Quantifying Amphibian Richness in Southeastern Forests

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Abstract: Despite numerous museum records and published range maps, gaps exist in current knowledge of the abundance and distribution of many amphibian species. Furthermore, because of the unique life histories among amphibians, several techniques conducted across several diurnal and seasonal time scales are needed to detect species presence. We conducted surveys at fixed points within forests on a military land base in east-central Mississippi to quantify amphibian richness using anuran call counts and time-constrained area searches, 1998–2000. Concomitantly, we completely enclosed 3 ephemeral pools with drift fence-pitfall arrays to monitor seasonal use by amphibians and gain further knowledge of local species richness. We detected 21 species of amphibians among 4 habitat types using anuran call counts and area searches at fixed points. Species richness at fixed points by habitat type was 12, 16, 17, and 9 for pine, pine-hardwood, riparian, and beaver (Castor canadensis) wetlands, respectively. Mean species richness by point ranged from 2.71 (SE = 2.52) in pines to 7.83 (SE = 0.75) in riparian hardwoods. Data from enclosed breeding pools added 2 additional species to the overall richness of the land base. We also conducted area searches away from fixed points and added 2 more species to overall richness. Thus, amphibian richness for the land base was 25 species though no technique detected >17 species alone. We documented 9 new counties records, including pine woods treefrog (Hyla femoralis) and four-toed salamander (Hemidactylium scutatum), which were extensions of documented and predicted ranges. We recommend that natural resource managers in the Southeast generate a list of all potential species in the area, based on historical and current information, then develop a sampling protocol that would confirm or deny the presence of each species based on specific habitat requirements and life histories. Furthermore, research technicians should be trained in amphibian identification in case new species are detected, and vouchers should be collected when new records are identified.

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Amphibians, in general, are considered indicators of ecosystem health, mainly because of their close association with aquatic and terrestrial habitats, and their susceptibility to toxins and radiation through their thin, permeable skin (Beebee 1996).

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Research suggests that amphibians are experiencing population declines at multiple scales due to habitat destruction, chemical contamination, disease, global warming, competition with invasive species, and commercial pet trade (Blaustein et al. 1994, Dodd 1997, Lips 1998, Wake 1998, Alford and Richards 1999, Carey et al. 1999).

The southeastern United States has the greatest diversity of salamanders in the world (Petranka 1998) and also is rich in anurans. Because of their abundance and richness, amphibians are integral components of many ecosystems, sometimes representing the greatest vertebrate biomass of a system (Burton and Likens 1975). However, amphibians are likely misrepresented in estimates of biological diversity due to their secretive nature, nocturnal habits, small ranges, or because of a general lack of understanding of their life histories. Many species of amphibians possess a biphasic life cycle where adults move to breeding ponds, court, deposit eggs, and return to their terrestrial habitats while others are adapted to permanent aquatic or terrestrial life (Duellman and Trueb 1994, Petranka 1998).

A variety of techniques are available to detect and/or capture frogs, toads, and salamanders (Heyer et al. 1994). However, applying a proven sampling technique at the wrong season or time of day will bias estimates of amphibian community composition and structure. Many species do not breed every year, including eastern narrow-mouth toad [*Gastrophryne carolinensis* (Dodd and Cade 1998)], marbled salamander [*Ambystoma opacum* (Petranka 1998)], and spotted salamander [*A. maculatum* (Phillips and Sexton 1989)]. Thus, the number of species detected at a breeding site may not represent the entire amphibian community. Furthermore, some species, such as the Northern crawfish frog (*Rana areolata*), only vocalize for a 1- to 2-week period in Mississippi (R. Altig, Miss. State Univ., pers. commun.), thereby reducing chances of detection compared to species with broader breeding seasons [i.e., spring peeper (*Pseudacris crucifer*), bronze frog (*Rana clamitans c.*), or southern leopard frog (*Rana sphenocephala*)].

Williams (Miss. GAP Project, unpubl. data), examined records from 16 museums in the United States and found 24 amphibian species recorded in Lauderdale County, Mississippi (Table 1). However, predicted distributions of 14 salamander, 11 frog, [12 if you separate gray treefrog (*Hyla chrysoscelis*) and Cope's gray treefrog (*H. versicolor*)], and 4 toad species including Lauderdale County [(Table 1); Miss. Herpetol. Atlas 2001]. Thus, there is a lack of knowledge regarding the true species richness of amphibians in Lauderdale County. To assist natural resource managers in assessing and maintaining biodiversity, we recorded presence or absence of amphibians in mature forests on Department of Defense (DOD) land in east-central Mississippi. Our objectives were to compare point richness of forest-dwelling amphibians based on current forest stand composition, arrangement, and silvicultural activities; evaluate the efficiency of multiple amphibian sampling techniques in quantifying local richness; and demonstrate how the same steps can be followed throughout the Southeast to initiate a biologically-based amphibian management program.

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Scientific name	Common name	Recorded	Predicted
Order Anura			
Family Bufonidae	Toads		
Bufo quercicus	Oak toad	Х	
Bufo terrestris	Southern toad	Х	Х
Bufo woodhousei fowleri	Fowler's toad		Х
Family Hylidae	Cricket frogs		
Acris crepitans c.	Northern cricket frog		Х
Acris gryllus g.	Southern cricket frog	Х	Х
Hyla avivoca	Bird-voiced treefrog		Х
Hyla chrysoscelis	Gray treefrog	Х	Х
Hyla cinerea	Green treefrog	Х	Х
Hyla squirella	Squirrel treefrog	Х	
Pseudacris crucifer	Northern spring peeper	Х	Х
Pseudacris triseriata feriarum	Upland chorus frog	Х	Х
Family Microhylidae	Narrow-mouthed toads		
Gastrophrvne carolinensis	Eastern narrow-mouthed toad	Х	Х
Family Pelobatidae	Spadefoot toads		
Scaphioppus holbrooki h.	Eastern spadefoot		Х
Family Ranidae	True frogs		
Rana catesbeiana	Bullfrog	Х	Х
Rana clamitans c.	Bronze frog	Х	Х
Rana palustris	Pickerel frog		Х
Rana sphenocephala	Southern leopard frog	Х	Х
Order Caudata			
Family Amphiumidae	Giant salamanders		
Amphiuma tridactylum	Three-toed amphiuma		x
Family Proteidae	Giant salamanders		24
Necturus alabamensis	Alabama waterdog	x	x
Family Sirenidae	Giant salamanders	21	24
Siren intermedia	Lesser siren		x
Family Ambystomatidae	Mole salamanders		24
Ambystoma maculatum	Spotted salamander	x	x
Ambystoma opacum	Marbled salamander	x	x
Ambystoma talpodium	Mole salamander	21	x
Ambystoma teranum	Smallmouth salamander	x	x
Family Plethodontidae	Lungless salamanders	21	24
Desmograthus auriculatus	Southern dusky salamander	x	
Desmognathus fuscus	Spotted dusky salamander	x	x
Furveea hislineata cirrigera	Southern two-lined salamander	x	x
Eurycea longicauda guttolineata	Three-lined salamander	x	x
Eurycea auadridigitata	Dwarf salamander	21	x
Plathodon alutinosus mississinni	Slimy salamander	x	X
Pseudotriton montanus flavissimus	Gulf Coast mud salamander	X	Λ
Pseudotriton rubber viosogi	Southern red salamander	X	x
Family Salamandridae	Newts	Λ	Λ
Notophthalmus viridescens	Central newt	x	v
louisianensis	Contrar new t	Δ	Δ

Table 1. Species of amphibians listed as county records and/or predicted to occur inLauderdale County, Mississippi.

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Methods

Study Area

This study was conducted on Naval Air Station Meridian (NASM) in Lauderdale County, Mississippi (3233N 8832E), from March 1998-June 2000. NASM was approximately 4,035 ha of DOD property located 24 km northeast of Meridian. It consisted of approximately 2,124 ha of forest, 33 ha of paved airfields and runways, and 300 ha of mowed and maintained grass cover bordering the runways and airfields for visibility and prevention of flight obstructions. The remainder of the installation was urban area. The forest cover on upland sites was comprised primarily of mature loblolly-shortleaf pine (*Pinus taeda-P. echinata*) stands, though many pine stands possessed relict hardwood trees which contributed to the upper canopies and total basal area. Alluvial floodplain or riparian sites consisted of mature hardwoods. Most of the larger riparian corridors were listed as separate stands in the NASM forest inventory, whereas many thin, unnamed riparian corridors existed throughout larger pine and pine-hardwood stands. Most forests ranged in age from 45 to 65 years (Jones et al. 1999). Intermediate forest management tools used included thinning, prescribed burning, and herbicide control of pest plants such as kudzu (Pueraria lobata). Salvage cutting of single trees and small selection patches was used often to prevent spread of southern pine beetle (Dendroctonus frontalis) outbreaks. When necessary, forests were regenerated through planting or natural regeneration. All pine stands over age 25 were thinned at least once (Jones et al. 1999) and hardwood stands were under custodial management. Slopes varied from rolling hills to steep narrow riparian slopes along creeks. Elevations ranged from 61 m above sea level in creek bottoms to 116 m above sea level on upland sites.

Fixed Point and Ephemeral Pool Selection

We selected 8 discrete areas on NASM which contained 4 habitat types: mature pine >45 years old, mature pine-hardwood >45 years old, mature riparian hardwoods >45 years old, and forested wetlands created by beaver damming activity. We established fixed sampling points using 2 criteria: 1) sample as many points as logistically possible and 2) sample in proportion to available habitat types across the land base. Point count stations (N = 39) were established at 250 m intervals to coincide with cooperating bird surveys (Taylor 2001). We permanently marked each point with a 1.3-m green metal fence post and a uniquely numbered sign on each side of the post (wetlands received signs only). We classified habitat types by the Society of American Foresters (SAF) code assigned to the respective stand in the NASM forest inventory.

Data also were collected at 3 ephemeral pools on NASM. Sampled pools were

separated spatially by \geq 800 m which exceeded the mean distance +1 standard deviation (198.5 m) reported for ambystomatid movement from ephemeral wetlands in 5 states (Semlitsch 1998). Pools varied in size, shape, soil type, surrounding forest characteristics, and hydroperiod (Taylor 2001). Thus, each pool was considered independent.

Fixed Point Sampling

We recorded species richness of salamanders, frogs, and toads in late winter and spring using time-constrained area searches (Crump and Scott 1994) and calling anuran point count surveys. A small pilot study of time-constrained area searches yielded poor results on warm, dry days. Therefore, we conducted all searches following a local rain event to improve search efficiency. Because the breeding season of all potential species in Lauderdale County included spring (Conant and Collins 1991), searches and calling surveys generally occurred from February–May focused around these wet periods.

Time-constrained area searches included visual observations under downed deadwood, rocks, and litter within a 100 m radius for 1 man-hour around each point. We used sweep nets to sample streams or ephemeral pools which occurred within sampling radii. We conducted 2 surveys of each point in late winters and springs of 1998 and 1999 and 1 at each point in spring 2000. Time-constrained area searches were not possible around beaver wetland points due to the presence of water >1 m deep at each point year round.

To determine if searches confined to the radii around fixed points were enough to quantify overall richness, we also conducted searches between and away from fixed locations using the same search techniques. This allowed technicians to systematically choose areas where amphibians were thought to be present. Some of the candidate species whose range includes Lauderdale County have habitat requirements which were not present within the sampling point radii. For example, southern dusky (*Desmognathus auriculatus*), spotted dusky (*D. fuscus*), southern red (*Pseudotriton ruber*), and gulf coast mud salamanders (*P. montanus flavissimus*) use forested seepages and clear water brooks; therefore, we searched for these special habitats away from the fixed points to determine if small, relict populations existed on NASM.

We conducted 5 anuran surveys (2 in 1998, 2 in 1999, and 1 in 2000). The first round of anuran call counts were conducted in spring 1998 by walking to each point during darkness and listening for anurans calling (10 minutes/point) within the sampling radius around each point, similar to the audio strip transect technique described by Zimmerman (1994). Because of the high level of danger in walking between points along a transect in the dark and lack of orienteering skills among volunteer support, we modified the call survey protocol. Thus, we established a series of listening points along roads, whereby calling anurans were recorded close to fixed points (≤ 100 m). Species composition lists were developed for each surveyed area and sites of high use were identified. We mapped habitat types with associated amphibian occurrences for delineation of areas high in biological diversity (Hayek and McDiarmid 1994).

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Ephemeral Pool Sampling

We enclosed basins of 3 ephemeral pools with drift fences made of galvanized metal flashing (Dodd and Scott 1994). Pitfall traps (19-liter plastic buckets) were placed on opposite sides of the flashing at approximately 20-m intervals, level with the ground. Holes were drilled in the bottom of each bucket to equalize pressure as water tables rose. Water was removed from buckets following rain events to prevent drowning of captured individuals. Pitfall traps were open on 21 April 1998 and closed on 27 June 2000. We checked traps daily following rain events or during observed seasonal breeding activity at pools. We checked buckets 3–4 times/week during other periods. We identified captured individuals to species and life stage (adult or juvenile), and generally released them on the opposite side of the fence from which they were captured.

Results

Fixed Points

Using a combination of anuran call counts (2,340 listening minutes) and timeconstrained area searches (10,200 search minutes) at fixed points, we detected 23 species of amphibians among 4 habitat types on NASM (Table 2). Species richness by habitat type using these 2 techniques was 12, 16, 17, and 9 for pine, pine-hardwood, riparian hardwood, and beaver wetlands, respectively (Table 2). Mean species richness by point was 2.71 (SE = 2.52) in pines, 5.78 (SE = 2.69) in pine-hardwoods, 7.83 (SE = 0.75) in riparian hardwoods, and 6.40 (SE = 2.19) in beaver wetlands.

We detected 11 species by call counts only at fixed points, including southern toad (*Bufo terrestris*), Fowler's toad (*B. fowleri*), southern cricket frog (*Acris gryllus g.*), bird-voiced treefrog (*Hyla avivoca*), gray treefrog, green treefrog (*H. cinerea*), spring peeper, pine woods treefrog, squirrel treefrog (*H. squirella*), eastern spade foot (*Scaphiopus holbrookii h.*), and bullfrog (*Rana catesbeiana*, Table 2). By area searches only at fixed points, we detected 8 species: eastern narrow-mouthed toad, spotted salamander, marbled salamander, southern two-lined salamander (*Eurycea bislineata cirrigera*), three-lined salamander (*E. longicauda guttolineata*), four-toed salamander, slimy salamander (*Plethodon mississippi*), and central newt (*Notoph-thalmus viridescens louisianensis;* Table 2). Bronze frog and southern leopard frog were detected by area searches and call counts at fixed points (Table 2).

Southern toad, spring peeper, southern cricket frog, bird-voiced treefrog, gray treefrog, and bronze frog were present in all 4 habitat types, whereas other species were found in \leq 3 stand types. We detected squirrel treefrog and eastern spadefoot in pine stands only, and found eastern narrow-mouthed toad and four-toed salamander only in riparian hardwoods.

Searches outside the fixed point radii yielded no new microhabitats such as seepages or coldwater brooks for southern dusky, spotted dusky, and southern red, and gulf coast mud salamanders. However, we detected lesser siren (*Siren interme-dia*) and three-toed amphiuma (*Amphiuma means*) in area searches not confined to

	Techniques(s)		
Scientific name	Common name	useda	Habitat(s) ^b
Order Anura			
Family Bufonidae	Toads		
Bufo terrestris	Southern toad	CC, DP	P, PH, RH, BW
Bufo woodhousei fowleri	Fowler's toad	CC, DP	PH, RH
Family Hylidae	Cricket frogs		
Acris gryllus g.	Southern cricket frog	CC, DP	P, PH, RH, BW
Hyla avivoca	Bird-voiced treefrog	CC	P, PH, RH, BW
Hyla chrysoscelis	Gray treefrog	CC, DP	P, PH, RH, BW
Hyla cinerea	Green treefrog	CC, DP	PH, RH, BW
Pseudacris crucifer	Northern spring peeper	CC, DP	P, PH, RH, BW
Hyla femoralis	Pine woods treefrog	CC	P, PH
Hyla squirella	Squirrel treefrog	CC	Р
Family Microhylidae	Narrow-mouthed toads		
Gastrophryne carolinensis	Eastern narrow-mouthed toad	AS, DP	RH
Family Pelobatidae	Spadefoot toads		
Scaphioppus holbrooki h.	Eastern spadefoot	CC, DP	Р
Family Ranidae	True frogs		
Rana catesbeiana	Bullfrog	CC, DP	PH, BW
Rana clamitans c.	Bronze frog	CC, AS, DP	P, PH, RH, BW
Rana palustris	Pickerel frog	DP	PH
Rana sphenocephala	Southern leopard frog	CC, AS, DP	PH, RH, BW
Order Caudata			
Family Amphiumidae	Giant salamanders		
Amphiuma tridactylum	Three-toed amphiuma	AS ^c	BW
Family Sirenidae	Giant salamanders		
Siren intermedia	Lesser siren	AS ^c	BW
Family Ambystomatidae	Mole salamanders		
Ambystoma maculatum	Spotted salamander	AS, DP	PH, RH
Ambystoma opacum	Marbled salamander	AS, DP	P, PH, RH
Ambystoma talpodium	Mole salamander	DP	P, PH, RH
Family Plethodontidae	Lungless salamanders		
Eurycea bislineata cirrigera	Southern two-lined salamander	AS	PH, RH
Eurycea longicauda guttolineata	Three-lined salamander	AS	PH RH
Hemidactylium scutatum	Four-toed salamander	AS	RH
Plethodon glutinosus	i our toed suluminder	110	Iui
mississinni	Slimy salamander	AS DP	Р РН RН
Family Salamandridae	Newts		.,,
Notophthalmus			
viridescens louisianensis	Central newt	AS. DP	P. RH
		,	-,

Table 2. Species of amphibians detected by techniques and habitat type on Naval Air Station Meridian from spring 1998 through summer 2000.

a. AS = area search; CC = call count; and DP = drift fence - pitfall.

b. P = pine; PH = pine-hardwood; RH = riparian hardwood, BW = beaver wetland.

c. Detected away from fixed points.

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the 100-m radius around the fixed points. Both specimens were detected near impounded beaver wetlands.

Ephemeral Pools

We monitored pitfall traps from 21 April 1998–27 June 2000 at 3 ephemeral pools on NASM (796 days; 47,760 bucket nights); and captured 2,398 amphibians. Total species richness of amphibians captured at the 3 pools was 17, whereas richness ranged from 11 to 15. However, these estimates include species with climbing adaptation (i.e., hylids and microhylids) and may bias comparisons between pools due to escape from buckets. Mole salamander (*Ambystoma talpoidium*) and pickerel frog (*Rana palustris*) were detected in pitfall traps at breeding pools only, thus adding 2 species to the overall richness of NASM.

Discussion

Results from this study are a contribution to the knowledge of amphibian richness in Lauderdale County, Mississippi, and can be used to make general comparisons between macro-habitat types on NASM. Moreover, our methods and rationale for combining techniques with respect to a wide range of species life history strategies can be used regionally to better understand amphibian diversity. Our findings confirm the Lauderdale County records (M. Williams, Miss. GAP Project, unpubl. data) and predicted ranges (Conant and Collins 1991, Miss. Herpetol. Atlas 2001) of 16 and 22 amphibians, respectively. Additionally, we added 9 new county records to the species list for Lauderdale County, including bird-voiced treefrog, eastern spadefoot, four-toed salamander, Fowler's toad, lesser siren, mole salamander, pickerel frog, pine woods treefrog, and three-toed amphiuma. These data set the framework for a comprehensive, long-term, biologically-based management plan for amphibians on NASM (Semlitsch 2000) and improve current knowledge of regional distributions of many species. While species may be declining regionally or globally, studies like this will provide information that support amphibian conservation with respect to regional changes in landscape patterns.

The combined use of anuran call counts and time-constrained area searches doubled the richness of amphibian species detected. Sampling across multiple habitat types and over multiple seasons also increased number of species detected. Observations of the pine woods treefrog and four-toed salamander are extensions of documented and predicted ranges (Conan and Collins 1991, Petranka 1998, Miss. Herpetol. Atlas 2001) and suggest that management recommendations for amphibians made solely on field guide maps or museum collection data are not sufficient. Well-designed, properly-executed sampling, conducted at the right time of day, season, and year are essential to obtain accurate information on amphibian community composition and structure. While our sampling was adequate to obtain estimates of point and local richness, the design was not intensive enough to obtain indices of relative abundance. These results however, establish baseline information on community richness, from which natural resource managers can obtain species-specific population parameters.

It is often common to compare the relative abundance of richness of species between habitat types. We did so in this study as part of a larger study quantifying the diversity of 3 guilds in mature forests managed for biological diversity: amphibians, birds, and small mammals (Taylor 2001). We recommend caution, however, to those who suggest that one forest type is "better" than another for amphibian conservation without examining information at the ecosystem scale. For example, ephemeral pools are often associated with bottomland hardwood forests in the Southeast, although they also occur within upland pine stands. While habitat loss and fragmentation of bottomland hardwoods have undoubtedly caused local extinction and isolation of many populations of forest-dwelling salamanders (Petranka 1998), the extent of the damage is unknown. Stands that have been converted from hardwood to pine should still be monitored and managed for amphibians.

We observed less species/point in pines than other habitats though amphibian richness in pines was 13. Nevertheless, 1 point contained 10 of the 12 species found in pines. In fact, 3 locally rare species (pine woods treefrog, squirrel treefrog, and 1 eastern spadefoot) were heard calling close to this point during only 1 anuran survey. The reason for the high richness at this particular point was likely because of the underlying soil type (somewhat poorly drained) and the presence of an intermittent drainage. Part of the stand near this point floods occasionally and forms ephemeral pools and generally mesic soil conditions. In fact, this point was ≤ 150 m from one of the enclosed ephemeral pools in this study. During the same time frame (1998–2000), 11 species were captured in the breeding pool, including 7 species which were not detected with area searches and call counts at the nearby fixed point. Conversely, area searches and call counts detected 6 species that were not detected with the drift fence and pitfall traps surrounding the breeding pool. Thus, microsite conditions are crucial to amphibian conservation and survey design should consider edaphic and hydrologic properties along with dominant forest cover.

Species richness within pine-hardwoods was greater than pines partially because of presence of streams-dwelling species, such as southern two-lined salamander and three-lined salamanders, which were found in and around perennial and intermittent streams intersecting some of these patches. Our success in locating these and other species around streams was enhanced by conducting sampling when water was present and utilizing multiple techniques: anuran call counts and area searches. Our area searched included dipnetting, thus larvae identification also was important to quantify species richness.

Riparian hardwoods had more species/point and greatest species richness among habitats sampled. All 7 salamander species detected with area searches were found in riparian hardwood patches, including the rare four-toed salamander. Age of the stands and absence of mechanical distrubance likely supported suitable habitat conditions (Taylor 2001) for forest-dwelling salamander described by Petranka (1998).

Though beaver wetlands were sampled with anuran call counts only, points av-

eraged 6.40 species (SE = 0.29). Bullfrogs prefer larger bodies of water than most other frogs (Conant and Collins 1991), and beaver wetlands were the only habitat where bullfrogs were heard calling. Additionally, bird-voiced treefrogs have an affinity for wooded wetlands (Conant and Collings 1991) and the largest choruses of birdvoiced treefrogs were recorded in beaver wetlands during the surveys. Generally, more species were heard calling closer to the banks than in the interior of the beaver wetlands. Because estimates were made at fixed points within the wetlands, richness of amphibians is likely biased low for the wetland ecosystem. Area searches conducted near the wetland yielded observations of other species, such as marbled salamander and three-toed amphiuma. Additionally, another area search on NASM yielded presence of lesser sirens where a beaver wetland was drained. Thus, future studies of amphibian richness in beaver wetlands should include anuran call count surveys, time-constrained area searches on the land surrounding the standing water, and basket traps for giant salamanders (sirens and amphiumas).

Conservation and Research Implications

Conservation of amphibian diversity must include biologically-based management plans which include coordination among ecologists (population, community, and landscape), natural resource managers, and policymakers (Semlitsch 2000). Quantification of biological diversity is laborious and requires observations at multiple temporal scales. Furthermore, techniques used to record abundance and richness must be species-specific. Several species may use the same area, yet partition their use diurnally and seasonally.

We submit that a quick assessment of habitat composition will not suffice to predict amphibian species richness. Likewise, field guide range maps are to be used as a guide to what might be present, not as a finite account of species richness. Successful amphibian conservation efforts must begin with an understanding of current and past species richness. We recommend that parties interested in amphibian conservation use all available tools at their disposal to generate a list of potential species within an area of interest, then design a species and time specific protocol to confirm or deny the presence of each species. Baseline knowledge of species richness can then be built upon to identify and monitor population parameters over time.

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