

A HIERARCHICAL STRATEGY FOR SAMPLING HERPETOFAUNAL ASSEMBLAGES ALONG SMALL STREAMS IN THE WESTERN U. S., WITH AN EXAMPLE FROM NORTHERN CALIFORNIA

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ABSTRACT: The advantages of amphibians and reptiles (herpetofauna) as vertebrate indicators of environmental change is increasingly recognized, and their use for this purpose is becoming more common. However, herpetofauna consists of a broad range of species with very diverse natural histories, making the design of a strategy for sampling entire assemblages challenging. We developed a hierarchical, 4-tiered strategy, designed to sample the complete herpetofaunal assemblage along tributary streams in an ecologically complex northern California watershed. Our approach was to first combine 3 complimentary methods for sampling the more diverse aquatic/riparian species assemblage, then to include an optional fourth method to sample upland (terrestrial) forms. To test this approach we sampled aquatic, riparian, and upland sites along 15 tributaries of the Mattole River watershed. We detected 1108 individual amphibians and reptiles, and 23 of the 28 potential species of reptiles and amphibians found in the Mattole. We discuss the merits of this approach as well as some specific considerations relative to sampling herpetofauna.

Key words: Amphibians, aquatic and riparian fauna, herpetofaunal sampling, Mattole watershed, monitoring, reptiles, small streams, vertebrate sampling methods.

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Riparian environments are of critical concern to resource managers in the United States, particularly in the arid west (e.g., Abell 1988, Shaw and Finch 1996). Riparian and aquatic resources are often scarce and always highly prized in the west, and conflicts over these resources are commonplace and often contentious. These environments often support the highest levels of biotic diversity in western landscapes (Abell 1988, Shaw and Finch 1996). An understanding of the components of riparian and aquatic systems and how they function is needed in order to arrive at reasonable strategies to meet both the needs of human users and dependent riparian and aquatic biota. A first step would be to compile accurate resource inventories. Many amphibians and reptiles (herpetofauna) are dependent on riparian or aquatic environments, and monitoring their populations can indicate the quality of these environments for these and other elements of the biota. Consequently, there is a great need for reliable, repeatable methods to sample amphibian and reptile species, especially those associated with riparian and aquatic environments.

Using appropriate species or species groups as indicators for other life forms or biotic communities can be a valuable strategy in natural resource management (e.g., Noss 1990, Barber 1994). However, the use of indicators is not without controversy and such applications must be carefully and properly applied and interpreted to be valid (Landres et al. 1988, Noss 1990, Kremen 1992, Murtaugh 1996). Amphibians and reptiles have been shown to be excellent indicators of environmental stress or change (e.g., Stafford et al. 1976, Hall 1980, Ohlendorf et al. 1988, Power et al. 1989, Vitt et al. 1990,

Hall and Henry 1992, Olson 1992). Monitoring amphibians and reptiles can help guide management decisions in order to maintain ecosystem functions as well as protect areas of critical habitat for other less tractable organisms.

Amphibians have recently gained attention as a result of widespread and significant declines in populations of many species (Blaustein and Wake 1990, Wake 1990). Although the debate continues on the relative roles of natural fluctuations and anthropogenic influences in these declines (Blaustein 1994, Pechmann and Wilbur 1994), many species are undergoing range-wide population crashes and local extinctions (Halliday 1993, Blaustein et al. 1994, Corn 1994, Drost and Fellers 1996). As a result of these declines, monitoring amphibian numbers is currently of great interest to scientists, resource managers, and the public.

Discerning differences between natural fluctuations and anthropogenic influences in amphibian populations requires rigorous sampling methods. Our methodology was developed and tested to derive repeatable, statistically reliable relative abundance estimates for a wide range of reptiles and amphibians that occur in both riparian and aquatic environments, and adjacent uplands. Our method was developed primarily to sample the amphibian and reptile assemblages associated with headwaters and small to medium tributary streams (first through fourth order [Strahler 1952]). In particular, we developed and applied this methodology for a landscape-scale study of the distribution and habitat associations of the herpetofauna of a northwestern California river basin. In addition, the watershed we sampled contains

elements of both the Madrean herpetofauna, associated with the relatively xeric oak woodlands and chaparral communities of the west, and the Pacific Northwest herpetofauna associated with cooler, moister forested areas to the north (see Welsh [1988] for the historical biogeography of this herpetofauna). Therefore, it offered a unique opportunity to develop a comprehensive sampling methodology that could be employed with a wide range of amphibian and reptile species. Although our approach was developed particularly for the western U. S., we think it may be applicable for sampling herpetofaunal assemblages throughout much of the United States. We conclude by outlining several critical issues related to amphibian and reptile biology that need consideration when designing a sampling strategy for these taxa.

STUDY AREA

Our sampling strategy was developed for and tested in the Mattole River watershed (hereafter referred to as the Mattole) of Humboldt and Mendocino counties in northwestern California. The Mattole covers 787 km² (304 mi²), and the river runs from southeast to northwest, between 39° 57' and 40° 25' latitude and 123° 52' and 124° 21' longitude, and ranges from sea level to 1245 m (4087 feet) in elevation. The Mattole lies in the North Coast Bioregion (Welsh 1994) and contains a diverse vegetation cover depending upon slope, aspect, elevation, and soil type. The following plant associations occur commonly within the Mattole: forests variously dominated by redwood (*Sequoia sempervirens*), mixed Douglas-fir (*Pseudotsuga menziesii*), hardwoods, (primarily tanoak [*Lithocarpus densiflorus*] and madrone [*Arbutus menziesii*]), with elements of coastal oak woodland (primarily black oak [*Quercus kelloggii*] and canyon live oak [*Q. chrysolepis*]), as well as mixed chaparral (primarily manzanita [*Arctostaphylos* spp.] mountain whitethorn [*Ceanothus cordulatus*], scrub oak [*Q. berberidifolia*], and coyote brush [*Baccharis pilularis*]), and both annual and perennial grasslands (see Mayer and Laudenslayer [1988] wildlife habitat relationships [WHR] types: redwood, Douglas-fir, montane hardwood-conifer, montane hardwood, coastal oak woodland, mixed chaparral, coastal scrub, perennial grassland, and annual grassland for more detailed descriptions of the common vegetation associations of the Mattole).

METHODS

Our approach incorporated a 4-tiered riparian/aquatic and upland sampling strategy that was applied to a 300-m segment of stream (Fig. 1). This 300-m segment (hereafter referred to as a reach) comprised the primary unit of analysis for our study, and defined the spatial limits

of the various tiers of our sampling. The reaches were intended to be representative samples of streams that we pre-selected based upon a stratification of the dominant upland vegetation types in the Mattole. Placement of the reaches within these streams was then determined by drawing a random number and pacing that distance in meters upstream from the point where an access trail or road first met or crossed each stream. A second question about the composition of the aquatic/riparian herpetofauna in intermittent and perennial reaches (not reported here) required selection of 2 reaches per stream where possible. We determined reach distances (the 300-m sampling units) based on wetted channel for all intermittent stream sections, so that the dry portions of these stream channels did not count toward the 300 meters. This reach selection and sampling was conducted only during the summer dry season to account for any possible seasonal variations. As a result, our intermittent reaches are somewhat longer in total length than the perennial reaches. Those animals observed along the dry portions of intermittent reaches (mostly lizards) were counted only as incidental observations (not reported here) to avoid biasing our comparisons.

The first tier--a distance-based visual encounter sample (Crump and Scott 1994) (Fig. 1a)--was conducted with 2 persons walking slowly from downstream to upstream in the streambed. The lead person watched carefully for animals in the water and on streambed and streamside substrates, and recorded these observations. The second person, using a 50-m measuring tape or hip chain, mapped the reach and classified the stream habitats into slow and fast water (main channel), also mapping seeps, springs, or side channels along the stream margins. The maps included landmarks and distances as well as animal observations. Fast or slow water was determined by the relative velocity of the water (e.g., pools are slow, and riffles, runs, and cascades are fast). More elaborate systems for classifying streams are available (e.g., Hawkins et al. 1993); however, we recommend this simplified approach as a good compromise because gathering finely subdivided microhabitat data can result in problems with small sample sizes and reduced statistical power (Green 1989, Anon 1995).

The second tier of sampling consisted of area-constrained searches (ACS) randomly placed within each 300-m reach (Fig. 1b). Three slow and 3 fast stream units per reach were randomly selected from the maps created in the first tier. This was done by assigning each stream unit a number and making selections with a random number generator. One-meter-wide, cross-stream, belts were then centered on each unit (Fig. 2). Stream widths were measured at the belts so animal captures could later be adjusted for the actual belt areas

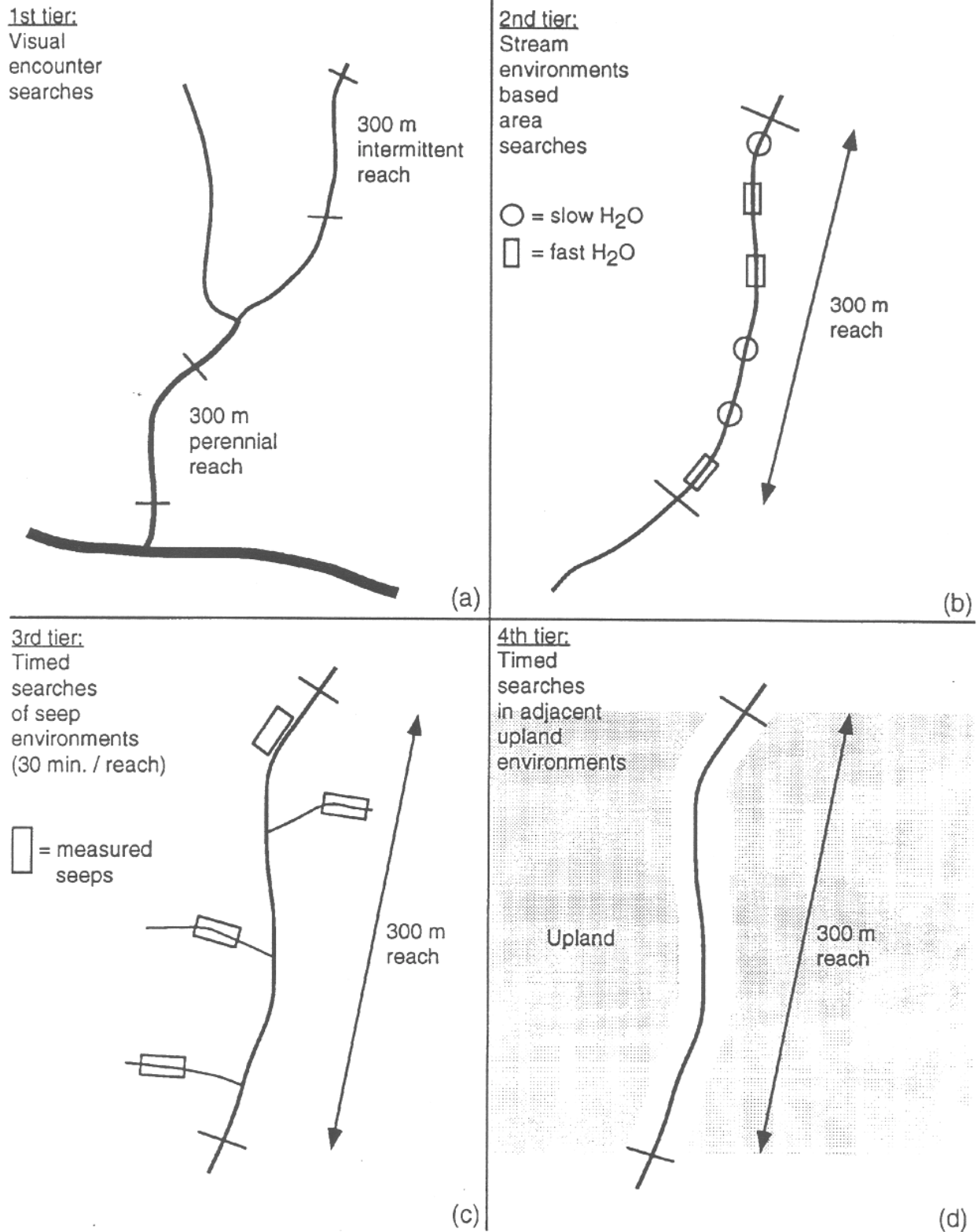


Fig. 1. Four-tiered strategy for sampling herpetofauna along small streams and adjacent uplands. The sample unit size is a 300-m length of stream (1 reach). (a) Visual encounter search; (b) aquatic (3 each in fast and slow water) area-constrained search using randomly placed belts (1-m wide); (c) time-constrained (per 30 min) search of seeps; (d) time-constrained search of adjacent upland environments (2 hours search time per bank). See text for details.

searched (so captures can then be corrected to densities per unit area and compared among belts and reaches). Belt placement was adjusted slightly up or down stream if necessary to allow for the most effective animal searching (e.g., to avoid working directly under fallen logs where observations were severely hampered). Stream units were measured from downstream to upstream, and belts were placed and searched in the same fashion to avoid disturbing animals upstream in areas that were yet to be sampled. ACS requires a systematic search of the entire belt area, removing all movable substrates with catch nets held downstream to capture dislodged animals (Welsh 1987, Bury and Corn 1991, Jaeger 1994, Jaeger and Inger 1994, Welsh et al. 1997). We used small, hand-held plexi-glass boxes (viewing windows) and hand nets to locate and capture animals. We released all animals after data were collected. To provide information on microhabitat associations, we recorded stream unit measurements including slope, aspect, water temperature; and belt measurements such as belt length, mean belt water depth, maximum belt water depth, substrate embeddedness (estimate of the percent of belt substrates that were embedded in fine substrates during animal sampling), maximum water velocity, and substrate composition (percent estimates) (see Platts et al. [1983] for substrate particle size standards).

Our approach is designed for extensive sampling or monitoring of herpetofauna. However, to examine differences within streams or among stream habitats (e.g., pools, riffles, runs), more intensive sampling would be required, including sampling more stream units and using more randomly placed belts per unit. Welsh et al. (1997) described a method that allows more rigorous analysis and estimations of minimum population sizes.

The third tier of sampling consisted of a 30-min area-constrained search in available seeps or springs (Fig. 1c). Seeps and springs noted on the maps created for each 300-m reach during tier 1 were censused if there were only 1 or 2, or randomly selected if there were >2, in order to allow for 30 min of search time. Seeps and springs were generally not common and small where they did occur in the Mattole. As a result, it often required more than 1 or 2 patches of this type to complete the required sampling effort under tier 3. Seeps were then thoroughly and systematically searched as above (ACS belts in tier 2) for a total search time of 30-min. Animals were collected for measurement, held to prevent resampling, and then released at the conclusion of the search. Springs, seeps, and shallow, wet marginal were searched as part of this tier, but if no such areas existed then no third tier sampling was conducted. Many small patches of seeps or springs could be sampled within a reach, so long as the total search time spent was 30-

min. Area measurements of each patch were recorded along with captures for later calculation of animal densities. We recorded substrate composition, water temperature, water depth, aspect, and slope at each site.

A fourth tier of sampling, using a timed visual encounter (Crump and Scott 1994) or time-constrained search (TCS; Welsh 1987, Corn and Bury 1990) was employed to sample species in the upland environments adjacent to the stream and riparian zone (Fig 1d.). We used a 4 person-hour TCS to sample each 300-m reach. The sampling effort was equally divided between the 2 sides of the stream, with a 2 person-hour TCS in the

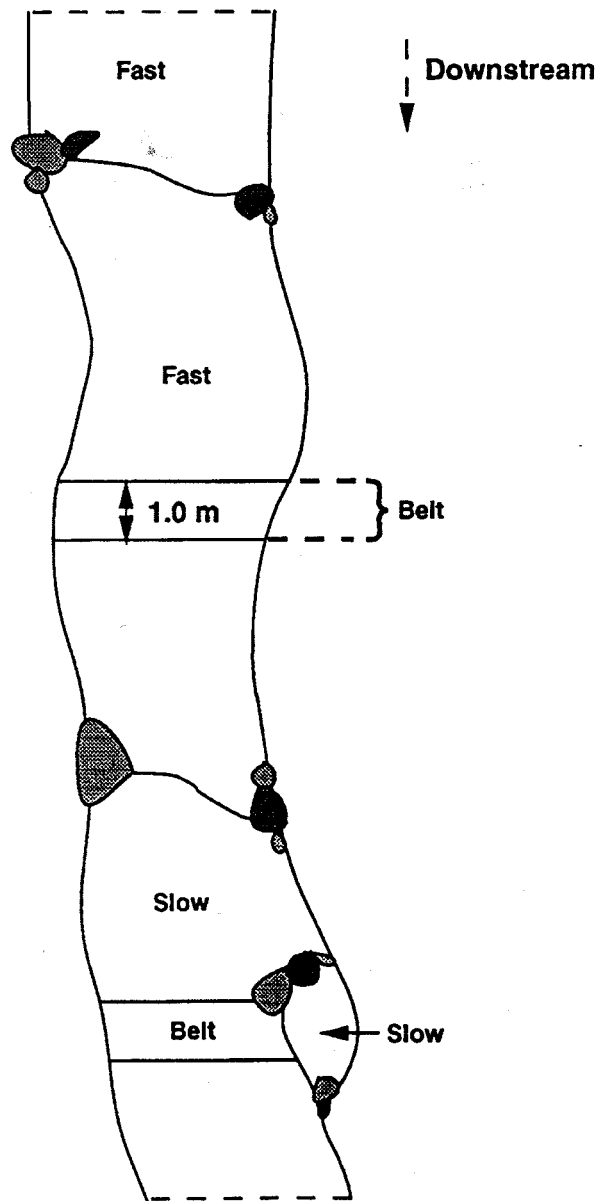


Fig. 2. Details of belt placement for the aquatic area-constrained search method (Fig. 1, b).

upland environments above each bank, along the 300-m length of each stream reach. For this method, we searched upslope >10 m from vegetation indicative of riparian environments (e.g., willows (*Salix* spp.), alders (*Alnus rubra*), and other aquatic and riparian obligates) to assure capture data were gathered only from within upland environments. Upslope distance from the riparian edge was limited to ≤ 100 m. While some differences in the overall area covered among sites result from this type of sampling (depending mostly on the thickness of the vegetation cover), the search effort is held constant by using a constant search time.

Comments on the Analysis of Data

Although the results of our statistical analyses are not reported here, we do wish to offer some direction in this regard for potential users of our method. The linking of complimentary sampling methods, as was done here, requires some particular considerations for analysis of the resulting data. Although the overall unit of analysis is the 300-m reach, each of the 4 tiered methods executed within each reach offers advantages for particular groups of species as noted above, and therefore it is advisable, and in most cases essential, that the data be subsetted by tier for analysis. The choice of which subset of data to use will be an obvious because each species will usually be detected best by only one of the methods. For example, Welsh (1990) used data gathered by 3 different methods to examine relationships between the abundances of 3 species of amphibians and forest seral stage. This approach assures that one does not inappropriately combine data across methods. This caution is necessary because each of the 4 tiers has a different sample effort, and thus the underlying units for the resulting data differ. The 1st tier visual encounter sampling results in numbers of individuals per 300-m reach, the 2nd tier ACS belts result in numbers of individuals per belt area (m^2), the 3rd tier ACS of seeps results in numbers of individuals per seep area (m^2), and the 4th tier TCS results in numbers of individuals per unit time. These differences in sampling effort and species detectability will usually require separate analyses of tiers. One exception might be when doing comparisons of species richness and evenness (diversity indices). Here, if the entire 4-tier protocol is executed at every site, the units of analysis would be the total number of species and/or individuals detected for each 300-m reach, with all tiers combined.

RESULTS

We sampled 18 reaches, along 15 Mattole tributaries, detecting 1087 individual animals of 23 species (8 salamanders, 4 frogs and toads, 1 turtle, 4 lizards, and 6

snakes), totaling 997 amphibians and 90 reptiles (Table 1). We failed to detect 2 species of relatively rare salamanders. The arboreal salamander (*Aneides lugubris*), a terrestrial species, is near the northern extreme of its range in the Mattole and is therefore very spotty in occurrence. The northwestern salamander (*Ambystoma gracile*), a pond breeding species, is known from a single record in the headwaters of Bear Creek, a large Mattole tributary where we did not sample. Natural ponds are rare in the Mattole which may limit the occurrence of this species in the watershed. We also failed to detect 3 of the 9 species of snakes that potentially occur in the Mattole (see Table 1, footnote 4). Many snake species are secretive and difficult to sample, requiring more specialized, species-specific methodologies (Lillywhite 1982, Fitch 1987, Welsh 1987). Overall, we detected 82% of the species possible in the watershed, missing only those that are rare or associated with environments we did not sample.

Each of our 4 methods showed biased results relative to particular species and life stage (Table 1). For example, the visual encounter method was superior for detecting diurnal frogs and aquatic salamanders (both adults and larvae), while the belt search method was best for detecting the more secretive amphibians like larval red bellied newts and larval tailed frogs. The seep search was the best technique for detecting black salamanders and the only technique that detected southern torrent salamanders, both of which are microhabitat specialists. Although overall similar numbers of reptiles were detected with the visual encounter and TCS methods, TCS detected more species of snakes and lizards. TCS also detected 3 species of terrestrial salamanders that were not detected by any of the other methods. The visual encounter method detected the highest number of individual animals; however, almost half of these were of the single most abundant species and life stage we sampled, tadpoles of the foothill yellow legged frog. Although each method clearly had its own strengths and weaknesses, linked together in this complimentary fashion, they functioned well to sample the overall diversity of the herpetofauna of the Mattole. These methods provide what we believe are reliable presence data for all species detected, relative abundance data for those common species, and density estimates for the few species best detected by the tiers that incorporated area-constrained searching (tiers 2 and 3).

DISCUSSION

Our 4-tiered sampling approach provides a framework for sampling amphibians and reptiles that could be readily adopted elsewhere because it can detect and sample high numbers of individuals from a wide range

Table 1. Captures of reptiles and amphibians (adults/larvae) from 18 reaches sampled in 15 tributaries of the Mattole watershed of northwestern California using the tiered strategy (Fig. 1). For captures of non-metamorphosing species juvenile and adult life stages are combined. Visual search, belt, and seep searches were conducted in the summer of 1995, and timed searches in the spring of 1996¹.

Species	Visual encounter	Belt search	Seep search	Upland timed search	Total
Clouded salamander	0	0	0	2	2
<i>Aneides ferreus</i>					
Black salamander	2	0	8	0	10
<i>Aneides flavipunctatus</i>					
Slender salamander	0	0	0	134	134
<i>Batrachoseps attenuatus</i>					
Pacific giant salamander ²	2/107	5/118	0/15	0	7/240
<i>Dicamptodon tenebrosus</i>					
Ensatina	0	0	0	13	13
<i>Ensatina eschscholtzii</i>					
Southern torrent salamander	0	0/1	2/6	0	2/7
<i>Rhyacotriton variegatus</i>					
Rough-skinned newt	57/1	7/6	0	0	64/7
<i>Taricha granulosa</i>					
Red-bellied newt	0	0/3	0	0	0/3
<i>Taricha rivularis</i>					
Total salamanders	61/108	12/128	10/21	149	232/257
Tailed frog	1/0	1/15	0	0	2/15
<i>Ascaphus truei</i>					
Western toad	0	1/0	0	0	1
<i>Bufo boreas</i>					
Pacific tree frog	1/23	0	0	0	1/23
<i>Hyla regilla</i>					
Yellow-legged frog	106/341	13/6	0	0	119/347
<i>Rana boylei</i>					
Total frogs	108/364	15/21	0	0	123/385
Pacific pond turtle ³	1	0	0	0	1
<i>Clemmys marmorata</i>					
Total turtles	1	0	0	0	1
Northern alligator lizard	0	0	0	2	2
<i>Elgaria coerulea</i>					
Southern alligator lizard	0	0	0	3	3
<i>Elgaria multicarinata</i>					
Western skink	4	0	0	3	7
<i>Eumeces skiltonianus</i>					
Western fence lizard	23	0	0	26	49
<i>Sceloporus occidentalis</i>					
Total lizards	27	0	0	34	61

Table 1. (cont.)

Species	Visual encounter	Belt search	Seep search	Upland timed search	Total
Ringneck snake	0	0	0	2	2
<i>Diadophis punctatus</i>					
Gopher snake	0	0	0	1	1
<i>Pituophis melanoleucus</i>					
Oregon garter snake	8	2	0	0	10
<i>Thamnophis atratus hydrophilus</i>					
Western terrestrial garter snake	0	0	0	1	1
<i>Thamnophis elegans</i>					
Common garter snake	6	0	0	1	7
<i>Thamnophis sirtalis</i>					
Racer	0	0	0	7	7
<i>Coluber constrictor</i>					
Total snakes ⁴	27	2	0	12	28
Total number of species	11	8	3	12	23
Total captures (all species and all life stages)	683	178	31	195	1087

¹ Visual searches = one transect of each 300 reach, belts searches = 3 each in fast and slow water per reach, seep searches = 1/2 hour timed search of seeps per reach, TCS (time constrained searches) in adjacent upland = 4 person-hours per reach (two hours above each bank).

² May include some neotenic (sexually mature) individuals.

³ One juvenile turtle captured in our pilot year (1994) was included to illustrate the comprehensiveness of this approach.

⁴ Three species of snake known to occur in the Mattole were not encountered during our sampling: the rubber boa (*Charina bottae*), the sharp-tailed snake (*Contia tenuis*), and the western rattlesnake (*Crotalus viridis*).

of species (Table 1). There are a variety of issues, however, that need to be considered when undertaking amphibian and reptile sampling (see especially papers in Scott [1982], as well as Scott and Seigel [1992] and Heyer et al. [1994]). We recommend consulting many available references to gain insight into the biology of potential target organisms before attempting to design a sampling strategy. (1) Consult standard field guides (e.g., Stebbins 1985, Conant and Collins 1991) to compile a list of potential species. (2) Review pertinent technical literature to save time and money, and improve the quality and applicability of results. (3) Consult knowledgeable experts to obtain the most current information relative to any proposed project or species. Below is a discussion of several universal issues that need consideration when designing a sampling strategy for any herpetofaunal assemblage.

Ectothermy

The ectothermic nature of amphibians and reptiles means that these organisms rely heavily on behavior to select appropriate temperatures and moisture levels within the environment in order to maintain body conditions amenable to foraging, reproduction, and escape from predation (Dough 1980, 1983; Stevenson 1985). Thus, amphibians and reptiles are generally more sensitive to fluctuations in temperature and moisture than are endothermic organisms. This poikilothermic physiology also allows them to withstand long periods without food and moisture, a distinct advantage over homeothermic organisms (see Scott and Seigel [1992]). However, this adaptation has important implications for designing a sampling strategy. The first implication is that a given species may act quite differently under differing conditions of temperature and moisture. Also, such differ-

ences can be cyclical depending on the time of day or year (see below). Therefore, sampling for a particular species must take into account behavioral differences relative to climatic variation. It is critical to sample under climatic conditions that are representative of conditions that favor typical behavior for the species and will permit periodic future sampling under similar conditions. This means gathering data within a pre-determined range or climatic window of temperature and moisture conditions and documenting those conditions with accurate measurements. These conditions should be determined based on the known preferred ranges, as well as daily and seasonal patterns of a given species as determined by a literature review or pilot field work. This approach will ensure consistency across sampling periods by eliminating biases interjected by animal response to climatic fluctuations.

Life History Stages

Age and life history stages within a species may have different characteristics and habitat requirements, and may require different sampling strategies. Amphibians with biphasic life histories (aquatic larvae/terrestrial adults) are an obvious example, but some reptiles can show strong differences in behavior and habitat use depending on life stage. For example, the Oregon garter snake, a highly aquatic species, feeds on juvenile salmonids and tadpoles of the foothill yellow-legged frog when young, but shifts to the much larger larval and neotenic Pacific giant salamander as they grow (Lind and Welsh 1994). This shift in prey species is accompanied by shifts in both behavior and habitat use on the part of these snakes as they grow larger and can utilize faster and deeper waters for foraging.

Annual Cycles

Amphibians and reptiles are similar to other vertebrates in so far as one can observe different behaviors among adults during different times of the annual cycle. For example, snakes of many desert and chaparral species show differences in their movements depending upon season (Lillywhite 1982), presumably in search of food or mates, or both. However, with amphibians and reptiles, reproductive behaviors are often also linked with particular conditions of moisture and temperature. Salamanders that migrate to breeding ponds from terrestrial habitats during late winter and early spring generally do so at night coincident with rainfall (e.g., Santa Cruz long-toed salamander [*Ambystoma macrodactylum croceum*] [Ruth 1988] and California tiger salamander [*A. californiense*] [Morey and Guinn 1992]). Such differences in behavior attributable to reproductive cycles is best accommodated by choosing sampling periods consistent with the known reproductive biology of the

species in question and conducting your work consistently from year to year. Some amphibians, particularly in arid regions, are often explosive breeders, able to successfully reproduce in 1-2 weeks; they can breed anytime between January-April (northern hemisphere) if weather conditions are suitable (e.g., Mayhew 1965, Zweifel 1968, Newman 1989).

Diel (24 hour) Cycles

Many species of reptiles and amphibians are largely nocturnal or crepuscular. Activity relative to the diel cycle can also differ for different life history stages within the same species. For example, both spadefoot toads (genus *Spea* or *Scaphiopus*) (Mayhew 1965) and tailed frogs (Metter 1964) have diurnally active larval stages and nocturnal adult stages. Diel cycle activity patterns may also change depending on the stage of the annual reproductive cycle or in response to temperature and moisture conditions. For example, in western North America there are few if any snakes active on the surface on a cold spring night compared to a warm spring night. This has obvious implications for choosing when to sample.

CONCLUSIONS

The 4-tiered sampling strategy we developed was very effective for addressing the challenges of sampling the diverse amphibian and reptile fauna of the Mattole River Basin. With careful consideration for the potential species present, this methodology could be adapted for other areas in North America. As it stands, this method provides a basis for multiple year, long-term monitoring for amphibians and reptiles by providing a consistent approach to gather comparable baseline data in many areas throughout the west. The creation of amphibian and reptile monitoring programs by preserve and parkland managers should take full advantage of those attributes of herpetofauna that make them excellent indicators of environmental change (i.e., their high site fidelity, longevity, relatively abundant, and ease of counting). Focusing on amphibians and reptiles would provide managers with a suite of vertebrate indicators that are readily surveyed and can provide valuable feedback on the status and trend of many ecosystem functions within our threatened and retreating natural environments. In addition, these species are attractive to, and can be readily identified by, an informed public that is often willing to participate in monitoring programs (e.g., Bishop et al. 1997, Lepage et al. 1997).

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