collection of additional specimens. In general, the larger universities and research institutions welcome additions to their collections if the animals are properly preserved and labeled.

Tissue Samples

Only small amounts of tissue are needed for mitochondrial DNA analysis. For example, one or two toe tips from an adult amphibian are sufficient for evaluating mitochondrial DNA for that individual. For small tadpoles, the entire tadpole is usually collected, but it would be possible to remove only the tip of the tail and release the tadpole.

The rationale for not removing the tail tip is that the resulting injury might provide a point of entry for a disease organism. Since the chance of survival (to reproductive age) for a young tadpole is remarkably small (often one in 100,000), it seems preferable to remove the tadpole from the population. The tadpole is also important as a voucher specimen since tadpoles can be difficult to identify.

For adults that are at or near reproductive age, the loss of an individual is much more biologically significant and hence the balance between taking the entire individual and removing a toe tip favors the latter. While several studies have indicated that removing toes (generally as part of mark and recapture studies, e.g., Clarke, 1972, Daugherty, 1976, Ferner, 1979) can reduce survivorship, collecting toe tissue is still biologically preferable to taking the entire animal (which reduces survivorship to zero). Cleaning clippers or scissors with 70% ethanol between use on each animal may help increase survivorship by eliminating the spread of disease from one animal to the next.

When an entire animal is not collected, it should be photographed so there is at least a photographic voucher available for verification of identification.

Tissue samples should be stored at a major university or museum so that they are available to biologists conducting research on amphibian genetics. Privately held samples are of limited value since their existence will not generally be known and they will not be included in taxonomic studies. It would be a good to check with an appropriate institution at the start of a study to determine where the best repository for tissue samples would be.

Photographic Vouchers

Photographs can be used to document the presence of amphibians at a particular location. These photographs must be of sufficient quality to allow for identification to at least the species level. The primary advantage of photography is that animals can be released unharmed. This consideration is important for amphibian populations that are in serious decline.

There are several disadvantages of photographs. They may not come out and hence the documentation will be lost. If the locality data are not recorded directly on the slide, it is necessary to keep meticulous notes and carefully label photographs once they are developed.

Usually, little information beyond species and locality are retained in a photo. Color and color patterns are retained better than in formalin preserved specimens, but

differences in lighting, color balance of film, and conditions under which an animal was photographed make detailed color analysis problematical at best.

Photos tend to be kept by the photographer or project manager and are frequently not available to other researchers. The existence of documentary photographs is often not known even amongst those actively engaged in amphibian research. These problems might be reduced if museums were to archive amphibian photographs along with their specimen collections.

Recommendations for the Collection of Vouchers

We do not support the routine collection of traditional, preserved voucher specimens. While this procedure may be acceptable for some large amphibian populations, it is no longer appropriate to collect amphibians routinely at each site. In general, we recommend that a combination of photographs and tissue samples be collected, but we also offer guidelines for collecting traditional voucher specimens.

Photographs

Photographs of the first three adults/subadults should be taken following the procedures in the Photography section. Tadpoles should be photographed as well, but it is much more difficult to confirm the identification of tadpoles based on a photograph. A high quality camera with a macro lens and flash is necessary for adequate documentary pictures.

Tissue samples

It is recommended that up to 25 tissue samples be collected from each population, depending on the size of the population. Specific guidelines for sampling populations of different sizes are given in Tissue Collection for DNA Analysis section.

Traditional voucher specimens

If traditional formalin-preserved vouchers are to be collected, we recommend that every effort be made to utilize all parts of the animal to the maximum extent possible. Each animal should be photographed while still alive, tissue should be collected for DNA analysis, and samples taken for disease and parasite analysis.

Tissue for DNA research should be preserved either in liquid nitrogen or 95% ethanol prior to fixing the specimen in formalin. This procedure will preserve a DNA sample that might provide the only means for identifying the specimen at a later time. At a minimum, take 3 - 4 entire toes and a piece of thigh muscle measuring approximately 5 x 5 x 5 mm.

Follow the procedures for collecting pathology samples from a recently dead specimen. At a minimum, this should include external and internal swab samples, a blood smear, and pieces of ventral thigh skin, heart, liver, and intestine preserved in formalin.

The number of adult frogs that could be taken from a population should follow the following guidelines:

Total Population	Number of Voucher Specimens
Less than 25	0
More than 25	5% of population

Unless a population is in an area that is certain to be flooded by a new dam, razed for shopping center construction, or subjected to a similar level of destruction, no more than 20 specimens should be collected regardless of population size.

Fish Identification and Collection

Any fish found at each locality should be identified, especially since the presence of non-native fish (e.g., trout, sunfish, bass) may be a significant factor affecting the decline of amphibians. Fish can be caught either with dip nets or a seine, then identified. Fish that are not readily recognized can be preserved for later identification. Normally this would involve the preservation of one or two vouchers for each species. These preserved specimens can be identified using one of the standard fish identification keys (e.g., McGinnis, 1984) and then used as comparative specimens during later surveys.

Fish are preserved by putting them directly in a jar with 10% formalin. If the specimen weighs more than a few grams, it is prudent to slit open the body cavity to allow rapid penetration of formalin into the internal organs. After 7 - 10 days, the tissues should be well preserved and specimens may be rinsed with water and transferred to 70% ethanol.

They can also be stored indefinitely in formalin, but ethanol is a more agreeable preservative for most people, and formalin is also a carcinogen. Note that some inks dissolve in ethanol and that film is quite sensitive to formalin.

Identification labels for preserved fish need to include locality, collector, date collected, and identification. If the identification is unknown at the time of collection, a second label can be added to each fish at a later time. Store fish of different species or from different localities in their own containers, so you can place a label in the jar without having to tie it to a specific fish. Labels should be made from a sturdy paper, preferably 100% rag, 28 lb. (see Appendices D and E).

Collecting Permits

Aquatic amphibian surveys involve the capture of amphibians or fish, procedures that generally require collecting permits. Contact the appropriate state fish and game agency to determine the permitting requirements. Depending on where the work is to be performed, a state collecting permit may be all that is required. In some areas, it may also be necessary to have permits from State or National Parks, Bureau of Land Management, a National Forest, or some other land management agency. Surveys on private land require verbal or written permission.

It is also necessary to determine whether any amphibians (or other wildlife) within the survey area are federally listed as threatened or endangered. If so, a permit from the U.S. Fish and Wildlife Service Office of Endangered Species will also be required. While obtaining permits is usually routine for the aquatic surveys described in this protocol, it may take 6 - 8 months to process an application so plan well in advance of each field season. Contact the nearest Fish and Wildlife Service office to determine what permits are needed and how to apply for them.

Data Analysis

Data from aquatic surveys can be analyzed at several levels of detail. The most basic information is presence/absence. This analysis might be adequate for mapping the distribution of a species within a local area, trying to determine whether a particular species is present within a project area, or making comparisons with limited historic information (such as museum records) which do not allow for more detailed comparisons.

On the other hand, a properly implemented program of aquatic surveys provides much additional valuable information. Even if comparative historic data are lacking, current studies will provide the baseline data for surveys in later years. We discuss some of the more basic aspects of data analysis below.

Number of Amphibians Found per Hour Searched

Time is calculated as the total time spent searching, multiplied by the number of fully qualified observers. All data summaries need to be broken down by species and life history stage (e.g., eggs, larvae, subadults, adults). For some species, however, it may not be practical to distinguish between adults and subadults.

Number of Amphibians per Area or Distance Searched

Meadows are generally evaluated on an area basis while streams and rivers are evaluated as linear features. Lakes that are occupied primarily around the edge can be considered linear, though smaller lakes and ponds should be evaluated on an area basis. All data should be analyzed on a species by species basis as well as for each life history stage (e.g., eggs, larvae, subadults, adults).

Number of Eggs, Larvae, Subadults, and Adults Found at Each Site

Though the distribution of these life history stages can vary greatly throughout the season, these data can be valuable for evaluating breeding success and survival either throughout the season or from year to year.

Further Analysis

Additional data on the Amphibian Survey Data Sheet can be used to gain a detailed understanding of the factors affecting the presence and numbers of amphibians. Some of these factors are fairly obvious, such as how amphibians are distributed with respect to habitat, elevation, substrate, associated plants, presence of fish, land use, weather, and water velocity. Any of these factors can be analyzed by comparing simple presence/absence or by comparing the number of amphibians per hour, area, or distance searched with the variable of interest.

Other possible analyses may be less obvious. Distance to mapped trails and roads can be used to evaluate how human accessibility to a site relates to the presence and/or abundance of amphibians.

In some cases, it is important to use only a subset of the data. For example, in analyzing the distribution of foothill yellow-legged frogs, only sites with suitable habitat should be included, e.g., the analysis should be restricted to localities with streams or rivers. Including springs, woodlands, grasslands, and meadows would be inappropriate. Similarly, water flow can be an important limiting factor. Fast moving streams are preferred by some species (e.g., tailed frogs), but are rarely, if ever, used by foothill yellow-legged frogs.

The weight and length data can be used to evaluate the general health of an amphibian population. The relative mass of an animal can provide an indication of its health, e.g., healthier animals are likely to weigh more for their size. Since weight has a curvilinear relationship with body length, it is necessary to calculate a regression as weight versus length^3. Differences in slope can then be evaluated statistically (Analytical Software, 1994; Norusis, 1990; Zar, 1974). Statistically significant differences in slope would suggest that one population was not as healthy as the other. It would be appropriate to compare weight-length relationships between different populations and between years at a single population. The data would also need to be examined to determine whether statistical differences were due to environmental or genetic factors.

Multivariate statistics can be used to evaluate the importance of several variables (e.g., water flow, substrate, elevation, turbidity) on the presence of amphibians. It is well beyond the scope of this protocol to detail specific methods, but such an analysis might prove to be quite valuable.

Data Base Files and Programs

The Aquatic Survey Data Sheet provides an opportunity to record detailed information about both the habitat and the amphibians located. These data are easiest to manage if they are entered into a data base file. Appendix I shows the file structure for the three files that we have used.

Limitations of Aquatic Surveys

Though aquatic surveys can provide a great deal of information, it is appropriate to understand both the strengths and weaknesses of this technique.

Aquatic surveys are designed to detect the presence of aquatic amphibians and provide an index of their abundance. Data are typically summarized in terms of number of each species found per unit time and/or for area searched. Summaries can be broken down by life history stage (eggs, larvae, subadults, and adults). More detailed analyses include calculations of relative weight, which can be used to evaluate the health of individuals at a given site.

There are biases in data obtained from aquatic surveys and, unfortunately, it is very difficult to evaluate how significant the bias actually is. For example, the number of tadpoles observed can be influenced by a wide variety of factors including time of day, air and water temperature, weather, age of the tadpole, species of amphibian, and habitat characteristics of the site. The conspicuousness of eggs and the behavior of both larvae and adults vary from one species to another. Many of these factors interact with each other. Tadpoles move into the warm, shallow areas during the day and back down into deeper water at night. This behavior is typical of the older/larger tadpoles, but less so for the younger/smaller individuals. These differences are related both to the type of habitat occupied and behavioral differences between species. All these factors interact to influence how many individuals of each species are found during aquatic surveys.

Some of the variables mentioned above can be minimized, but not eliminated. Use of a standardized protocol by well-qualified and well-trained field biologists will substantially decrease observer variability. Comparison of two sites or year-to-year comparisons are most appropriate when surveys are conducted at the same time each year (see Monitoring of Amphibian Populations).

Few amphibian survey techniques have been evaluated in sufficient detail to determine how indices of abundance relate to the actual numbers of animals present. This is largely a reflection of the difficulty of evaluating survey techniques. To fully validate a particular technique, one would need to know the actual number of frogs, tadpoles, and egg masses in a pond during a survey. Because of changes in habitat that naturally occur throughout the season and changes in behavior at different ages, the validation would need to be repeated throughout the season for each species of interest. It would also need to be carried out in each habitat of interest. Clearly this is a difficult job. A more attainable goal would be an assessment of repeatability of aquatic surveys, i.e. how similar are the results from day to day.

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Appendix A. Evaluation Criteria for GS-5 Field Biologists

Knowledge, Skills, and Abilities (KSAs)

KSA 1. Knowledge of the biology, natural history, and field identification of amphibians and reptiles. (Describe research experience with field studies, emphasizing any work experience with amphibians in the western U.S.).

Level 4: Extensive field work on the ecology, distribution, or status of amphibians (not reptiles). This would be demonstrated by working on a long-term project (or several shorter ones) involving more than one species of amphibian and at least three different areas or habitat types. Experience must be entirely field work, not museum or laboratory research.

Level 3: Moderate amount of field experience with more than one species of amphibian and/or reptile over an extended length of time or experience working on several shorter projects. Experience must be entirely field work, not museum or laboratory research.

Level 2: 1) Limited field experience (typically with one or a few species) of amphibians or reptiles such as a senior project or working as an assistant for only a limited amount of time, or 2) a moderate amount of field experience with more than one species of fish, bird, or mammal over an extended length of time or experience working on several shorter projects. Experience must be entirely field work, not museum or laboratory research.

Level 1: Meets basic job requirements.

KSA 2. Ability to implement standard capture and census techniques for amphibians and reptiles in both terrestrial and aquatic habitats.

Level 4: Extensive experience with more than two standard capture and census techniques for amphibians or reptiles. Techniques could include time-constrained searches (TCS); pitfall trapping; systematic stream surveys; mark and recapture studies; and seining of ponds, lakes, or streams. This experience typically would be gained by working on a long-term project or several shorter ones.

Appendix A. Evaluation Criteria for GS-5 Field Biologists - continued

Level 3: A moderate amount of experience with at least two capture and census techniques for amphibians and/or reptiles over an extended length of time or experience working on several smaller projects. This experience typically would be gained by working on one or two short-term projects.

Level 2: Experience with at least one capture and census technique for amphibians or reptiles. This experience would typically be obtained while working on a senior project or as a field assistant for a limited amount of time. Has worked primarily on one or a few species.

Level 1: Meets basic job requirements.

KSA 3. Ability to take reliable, scientific notes with a high degree of accuracy, sometimes under difficult field conditions.

Level 4: Extensive experience recording data (e.g., field notes, data loggers, scientific data forms) as part of a long-term project (or several shorter ones) for scientific research on terrestrial vertebrates (e.g., mammals, birds, amphibians, reptiles). There must be significant experience in recording data in the field, not just a laboratory or museum setting.

Level 3: Modest experience recording data for scientific research (of any kind). There must be significant experience in recording data in the field, not just a laboratory or museum setting.

Level 2: Limited experience with scientific research (of any kind) where they participated in recording data. This experience could be gained in a laboratory, museum, or field setting.

Level 1: Meets basic job requirements.

KSA 4. Skill at reading topographic maps. Ability to perform work in difficult terrain and under extreme weather conditions, sometimes for long hours. Ability to use good judgment and resourcefulness, and to be flexible in carrying out field work.

Appendix A. Evaluation Criteria for GS-5 Field Biologists - continued

Level 4: Extensive experience with topographic maps, though not necessarily in a research setting, which would clearly indicate a strong ability to follow topographic maps and accurately map locations in remote areas (e.g., proficient at orienteering, cross country backpacking, mapping of field sites for GIS or research applications). Extensive work experience in outdoor situations as part of a long-term research/resource management project or several shorter ones. Has also worked in situations were good judgment and resourcefulness were needed (e.g., unsupervised field work, difficult or dangerous terrain, map reading skills needed).

Level 3: Moderate experience with topographic maps, not necessarily in a research setting, which would indicate at least a modest ability to follow topographic maps and accurately map locations in remote areas. Moderate experience in outdoor situations in a work setting (e.g., outdoors work, backpacking, search and rescue). Has had at least some experience in situations were good judgment and resourcefulness were needed (e.g., unsupervised field work, difficult or dangerous terrain, map reading skills needed).

Level 2: Limited experience with topographic maps that would indicate some ability to follow topographic maps and accurately map locations in remote areas. Has some experience in outdoor situations either in a work or recreation setting (e.g., outdoors work, recreational backpacking, search and rescue).

Level 1: Meets basic job requirements.

Appendix B. Training Outline

Introductions and past experience as biologists

Overview of project - why are we doing this
- Historic and recent research
- Goals of current study
  - Evaluate distribution and status of amphibian populations
  - Collect data on genetic variability, diseases, effects of pesticides, acid precipitation, etc.
  - Monitoring of key populations
- Experimental Reintroductions
  - Evaluate causes of decline
  - Establish new populations

Identification, natural history, measurements
- Use of dichotomous identification keys
- Amphibian and reptile identification, preferred habitat, and natural history
  - Frogs and salamanders
    - Eggs
    - Larvae
    - Adults
  - Garter snakes
  - Turtles
- Tree identification
- Fish identification
- Determining sex of salamanders, frogs, snakes, turtles
- Measuring frogs, salamanders, snakes, turtles
- Taking snake food samples

Surveying techniques
- Basic survey techniques
  - Scan with binoculars
  - Walk in water when practical
  - Use dip net
- Estimating numbers of animals - Wildlife Count software

Appendix B. Training Outline - continued

- Special considerations
  - Meadows
  - Streams
  - Ponds and lakes
  - Cold weather
  - Footwear
- Dip nets
  - Wooden versus aluminum dip nets
  - D-shape versus round dip nets
  - Reach out and sweep along bottom for tadpoles
    - Do this even in clear water
  - Can wave from side to side to get adults to move
  - Catching garter snakes and turtles
  - Use net when hanging food from bears
- Seine
  - Seining techniques
  - Trap animals against shore
  - Dealing with logs and rocks
  - Count seine hauls
  - Keep specimens in bucket
- Nocturnal searches
  - Listening for frogs and toads
  - Triangulation techniques
  - Spotlighting
    - How to hold light
    - Frog color
    - Eye shine
    - Spider eye shine
    - Which species are easiest to find

Maps, orienting, and compasses

- Map reading
  - Contours
  - Slopes
  - Drainages
  - Reliability of USGS maps

Appendix B. Training Outline - continued

- Universal Transverse Mercator (UTM)
  - How to get UTMs from USGS maps
  - Extrapolating coordinates
  - UTM zones
- Compasses
  - Declination
- Global Positioning (GPS)
  - Satellites
  - Reliability of readings

Data forms

- Use black waterproof pens
- Aquatic surveys
  - Habitat descriptions
  - Areas searched
  - Physical measurements
    - Temperature, depth, substrate, other
- Photocopy field forms weekly
- Retain original - mail copy
- Collecting permits
- Incidental wildlife observations

Photography

- Pictures are important
- Basics of photography
  - Be familiar with camera - all buttons and knobs
- Macro photography
  - Depth of field
  - Use of flash and angle of flash
  - Get at same level as frog
  - Move camera not animal
  - Wet animal - carry water
  - Allow animal to position itself
  - Cover specimen with hand
  - Larvae difficult to photograph
    - Use plastic lid, Ziploc bag, or in net

Appendix B. Training Outline - continued

- Document all aspects of research
  - Habitat photos
  - People at work
  - Document amphibians
    - What specimens to photograph
    - Use of photo cards
    - Photo card versus in-hand versus natural pose
  - Unusual situations
    - Congregations of frogs
    - Egg masses with fungus
    - Mass mortality
    - Predation
- Keep notes on photographs taken
- Label slides as soon as possible
  - Park name or county (top)
  - Month/Year (top)
  - Species (bottom)
  - Locality (bottom)
  - Label right side up for sorting
- Practice photography of amphibians
- Review of practice photographs

Genetic samples

- Number of samples/locality
- Preserving specimens
  - Advantages of liquid nitrogen versus ethanol
  - Collecting samples in liquid nitrogen
  - Collecting samples in ethanol
  - What to collect
    - Frogs
      - Larvae
      - Adults
    - Salamanders
- Specimen labels
  - Label information
  - How and where to tie

Appendix B. Training Outline - continued

Pathology/disease
- Collect all sick/diseased animals
  - Express mail to pathology lab
- What samples to collect
- How to take swab samples
- Contamination of samples
- Taking samples from eggs

Estrogenic study
- Sample sites
- Sample size
- Collection of blood samples
- Shipping

Backcountry field work
- Tour of duty
- Special equipment
- Radio communication
- Safety

Safety and storage of chemicals
- Formalin
- Ethanol
- Liquid nitrogen
- Benzocaine
- Other chemicals

Safety/accidents
- Be prepared
  - Anticipate problems
  - Don't take risks
  - Let someone know where you are working
- Life threatening situations
  - Avoiding trouble
  - Being lost
  - Solving bad situations - thinking your way out of trouble

Appendix B. Training Outline - continued

- First aid
  - Mountain sickness
  - Extractor kit
  - Backcountry first aid
- Park/Forest radios
  - Call numbers, protocol (10-codes)
  - Know where repeaters are
  - Radio dead spots
- Helicopter safety
  - Carry or hold light gear - sleeping bag, hat, day pack
  - Wait for pilot's signal
  - Come and go from down hill
  - Do not go around back of helicopter
  - Wear something warm
  - Do not wear synthetic fabrics that would melt in crash
- Accident and injury paper work
  - Call supervisor if injured
  - Fill out forms

Dangerous animals
- Salamander and frog toxins/diseases
- Bears
  - Food container
  - Hanging food - use dip net, take two ropes
  - What to keep from bears
- Rattlesnakes
  - Avoid likely places
  - Stay calm, see a doctor - not life threatening
- Ticks
  - Lyme disease risks and symptoms
- Giardia - water purifiers

Administrative procedures
- Solicit frog observations
- Procurement Procedures
- Account numbers
- Hours worked and comp time
- Payday, holidays, overtime

Appendix B. Training Outline - continued

- Tour of duty
- Annual and sick Leave
- Travel
  - Overnight travel
    - Lodging & per diem
  - Backcountry travel
  - Travel forms - authorization and vouchers
- Use of government (GSA) vehicle
  - Definition of official travel
  - Appropriate use of vehicle
  - What not to do
  - Use of personal vehicle
- Government mail privileges
  - Official correspondence versus personal mail
- General conduct as government employee
  - Appropriate behavior
  - Drinking and drugs
  - Sexual harassment

Communication skills

- Keep others informed of research progress
  - Supervisor/project manager
  - Park/Forest staff
  - Other biologists
- Problems
  - Study design
  - Maintain gear for optimum performance
  - Lost/damaged equipment

Appendix C. Identification of Selected Western Amphibians

Field guides to amphibians tend to focus on the adult life stages of amphibians. In many ways, this emphasis is quite reasonable since these are the forms that are most likely to be found by both amateur and professional herpetologists. Larval amphibians are of particular interest to those conducting aquatic surveys because this is the life history stage that is most frequently encountered when surveys are conducted using a dip net or seine.

Though some guides have excellent illustrations of larval amphibians (e.g., Nussbaum et al., 1983; Stebbins, 1985; Leonard et al., 1993), they typically depict larvae at only one stage of development. It is easy to confuse species when they are very young or just before metamorphosis. For example, most biologists would readily recognize a bullfrog tadpole as being conspicuously larger than other tadpoles in the same area (at least in western U.S.). Problems arise, however, with very young bullfrog tadpoles since they can look remarkably similar to the rather ubiquitous Pacific treefrog.

Though experienced field biologists can learn to recognize most species of larval amphibians readily, it is often helpful to carry a good quality hand lens or have a dissecting scope available to look at mouthparts. Oral papillae and tooth rows are the two main features of interest in distinguishing several species.

The following two pages are intended to supplement information provided in field guides and also to point out some possible areas of confusion for the identification of larval amphibians. This information is based on observations of amphibians in California and may need to be modified when additional species occur in an area. Also, not all species are covered, notably desert anurans that have not been part of our recent work.

Species	Habitat	Eggs	Larvae	Activity
Ambystoma californiense	Central Valley and south coastal California. Ponds, lakes, quiet streams.	Single or small clusters attached to twigs, weeds, etc.	Pond type. Olive or greenish, mottled with dark brown or black.	Adults migrate and breed Dec - Mar depending on warm rains.
Ambystoma macrodactylum	Northern mountain ponds, lakes, and quiet parts of streams.	Single or cluster of 8-10, attached to vegetation or free on bottom.	Pond type. Olive-gray to brownish gray, mottled with brownish black.	Breed April - July in mountain areas.
Ambystoma gracile	Northwestern California. Ponds, lakes, and slow streams.	Round, firm cluster 2-6" across. Individual eggs in large chambers. Attached to submerged branches and other firm supports.	Pond type. Deep brown, olive-green or light yellow above, blotched with sooty and spotted with yellow on sides. Granular stripe along top of tail fin.	Breed Jan. - July.
Dicamptodon ensatus & D. tenebrosus	Coastal springs and streams.	Rarely seen, Single, but close together. Attached by short stalks to objects in water. Unpigmented.	Moderate stream type. Smoky dark and light mottling on back and fins. Light stripe behind eye.	Breed Dec. - Mar. Some larvae metamorphose second yr. Some adults neotenic.
Rhyacotriton olympicus	North coast. Cold streams and seepages.	Rarely seen. Single, attached to roots and other supports beneath stones and other objects.	Stream type. Gills greatly reduced. Olive or brown speckled with black.	Breeds in spring and early summer.
Taricha torosa	Coastal and mid- to lower mtns. Only newt in Sierra. Quiet or flowing water.	Round, firm clusters about 1" diameter. Attached to sticks, underside of stones, and vegetation.	Eyes at outline of head. Dorsal fin reaches shoulders. Two irregular black stripes on back.	Breeds Dec - May, depending on rains.
Taricha granulosa	Coastal and NE California. Quiet or flowing water.	Usually single in firm, gelatinous capsules. Attached to vegetation and other objects.	Eyes at outline of head. Dorsal fin reaches shoulders. No black stripes, light spots on sides often in longitudinal rows which may join to form light	Breeds Dec - Jan.
Taricha rivularis	North coast. Streams.	Flattened firm clusters of about 1" diameter, often only one egg thick. Usually attached to underside of stone.	Eyes at outline of head. Dorsal fin does not reach shoulders. Dark color rather evenly distributed over back and sides, no stripes as with other	Breeds March to May.
Rana catesbeiana	Widespread at lower elevations. Ponds, lakes, slow sections of streams. Generally below 4,500.'	Floating clusters usually one egg thick, 1-5 ft. across.	Eyes toward side of head, intermediate between H. regilla and R. aurora. Dorsal color olive-green with dark, defined spots (not mottled). Up to 150 mm. See below for first year tadpole ID.	Generally breed Mar - July (in the west). Tadpoles transform after two winters.
			First year tadpoles may look like "funny" H. regilla. R. catesbeiana have eyes almost to outline of head, but they are not "bug-eyed." Dorsal color black and olive-green (almost striped). Venter creamy white to pale yellow, not pinkish.	

Species	Habitat	Eggs	Larvae	Activity
Ascaphus truei	Coastal and NE California. Cold running water.	Rosary-like strings arranged in globular clumps and attached to the underside of stones. Eggs unpigmented.	Unique large round mouth occupying 1/2 of ventral surface. Tail tip often white or rose-colored set off with dark band. Metamorph. in fall after 2-3 yr.	Eggs laid from June - Aug. and hatch Aug. - Sept.
Hyla (=Pseudacris) regilla	Everywhere except deserts. Shallow quiet water.	Loose irregular clusters attached to plants, sticks, or other objects in the water.	Eyes on outline of head as seen from above; looks "bug-eyed." Venter often coppery or bronze. May look "pot-bellied." 35 mm	Breed Jan. - July.
Bufo canorus	Above 4,800' in central Sierra. Shallow pools in meadows.	Bead-like strings and clusters, often covered with silt.	Eyes on top of head. Always jet black. Short spatulate tail, compared with Bufo boreas. Oral papillae confined to sides of mouth. Not "toothy." 35 mm	Breed May to July, just as the snow melts.
Bufo boreas	Everywhere except higher elevation of central Seirra and deserts. Margins of ponds, streams and reservoirs.	Tangled strings with eggs in 1-3 rows, often greatly entwined in vegetation.	Eyes on top of head. Dark to jet black in early stages. Later, color may vary from uniform dark to gray or tan and mottled as in Rana boylii. Oral papillae confined to sides of mouth. Not "toothy." 55 mm, but often much smaller.	Breed Jan. - July, depending on location. Tadpoles transform April - Aug.
Rana boylii	Foothill and coastal streams. Below 6,000'. Not known to overlap elevationally with R. muscosa.	Compact grape-like cluster 2-4" across. Envelopes firm. In shallow water near margins of clear streams. Attached to stones, often on downstream side. May become coated with silt.	Eyes on top of head. Color varies from dark brown with gold flecks to mottled brown or olive. Oral papillae not well developed. "Toothy." 50-60 mm May be confused with late stages of Bufo boreas.	Breed Mar - May. Tadpoles transform in June - Sept. Edges of glottis (opening to lungs in the throat) not black in adults/subadults.
Rana muscosa	Mountain ponds, lakes, and streams. Above 4,500.'	Flattened clumps 1-2" across, attached to stems of sedge, other vegetation, or to the bank itself.	Eyes on top of head. Dark brown to mottled brown by end of first season. Venter may be flecked with gold. Oral papillae not well developed. "Toothy." 50 mm	Breed Mar - June, depending on snow. Tadpoles transform 1-2 years later in July - Aug. Edges of glottis always black.
Rana aurora	Coast and foothills. Lake margins, slow streams, and permanent pools. Generally below 5,000.'	Irregular grape-like cluster, 3-10" across. Jelly loose and viscous, attached to vegetation at or just below surface of water.	Eyes on top of head. Dorsal color brownish with darker marbling. Rana catesbeiana is spotted rather than marbled. Venter pinkish iridescence. Oral papillae well developed on sides of mouth; not "toothy." 75 mm	Brief period of breeding during Jan. - Mar. Tadpoles transform May - Aug.
Rana cascadae	Cascade mountains. Streams, lakes, ponds, meadows. 2,600 - 9,000'	Similar to R. aurora, but clusters usually smaller, deposited in shallow water of pools and lake margins.	Eyes on top of head. Similar to Rana aurora, but oral papillae not well developed. 30 mm.	Breed May - Aug., depending on snow. Tadpoles transform 80 - 95 days later, but a few may overwinter.
Rana catesbeian	****	See other page	****	****
Prepared by: Gary M. Fellers, National Biological Service, Point Reyes National Seashore, Point Reyes, CA 94956				Revised April 1995

Appendix D. Equipment

The use of trade names and the identification of vendors here or elsewhere in the document does not imply a U.S. Government endorsement.

Basic Field Equipment

Binoculars, 7 x 26 Baush & Lomb Custom - these are small, light, and have great close focus and clarity
Cloth bags - temporary storage of snakes, frogs
Cloth sewing tape, 1 m long - for measuring snakes & turtles
Compass - high quality sighting compass with adjustable declination
Dip net, spare bags - for both types of net
Dip nets, aluminum - lighter weight for backpacking

Dip nets, wooden - standard
Federal collecting permits
Field guides - see below
Hand lens for examining larvae
Knapsack
Ruler for SVL - preferably with wooden end stop to facilitate measuring frogs
Sandals, dive booties, felt-bottom boots, or waders
Scales for weighing animals
State collecting permits
Thermometers - preferably Celsius
Topographic maps
Watch with stopwatch function
Ziploc bags - temporary storage of larvae and frogs, and for holding salamanders while measuring

Data Recording Supplies

Amphibian survey forms
Clipboard
Pocket notebooks for photo notes - yellow GSA notebooks work well
"Rite in the rain" paper - photocopy field forms onto this if working in wet areas
Technical pen with waterproof ink
Transparent UTM overlay grid

Appendix D. Equipment - continued

Photographic Equipment

Single lens reflex Camera
Macro lens - for close up photography
Camera flash - to ensure adequate light and good depth of field
Film, 35 mm color slide, ISO/ASA 64
Spare camera batteries
Spare flash batteries

Identification References

Amphibian field guides

A Field Guide to Western Amphibians and Reptiles by Robert C. Stebbins
Amphibians and Reptiles of the Pacific Northwest by R.A. Nussbaum, E.D. Brodie, and R.M. Storm
Amphibians of Washington and Oregon by William P. Leonard, Herbert A. Brown, Lawrence L.C. Jones, Kelly R. McAllister, and Robert M. Storm

Tadpole/larvae identification guide

Amphibian field guide listed above and the tadpole identification in Appendix C

Tree field guide

California Tree Finder by Tom Watts
Pacific Coast Tree Finder by Tom Watts
Western Trees by George A. Petrides and Olivia Petrides

Fish field guide

Freshwater Fishes of California by Samuel M. McGinnis

Appendix D. Equipment - continued

Safety Equipment

Extractor - for poisonous bites
First aid kit - for minor first aid
Flashlight - for safety and for nocturnal surveys
Spare flashlight batteries
Two-way radio
Whistle
Safety glasses - for use around liquid nitrogen and formalin

Pathology Supplies

Cold packs/instant ice - for sick amphibians in field
Critoseal - to seal capillary tubes
Cryovials - for samples to be stored in nitrogen
Dissecting scissors, fine point
Ethanol, 70% - for cleaning equipment and skin surface
Formalin - sold as 37% formaldehyde and diluted to 3.7% formaldehyde (=10% formalin) for use as a fixative
Hematocrit capillary tubes, 1.1 - 1.2 mm diameter
Hematocrit centrifuge - optional, battery powered is ideal
Ice chest - for shipping live frogs or culture samples
Latex surgical gloves or soap for washing hands
Liquid nitrogen
Microscope slide container
Microscope slides (1 mm) with frosted end for labels
Microtainer capillary blood serum separator
Mini-Tip Culturette Collection and Transport System
Nalgene bottles - shipping live animals
Photographic equipment (see above)
Ruler for SVL - preferably with wooden end stop to facilitate measuring frogs
Sharps disposal containers - for syringes
Small file - for scoring capillary tubes prior to breaking
Syringes, 0.5 cc with 1/2" 28 gauge needles (insulin syringes) for collection of blood
Waterproof, fine-point Sharpie - to mark cryovials
Ziploc bags, heavy duty, sandwich-size, freezer type

Appendix D. Equipment - continued

Blood Collection Supplies

Benzocaine solution, 0.02% - for anesthesia
Bowl of fresh water - for rinsing anesthetized frogs
Critoseal - to seal capillary tubes
Cryogenic vials - necessary for samples that are stored in liquid nitrogen
Hematocrit centrifuge - battery powered is ideal
Heparinized hematocrit capillary tubes (75 mm length)
Heparinized saline (100 units heparin added to 250 cc normal saline)
Ice chest - for anesthetic recovery
Latex surgical gloves
Liquid nitrogen
Nalgene bottle, 1-liter - for benzocaine
Paper towels - to line ice chest
Pesola spring scale
Ruler for SVL - preferably with wooden end stop to facilitate measuring frogs
Sharps container - for used syringes
Syringes, 0.5 cc with 1/2" 28 gauge needles (insulin syringes)
Waterproof, fine-point Sharpie - to mark cryovials

Genetic tissue supplies

Cryogenic vials - necessary for samples that are stored in liquid nitrogen
Cryovial rack - handy when labeling and filling with tissue
Dewar flask - holds liquid nitrogen
Dowel for checking nitrogen level
Eppendorf vials (=microcentrifuge tubes), 1.5 ml - used to store tissue preserved in ethanol; cheaper than cryovials
Ethanol, 95% - used to preserve tissue in the absence of nitrogen, e.g., in the backcountry
Fingernail clippers - for removing toes from adults; scissors work, but the toes bleed much more
Labels, 5 x 15 mm, 100% rag, 28 lb. weight - for inside both cryovials and Eppendorf vials; this double labeling is very important
Strainer/aquarium net - used to scoop vials out of nitrogen in ice chest
Styrofoam ice chest - pour nitrogen into ice chest and use strainer or aquarium net to retrieve

Appendix D. Equipment - continued

Waterproof technical pen - Sharpies work well for labeling vials, but too blunt for notes.
Waterproof, fine-point Sharpie - to mark vials

Voucher Specimen Preservation

Alcohol hydrometer - to confirm ethanol concentration
Benzocaine solution (0.02%) - for anesthesia
Ethanol, 70% - preservative
Forceps, 30 cm (12") - for removing specimens from bottles
Formalin - sold as 37% formaldehyde and diluted to 3.7% formaldehyde (=10% formalin) for use as a fixative
Nalgene bottle, 1-liter - for benzocaine
Paper towels - to cover specimens being fixed
Plastic specimen jars
Specimen labels, 100% rag, 28 lb. weight
Straight Pins - for holding specimen in position while fixing
Thread, heavy white linen - to attach specimen label
Trays - for use while fixing specimens

Seining supplies

Buckets - to hold amphibians while more seining takes place
Closet rods - two 4' long with 6 mm (1/4") hole drilled at each end, used as seine poles
Seines - 3 or 6 mm (1/8" or 1/4") mesh works well
Seining forms

Nocturnal Survey Equipment

High intensity light, e.g., 250,000 candle power rechargeable light from Lectro Scientific Inc.
Spare batteries, if not rechargeable

Appendix D. Equipment - continued

Federal Government Forms

Acquisition Request forms
CA-1 personal accident form
Government drivers license
GSA vehicle accident form

Optional Field Equipment

Global Positioning System (GPS) - optional, but often quite useful
Spare batteries for above equipment

Backcountry supplies - partial list

Aluminum dip nets with two-piece handles
Water filter
Tent, pots, pack frame, sleeping bag, ground cloth
Bear-proof food containers and/or rope for hanging food

Appendix E. Suppliers

The use of trade names and the identification of vendors here or elsewhere in the document does not imply a U.S. Government endorsement.

Alt Air
2678 Bishop Dr.
San Ramon, CA 94583

510-277-2100

3XTL	3 liter, Taylor-Whatron extended time refrigerator canister (= dewar for storing liquid nitrogen)
10XT	10 liter, Taylor-Whatron extended time refrigerator canister

The Bag Lady
1391 N. Walter Dr.
Tucson, AZ 85743

602-682-77-9

Regular material, cloth "snake bags" - 25 x 65 cm (10 x 24"), approximately $1.50 each.

Appendix E. Suppliers - continued

Baxter Diagnostic Inc., Scientific Products Division
1430 Waukegan Road
McGaw Park, IL 60085-6787

1-800-964-5227

C8852-3	Mini-Tip Culturette Collection and Transport System (Becton Dickinson)
B3023-13A	Sharps Disposal Containers
M6147-1	Microscope slides (1 mm), frosted
B2975-1	Microtainer capillary blood serum separator
S9505-1	Single use insulin syringes, 28 g x 1/2"
M6270-10	SP Five Slide Container
C1801-1	Hematostat II - hematocrit centrifuge
C1801-10	Centrifuge replacement battery

BioQuip
17803 LaSalle Ave.
Gardena, CA 90248

310-324-0620

7322AG Gambusia net, aluminum dip net for backpacking
Spare net bags

Bureau of Land Management State Office
2800 Cottage Way Room E
Sacramento, CA 59825-1889

916-978-4754

Surface management status maps (provide public lands managed by BLM)

Appendix E. Suppliers - continued

Butler Company (formerly VCA)
30508 Whipple Rd.
Union City, CA 94587

1-800-551-3861

7702	Normal saline solution
14113	Latex exam gloves
2119	Heparin
	Sterile water
	Hematocrit capillary tubes
	Critoseal
	Microscope slides and slide holders

Cabela's
812 13th Ave
Sidney, Nebraska 69160

1-800-237-444

HD-50783-250	250,000-candle power cordless spotlight
HD-50752-802	Carrying strap for spotlight

Carolina Biological Supply Company
2700 York Road
Burlington, NC 27215

1-800-334-5551
910-584-0381

86-1283	95% Ethanol - dilute to 70% and check with hydrometer
85-3900	Chloretone, 100 g

Appendix E. Suppliers - continued

Drug store

Cloth measuring tape for snakes & turtles
Pins
Thread
Plastic trays
Paper towels
Ziploc bags
Kodachrome 35 mm slide film (64 or 200 ISO/ASA)

Fisher Scientific
2170 Martin Ave.
Santa Clara, CA 95050

1-800-766-7000

03-337-7D	Sterile Nalgene Cryovials, 2.0 ml (only sterile vials have white marking area)
05-47-10	Microcentrifuge tubes (=Eppendorf vials), 1.5 ml, natural color
02-893AA	Polyethylene bottles, 1 oz wide mouth
02-893BB	Polyethylene bottles, 2 oz wide mouth
02-893D	Polyethylene bottles, 32 oz wide mouth
03-337-7E	Cryovial holder (we made ours out of wood)
11-590	Alcohol hydrometer - to confirm concentration
	30 cm (12") long forceps

Appendix E. Suppliers - continued

Forestry Suppliers
205 W. Rankin St.
P.O. Box 8397
Jackson, MS 39284

1-800-647-5368

53190	Storage access holder (clipboard) - this is by far the best clipboard for field work
49247	"Rite in the Rain" waterproof paper
	Pesola scales
	Thermometers
	Compasses
	10 m tape

Garcia Machine
14097 Avenue 272
Visalia, CA 93277

209-732- 3785

Bear canisters

Hardware store

Closet rods for seine poles - drill one 6 mm (1/4") hole near each end of 4' rod (need one rod for each end of seine)

Appendix E. Suppliers - continued

The Map Center
2440 Bancroft Way
Berkeley, CA 94704

510-841-6277

USGS topographic maps
US Forest Service maps

Mountain Safety Research
P.O. Box 24547
Seattle, WA 98124

1-800-877-9677
206-624-8573

Whisperlite stove; Waterworks water purifier; repairs

Nichols Net and Twine Co., Inc.
2200 Highway 111
Granite City, IL 62040

1-800-878-6387

ACE 24 lb. 1/8" nylon minnow seine, 4 ft tall
Knotless No. 42. 25 lb. 1/4" nylon minnow seine, 4 ft tall

Office supply store
Waterproof ink
Notebooks
Transparencies for making UTM overlay grids

Appendix E. Suppliers - continued

Olympus Corporation
Consumer Product Group
Crossways Park
Woodbury, NY 11797

516-364-3000

Olympus OM-4T body
Olympus 50 mm F-3.5 macro lens
Olympus T32 flash

Out of This World
45050 Main St.
P.O. Box 1010
Mendocino, CA 95460

707-937-3335

61-7761 7 x 26 Baush & Lomb Custom binoculars

Trimble Navigation Limited
645 North Mary Ave.
P.O. Box 3642
Sunnyvale, CA 94088

1-800-827-8000

Trimble Scout GPS

Appendix E. Suppliers - continued

University Products, Inc.
P.O. Box 101
517 Main St.
Holyoke, MA 01041

1-800-628-1912

219-288511100% rag, 28 lb, 8.5 x 11", 100 sheets

Ward's Natural Science Establishment, Inc.
815 Fiero Ln.
P.O. Box 5010
San Luis Obispo, CA 93403

1-800-872-7289

10 W 0620	Dip net, D-Frame
10 W 0600	Dip net, 10" round
	Spare dip nets
39 W 1340	Formalin, 4 liters (37% formaldehyde solution, diluted to 3.7% formaldehyde (=10% formalin) for use as a fixative

Wildlife Counts
2215 Meadow Ln.
Juneau, Alaska 99801

907-789-0326

Wildlife Counts software

Appendix E. Suppliers - continued

US Geologic Survey
Map Distribution
P.O. Box 25286
Denver, CO 80225

303-236-7477

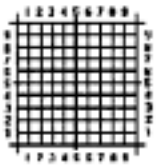
USGS topographic maps

Appendix F. Transparent UTM Overlay Grids

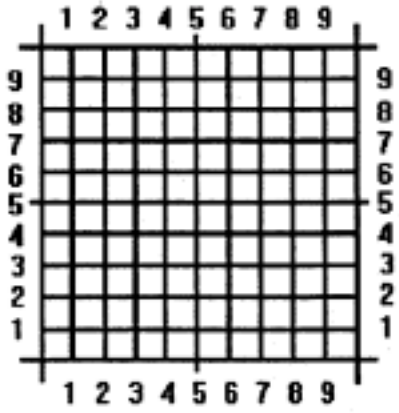
The two grids below can be used for determining UTM coordinates for either 7.5' or 15' topographic maps. Copy this page onto an overhead transparency and then cut out the grids with about a 1 - 2 cm margin around each one. Check the resulting transparency with the scale printed at the bottom of a 7.5' or 15' topographic map to make sure that the scale has reproduced appropriately.

It is helpful to use a colored transparency (such as yellow) so the overlay is not so easily lost or misplaced. Wrapping a piece of masking tape along one edge also helps to make an overlay more conspicuous.

15' grid



7.5' grid



Appendix G. Aquatic Survey Data Sheet

The Aquatic Survey Data Sheet printed on the next two pages is the form that we have used for several years. Normally, the data sheet is printed on both sides of one page.

The form may require some modifications to meet local needs. For example, beaver ponds are a prominent feature in some parts of the Rocky Mountains, but are relatively uncommon in the areas where we have worked. It may be necessary to add or substitute a particular habitat to better meet your needs.

The data sheet was created using an Excel spreadsheet. A copy of the file used to generate the data sheet can be obtained by sending a disk formatted for an IBM PC to the senior author (GMF).

Aquatic Survey Data Sheet

Site: ______________

Date: (mm-dd-yy)	Begin Time:	Total Time: min	Observer(s): 1 2 3 4

Locality:	Owner: ? NPS FS BLM St. Pvt. Oth.

County:	Elevation: m ft	North UTM: GPS Map	East UTM: 10 11 3 4 5 6 7 8
Topographic Map: 7.5 ' 15 '		North UTM: GPS Map	East UTM: 10 11 3 4 5 6 7 8

Distance to Mapped trail: km	Distance to Public dirt road: km	Distance to Public paved road: km

Weather: Clear Overcast Rain Pt. Cloudy Mostly Cloudy Snow	Wind: (mph) 0 5 - 20 < 5 > 20	Air Temp.: (at 1 m) C F	Water Temp.: (0.5 m out) C F

Habitat: Natural Altered 1 2 3 4 5	Description: Lake River Woodland Meadow/Wetl. Ditch Pond Stream Grassland Spring	Drainage: Permanent Seasonal

Site Length: m	Aver. Width: m	Aver. Depth: m	Max. Depth: m	Water Flow sec./10 ft.: 0 7-11 sec. < 7 sec. >11 sec.

Water Turbidity: Clear Turbid 1 2 3 4 5	Mid-day Shade: %	Emergent Vegetation: %	Floating Vegetation: %

Water-shed: ____ Natural ____ Grazed ____ Logged (last 15 yr.) ____ Urban ____ Agricul. ____ Other ________	Substrate: ____ Silt ____ < 2 mm ____ 75 - 300 mm ____ Bedrock ____ 2 - 75 mm ____ >300 mm

Predominant Vegetation:

Fishing Tackle: Yes No	Fish Present: Yes No ?	Species and Approx. Number:

Species	Adults	Subadults	Larvae	Eggs	DNA #	Survey Method(s)	Other
					N A	Visual Hand Aural TCS Dip Net Seine	Voucher Pathology Photo
					N A	Visual Hand Aural TCS Dip Net Seine	Voucher Pathology Photo
					N A	Visual Hand Aural TCS Dip Net Seine	Voucher Pathology Photo
					N A	Visual Hand Aural TCS Dip Net Seine	Voucher Pathology Photo
					N A	Visual Hand Aural TCS Dip Net Seine	Voucher Pathology Photo
					N A	Visual Hand Aural TCS Dip Net Seine	Voucher Pathology Photo

Site: _____ Date: _____

	Species	Tiss.	Sex	Weight (g)	Length (cm)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					

	Species	Tiss.	Sex	Weight (g)	Length (cm)
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					

Comments:

Return to: Dr. Gary M. Fellers, National Biological Service, Point Reyes National Seashore, Point Reyes, CA 94956

Revised: April 1995

Appendix H. Sample Photo Board

The photo board on the next page can be copied onto a onto dark gray or light charcoal paper which is cut to size and then laminated with heavy plastic to make it waterproof. A black waterproof pen can be used to write locality data on the card and then erased with a dry paper towel.

Paper color is important since automatic cameras adjust the exposure to average out the dark and light features in a photograph. Pictures taken on a white background will result in specimens that are so dark as to be unidentifiable in many cases.

See the section on Photography for a more detailed discussion of the use of photo boards and photography in general.

Site _____ **Date** _____

Observer(s) _____

Locality _____

_____ _____

Topo. Map _____ **7.5 15**

UTM North _____ **UTM East** _____

Scientific name _____

Dr. Gary M. Fellers, National Biological Service, Point Reyes National Seashore, Point Reyes, CA 94956

Appendix I. Data Base Files

Data from the aquatic surveys are stored in "dbf" files that can be read by data base programs (e.g., dBase and FoxPro), but also by many spreadsheet programs (e.g., Excel). It is most convenient to use three, related files to store the data. The first file (Survey.dbf) contains all the descriptive information for each locality. The fields follow in the exact order of the Aquatic Survey Data Sheet. This makes for relatively easy data entry. Some fields such as elevation could be numeric fields, but sometimes it is necessary to enter a range of elevations. This can only be done in a character field. Similarly, some fields such as fish would seem to work well as logic fields which accept either T or F, but it is sometimes useful to enter a ? when the observers are not sure. Hence a character field works better since it can accept ?, T, or F.

The Survey-a.dbf file contains data on which amphibians and reptiles were found. It is related (referenced) to the Survey.dbf file by the combination of date and site. In the unlikely event that sites are visited more than once on a single day, a time field could be added to distinguish repeat visits. The species field is used to enter the four letter codes for species names. The field in the survey-a.dbf file is longer than four characters because it is occasionally necessary to describe an animal in more detail (e.g., unconfirmed RABO sighting).

The name field is used to expand the four letter codes in the species field to their full scientific name. These fields are kept separate in case one needs to go back and review the original codes or descriptions. The name field is quite handy since it is often easier to use full names than codes.

The Survey-b.dbf file contains the measurements of amphibians and reptiles from the back of the data sheet. Like the Survey-a.dbf file, it is related to the Survey.dbf file with the site and date fields.

Though they are not shown in the file listings, it is often handy to add a few extra fields. A field called Code (either numeric or character) can be used to flag data records of particular interest in conducting data summaries. For example, when using a data base program, it is convenient to mark all the records of interest with a 1 on the code field and then select all records for code=1.

The use of three files is the most efficient way to store the type of data obtained from aquatic surveys. On the other hand, it can be cumbersome to extract some combinations of data if one lacks expertise in data base file manipulation. Using a report generator such as R&R Report Writer can greatly simplify tabulating some types of data.

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Appendix I. Data Base Files - continued

With such a program it is a fairly simple matter to generate a list of localities where a certain species of frog was found. Such reports can either be sent to a printer or stored in a file.

A copy of the dBase files listed below can be obtained by sending a disk formatted for an IBM PC to the senior author (GMF).

Structure for Survey.dbf

Field	Field Name	Type	Width	Dec
1	SITE	Character	6	
2	DATE	Date	8	
3	BEGIN	Character	8	
4	TIME	Numeric	3	
5	OBSERVER	Character	40	
6	OBS_NO	Numeric	1	
7	LOCALITY	Character	150	
8	OWN	Character	8	
9	COUNTY	Character	10	
10	ELEVATION	Character	14	
11	TOPO	Character	20	
12	SCALE	Character	4	
13	UTMN	Numeric	7	
14	UTME	Numeric	6	
15	GPS	Character	1	
16	MAP	Character	1	
17	ZONE	Numeric	2	
18	SAT	Numeric	1	
19	UTMN2	Numeric	7	
20	UTME2	Numeric	6	
21	GPS2	Character	1	
22	MAP2	Character	1	
23	ZONE2	Numeric	2	
24	SAT2	Numeric	1	

Appendix I. Data Base Files - continued

Field	Field Name	Type	Width	Dec
25	DIST_TRAIL	Character	10	
26	DIST_DIRT	Character	10	
27	DIST_PAVED	Character	10	
28	CLEAR	Logical	1	
29	PARTLY	Logical	1	
30	OVER	Logical	1	
31	MOSTLY	Logical	1	
32	RAIN	Logical	1	
33	SNOW	Logical	1	
34	CALM	Logical	1	
35	LIGHT	Logical	1	
36	MOD	Logical	1	
37	STRONG	Logical	1	
38	TEMP_AIR	Character	8	
39	TEMP_WATER	Character	8	
40	HABITAT	Numeric	1	
41	DITCH	Logical	1	
42	LAKE	Logical	1	
43	POND	Logical	1	
44	RIVER	Logical	1	
45	STREAM	Logical	1	
46	WOODLAND	Logical	1	
47	GRASS	Logical	1	
48	MEADOW	Logical	1	
49	SPRING	Logical	1	
50	PERM	Logical	1	
51	SEASON	Logical	1	
52	LENGTH	Character	14	
53	WIDTH	Character	14	
54	DEPTH	Character	10	
55	MAX_DEPTH	Character	10	
56	FLOW	Character	10	
57	STILL	Logical	1	
58	SLOW	Logical	1	
59	MED	Logical	1	
60	FAST	Logical	1	

Appendix I. Data Base Files - continued

Field	Field Name	Type	Width	Dec
61	TRUBIDITY	Numeric	1	
62	SHADE	Character	8	
63	VEG_ROOT	Character	8	
64	VEG_FLOAT	Character	8	
65	WSHED_NAT	Character	8	
66	WSHED_URB	Character	8	
67	WSHED_GRAZ	Character	8	
68	WSHED_AGR	Character	8	
69	WSHED_LOG	Character	8	
70	WSHED_OTH	Character	8	
71	OTHER	Character	15	
72	SILT	Character	8	
73	BED	Character	8	
74	S_2	Character	8	
75	S_2_75	Character	8	
76	S_75_300	Character	8	
77	S_300	Character	8	
78	VEGETATION	Character	75	
79	TACKLE	Logical	1	
80	FISH	Character	1	
81	FISHES	Character	40	
82	SPECIES	Numeric	1	
83	COMMENT	Character	240	
Total			933	

Structure for Survey-a.dbf

Field	Field Name	Type	Width	Dec
1	SITE	Character	6	
2	DATE	Date	8	
3	SPECIES	Character	25	
4	NAME	Character	25	

Appendix I. Data Base Files - continued

Field	Field Name	Type	Width	Dec
5	ADULT	Character	30	
6	SUBADULT	Character	30	
7	LARVAE	Character	30	
8	EGGS	Character	30	
9	VISUAL	Logical	1	
10	AURAL	Logical	1	
11	DIP	Logical	1	
12	TCS	Logical	1	
13	HAND	Logical	1	
14	SEINE	Logical	1	
15	BLOOD	Logical	1	
16	TISSUE	Logical	1	
17	VOUCHER	Logical	1	
18	PHOTO	Logical	1	
19	COMMENT	Character	200	
Total			395	

Structure for Survey-b.dbf

Field	Field Name	Type	Width	Dec
1	SITE	Character	8	
2	DATE	Date	8	
3	SPECIES	Character	4	
4	TISSUE	Character	40	
5	SEX	Character	40	
6	WEIGHT	Numeric	6	2
7	LENGTH	Numeric	6	2
8	COMMENT	Character	100	
Total			213	

Appendix J. Sample dBase Programs

The following dBase program (Update.prg) is a sample of a program used to standardize the data within the three dbf files listed in Appendix I. The program converts a series of fields to upper case, puts all the site numbers into the same format, converts species names to a standard common name, and then lists any species names which could not be found. Clearly this file would need to be modified to reflect the local species.

The program uses one field which is not included in the above listing, a 6-digit numeric field called elev. This field is used to convert all elevations to feet and average any ranges of elevations that are given in the elevation field. Hence to run this program with the files listed above, it would be necessary to add a 6-digit numeric field called elev to the file survey.dbf. Alternatively, the program lines in the update.prg program below could be deleted.

A copy of the program below can be obtained by sending a disk formatted for an IBM PC to the senior author (GMF).

Update.prg listing

```
close all
set talk on
set safety off
clear
use survey
replace all gps with upper(gps)
replace all map with upper(map)
replace all wshed_nat with upper(wshed_nat)
replace all wshed_urb with upper(wshed_urb)
replace all wshed_graz with upper(wshed_graz)
replace all wshed_agr with upper(wshed_agr)
replace all wshed_log with upper(wshed_log)
replace all wshed_oth with upper(wshed_oth)
replace all silt with upper(silt)
replace all s_2 with upper(s_2)
```

Appendix J. Sample dBase Programs - continued

Update.prg listing - continued

```
replace all s_2_75 with upper(s_2_75)
replace all s_75_300 with upper(s_75_300)
replace all s_300 with upper(s_300)
replace all observer with upper(observer)
replace all fish with upper(fish)
replace all elev with val(elevation) for 'ft' $ elevation
replace all elev with val(substr(elevation, 3, 10)) for 'ft' $ elevation
replace all elev with 39.37*val(elevation)/12 for 'm' $ elevation
replace all elev with ;
   (val(substr(elevation,1, at('-', elevation)))- ;
   val(substr(elevation, at('-', elevation), 6)))/2 ;
   for "-" $ elevation .and. 'ft' $ elevation
replace all elev with ;
   39.37*((val(substr(elevation,1, at('-', elevation)))- ;
   val(substr(elevation, at('-', elevation), 6)))/2)/12 ;
   for "-" $ elevation .and. 'm' $ elevation
replace all elev with ;
   (val(substr(elevation,1, at('-', elevation)))- ;
   val(substr(elevation, at('-', elevation), 6)))/2
   for "-" $ elevation .and. 'ft' $ elevation
replace all elev with ;
   39.37*((val(substr(elevation,1, at('-', elevation)))- ;
   val(substr(elevation, at('-', elevation), 6)))/2)/12 ;
   for "-" $ elevation .and. 'm' $ elevation
replace all site with substr(site,1,2)+'00'+substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=3 ;
   .or. len(trim(site))=4 .and. asc(substr(site,4 ,1))>60)
replace all site with substr(site,1,2)+'0' +substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=4 ;
   .or. len(trim(site))=5 .and. asc(substr(site,5 ,1))>60)
replace all site with substr(site,1,2)+'00'+substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=3 ;
   .or. len(trim(site))=4 .and. asc(substr(site,4 ,1))>60)
```

Appendix J. Sample dBase Programs - continued

Update.prg listing - continued

```
replace all site with substr(site,1,2)+'0' +substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=4 ;
   .or. len(trim(site))=5 .and. asc(substr(site,5 ,1))>60)
use survey-a
replace all site with substr(site,1,2)+'00'+substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=3 ;
   .or. len(trim(site))=4 .and. asc(substr(site,4 ,1))>60)
replace all site with substr(site,1,2)+'0' +substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=4 ;
   .or. len(trim(site))=5 .and. asc(substr(site,5 ,1))>60)
replace all site with substr(site,1,2)+'00'+substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=3 ;
   .or. len(trim(site))=4 .and. asc(substr(site,4 ,1))>60)
replace all site with substr(site,1,2)+'0' +substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=4 ;
   .or. len(trim(site))=5 .and. asc(substr(site,5 ,1))>60)
replace all species with upper(species)
replace name with '??' for species='UNIDENTIFIED FROG'
replace name with 'Escaped frog' for species='ESCAPED FROG'
replace name with 'Unknown tadpoles' ;
   for species='UNKNOWN TADPOLES'
replace name with 'Unknown salamander larvae' ;
   for species='UNKNOWN SALAMANDER LARVAE'
replace name with 'Ambystoma californiense' for species='ABCA'
replace name with 'Ambystoma gracile' for species='AMGR'
replace name with 'Ambystoma macrodactylum' for species='AMMA'
replace name with 'Aneides ferreus' for species='ANFE'
replace name with 'Aneides flavipunctatus' for species='ANFL'
replace name with 'Aneides lugubris' for species='ANLU'
replace name with 'Ascaphus truei' for species='ASTR'
replace name with 'Batrachoseps attenuatus' for species='BAAT'
replace name with 'Batrachoseps nigriventris' for species='BANI'
replace name with 'Bufo boreas' for species='BUBO'
replace name with 'Bufo canorus' for species='BUCA'
replace name with 'Clemmys marmorata' for species='CLMA'
```

Appendix J. Sample dBase Programs - continued

Update.prg listing - continued

```
replace name with 'Coluber constrictor' for species='COCO'
replace name with 'Contia tenuis' for species='COTE'
replace name with 'Cnemidophorus tigris' for species='CNTI'
replace name with 'Crotalus viridis' for species='CRVI'
replace name with 'Diadophis punctatus' for species='DIPU'
replace name with 'Dicamptodon ensatus' for species='DIEN'
replace name with 'Dicamptodon sp.' for species='DICAMPTODON SP'
replace name with 'Dicamptodon tenebrosus' for species='DITE'
replace name with 'Elgaria sp.' for species='ELGARIS SP.'
replace name with 'Elgaria multicarinatus' for species='ELMU'
replace name with 'Elgaria coeruleus' for species='ELCO'
replace name with 'Ensatina eschscholtzi' for species='ENSATINA'
replace name with 'Ensatina eschscholtzi' for species='ENES'
replace name with 'Eumeces gilberti' for species='EUGI'
replace name with 'Eumeces skiltonianus' for species='EUSK'
replace name with 'Hydromantes brunus' for species='HYBR'
replace name with 'Hydromantes platycephalus' for species='HYPL'
replace name with 'Pituophis melanoleucus' for species='PIME'
replace name with 'Plethodon elongatus' for species='PLEL'
replace name with 'Hyla regilla' for species='PSRE'
replace name with 'Hyla regilla' for species='HYRE'
replace name with 'Hyla regilla' for species='HYLA'
replace name with 'Rana aurora' for species='RAAU'
replace name with 'Rana boylii' for species='RABO'
replace name with 'Rana catesbeiana' for species='RACA'
replace name with 'Rana catesbeiana' for species='RACT'
replace name with 'Rana cascadae' for species='RACAS'
replace name with 'Rana cascadae' for species='RANA CASCADAE'
replace name with 'Rana muscosa' for species='RAMU'
replace name with 'Rana sp.' for species='RANA ?'
replace name with 'Rana sp.' for species='RANA SP.'
replace name with 'Rhyacotriton variegatus' for species='RHVA'
replace name with 'Rhyacotriton olympicus' for species='RHOL'
replace name with 'Scaphiopus couchi' for species='SCCO'
replace name with 'Scaphiopus hammondii' for species='SCHA'
```

Appendix J. Sample dBase Programs - continued

Update.prg listing - continued

```
replace name with 'Sceloporus occidentalis' for species='SCOC'
replace name with 'Taricha granulosa' for species='TAGR'
replace name with 'Taricha torosa' for species='TATO'
replace name with 'Taricha sp.' for species='TARICHA SP'
replace name with 'Thamnophis atratus' for species='THAT'
replace name with 'Thamnophis atratus' ;
   for species='THAMNOPHIS ATRATUS'
replace name with 'Thamnophis couchi' for species='THCO'
replace name with 'Thamnophis hammondii' for species='THHA'
replace name with 'Thamnophis hammondii' ;
   for species='THAMNOPHIS HAMMONDII'
replace name with 'Thamnophis elegans' for species='THEL'
replace name with 'Thamnophis elegans' ;
   for species='THAMNOPHIS ELEGANS'
replace name with 'Thamnophis infernalis' for species='THIN'
replace name with 'Thamnophis ordinoides' for species='THOR'
replace name with 'Thamnophis ordinoides' ;
   for species='THAMNOPHIS ORDINOIDES'
replace name with 'Thamnophis sirtalis' for species='THSI'
replace name with 'Thamnophis sirtalis infernalis' ;
   for species='T. SIRTALIS IN'
replace name with 'Thamnophis sirtalis' ;
   for species='THAMNOPHIS SI'
replace name with 'Thamnophis sp.' for species='THAMNOPHIS SP'

use survey-b
replace all site with substr(site,1,2)+'00'+substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=3 ;
   .or. len(trim(site))=4 .and. asc(substr(site,4 ,1))>60)
replace all site with substr(site,1,2)+'0' +substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=4 ;
  .or. len(trim(site))=5 .and. asc(substr(site,5 ,1))>60)
replace all site with substr(site,1,2)+'00'+substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=3 ;
   .or. len(trim(site))=4 .and. asc(substr(site,4 ,1))>60)
```

Appendix J. Sample dBase Programs - continued

Update.prg listing - continued

```
replace all site with substr(site,1,2)+'0' +substr(site,3,9) ;
   for substr(site,2,1)='-' .and. (len(trim(site))=4 ;
   .or. len(trim(site))=5 .and. asc(substr(site,5 ,1))>60)
replace all tissue with upper(tissue)
replace all sex with upper(sex)

use survey-a
index on species to temp
?
? 'Missing species names'
?
list off fields species for name='   '
?
set safety on
close all
```