Efficacy of Sampling Techniques for Determining Species Richness Estimates of Reptiles and Amphibians

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Original article

Efficacy of sampling techniques for determining species richness estimates of reptiles and amphibians

Stan J. Hutchens & Christopher S. DePerno

The ability to detect reptiles and amphibians is influenced by environmental and behavioural variables and detection probabilities, but studies to determine herpetofauna species richness often employ only a small number of sampling techniques, primarily drift fence arrays, visual encounter surveys, and coverboards (i.e. primary techniques). However, using only two or three sampling techniques can underestimate species richness. To evaluate the efficacy of sampling methodologies in determining the species richness of herpetofauna, we employed 11 different sampling techniques. We hypothesized that adding standardized road searches, polyvinyl chloride (PVC) piping grids, line transects, auditory surveys (i.e. secondary techniques), opportunistic encounters, aquatic funnel traps, crayfish traps and basking traps (i.e. tertiary techniques) would better portray species richness. Observed species richness (S_{obs}, species physically detected or observed), Chao2 estimates of species richness (S), unique species captured (i.e. species detected by only one technique), cost, and cost-per-species-captured for individual techniques and categories (i.e. primary, secondary and tertiary) were used to determine efficacy. Primary capture methodologies detected 13 species ($S=14$). Secondary and tertiary sampling techniques captured 18 and 24 species, respectively ($S=29$ and 25). All sampling methodologies combined captured 33 species for a Chao2 estimate of 34. More unique species were captured by tertiary techniques than by primary or secondary methodologies. Costs for primary techniques were much higher than for secondary and tertiary methodologies. To better determine species richness, we recommend that future research incorporate multiple sampling methodologies in addition to more common techniques.

Key words: amphibians, capture techniques, efficacy, reptiles, species richness

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Inventory, or monitoring, of biodiversity is becoming increasingly important and more widely used for scientific and management objectives (Yoccoz et al. 2001). However, reptiles and amphibians can be difficult to inventory due to environmental and behavioural variables and differing capture probabilities between sampling techniques (Vogt & Hine 1982, MacKenzie et al. 2002, Williams & Berkson 2004). Environmental variables such as temperature, humidity, wind and season can influence activity and detectability (Vogt & Hine 1982, Williams & Berkson 2004). Similarly, sedentary and fossorial behaviours, and cryptic capabilities can limit the detectability of certain species (Fitch 1992, Flint & Harris 2005). Sampling techniques can affect the probability of detecting certain species by biasing for or against size, behaviour or taxon (Gibbons & Semlitsch 1981, Enge 1997). However, most herpetofaunal species richness studies use only two or three sampling methodologies, which limits the reliability of estimates due to low, or zero, detection probabilities (MacKenzie et al. 2002, Bailey et al. 2004).

The two or three sampling methodologies most commonly employed include drift fence arrays (with pitfall and/or funnel traps), visual encounter surveys (VES), and coverboards. We designated these techniques as 'primary' due to their prevalence in reptile and amphibian research (Bury & Corn 1988, 1990, Mitchell et al. 1993, Fair & Henke 1997, Kjoss & Litvaitis 2001). Additionally, we incorporated 'secondary' and 'tertiary' sampling techniques in this study.

Generally, secondary techniques, such as standardized road searches, polyvinyl chloride (PVC) piping grids, line transects and auditory surveys were not reported in the literature as frequently as primary techniques, but might have been used in conjunction with primary methodologies (Jones 1988, Lacki et al. 1994, Moulton et al. 1996, Sullivan 2000, Turner et al. 2003). Techniques in primary and secondary categories employed a standardized, quadrat sampling design (Williams et al. 2002). Tertiary techniques (i.e. opportunistic encounters, and aquatic funnel, crayfish and basking traps) were infrequently mentioned in the literature (Fair & Henke 1997, Hanlin et al. 2000, Metts et al. 2001, Johnson & Barichivich 2004) and used a non-standardized, empirical species abundance distribution design (Williams et al. 2002).

We evaluated observed species richness (S_{obs}, those species physically observed or captured), species richness estimates (S), unique species captured, cost, and cost-per-species-captured among primary, secondary and tertiary techniques to determine efficacy. Unique species were defined as those species detected or observed by only one sampling technique. Specific objectives of our study were to determine: 1) if primary techniques alone were effective at obtaining accurate species richness, 2) whether secondary and tertiary techniques increased species richness enough to justify their time and cost, and 3) the trade-off of cost versus success among techniques for use in short- or long-term studies.

Material and methods

Study area

We conducted our study at Bull Neck Swamp (BNS) in Washington County, North Carolina (35.96667° N, 076.41667° W; Fig. 1). The property was a 2,428 ha pocosin wetland owned by North Carolina State University's Department of Forestry and Environmental Resources and managed by the Fisheries and Wildlife Sciences Program. Five habitats were recognized at BNS, including four habitat preserves (non-riverine swamp, Atlantic white-cedar *Chamaecyparis thyoides*, pond pine *Pinus serotina*, and shoreline/islands) and a 'manageable' area. The property was bordered by the Roanoke River delta and Albemarle Sound on three sides. Bottomland forest and hardwood swamps with patchy cultivated areas comprised the southern border of the property.

Sampling techniques

During two field seasons (May-August in 2005 and 2006), 11 sampling methodologies were employed to determine species richness. We categorized techniques based on their prevalence in published research and sampling design. Primary capture techniques consisted of drift fence arrays with pitfall and/or funnel traps, VES and coverboard arrays. Standardized road searches, PVC piping grids, line transects and auditory surveys were designated as secondary methodologies. Tertiary techniques consisted of opportunistic road cruises, aquatic funnel traps, crayfish traps and basking traps. Further distinction of tertiary techniques was made based on their non-standardized nature and disparate sampling design (Williams et al. 2002). Within the five habitats, we evenly distributed all sampling techniques and ran all techniques simultaneously except for drift fence arrays and visual encounter surveys, which employed a robust sampling design.

Primary sampling techniques

Drift fences with pitfall traps and/or funnel traps of several designs are widely employed in reptile and amphibian research (Gibbons & Semlitsch 1981, Mitchell et al. 1993, Hanlin et al. 2000, Metts et al. 2001, Enge 2001). Ten drift fence arrays were distributed in a systematically random design, at least 30 m from other capture techniques. Drift fences were arranged in 'Y'-formations with six funnel traps and a pitfall trap in the center where possible (e.g. pitfall traps could not be placed in areas inundated with water). Arrays were checked every morning for two 3-week periods during May - August in 2005 and 2006.

VES ($N = 25$) were an active capture technique where 10×10 m standardized plots were thoroughly searched for 30 minutes (Jung et al. 2000, Flint & Harris 2005). Plots were established in a systematically random distribution. All natural cover and vegetation was searched by two observers following perpendicular paths through the plots. Captured amphibians were placed in individual plastic bags with substrate and water for moisture, and captured reptiles were placed in individual cotton bags until

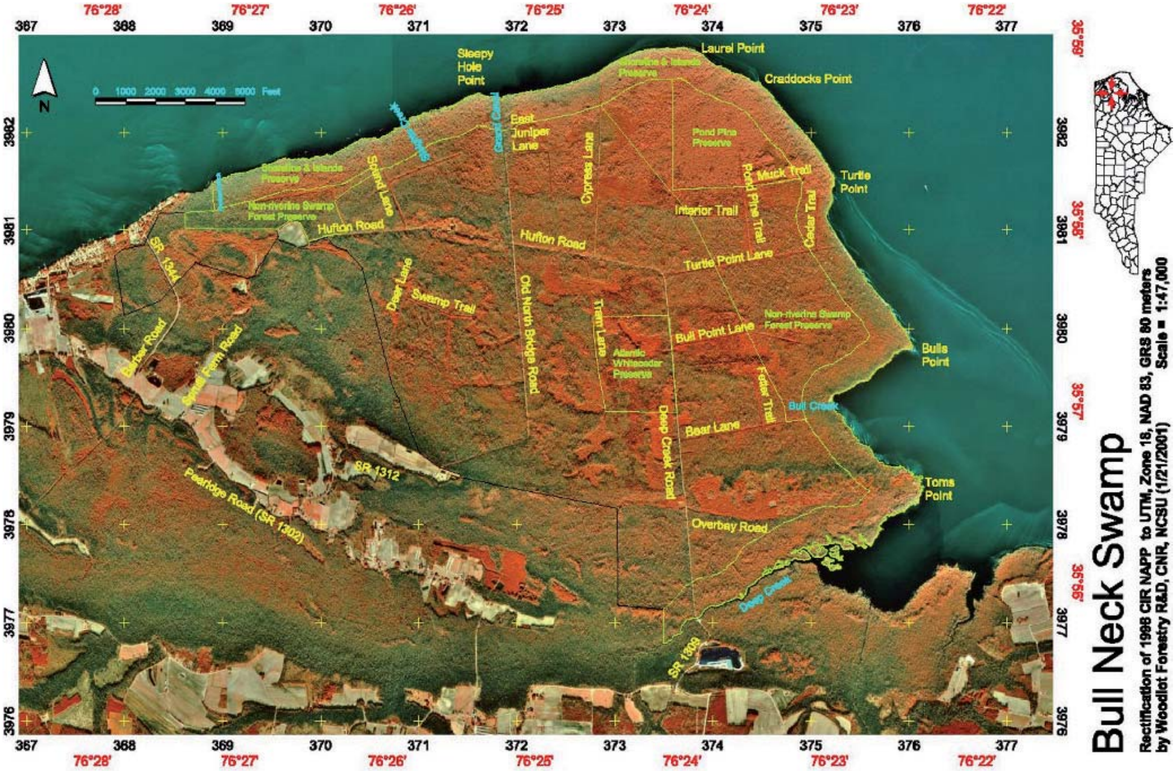

Figure 1. Colour infrared photography (CIR) of Bull Neck Swamp, Washington County, North Carolina. Outlined are four habitat preserves (non-riverine swamp, Atlantic white-cedar *Chamaecyparis thyoides*, pond pine *Pinus serotina*, and shoreline/islands) designated by the Natural Heritage Trust Fund.

search time was completed. Surveys were conducted in the morning between 09:00 and 11:00 during June 2005 and July 2006. All plots were visited twice.

Coverboards, or artificial refugia, are passive sampling techniques that use several materials (e.g. plywood sheets and tin), and different designs to simulate natural cover (Mitchell et al. 1993, Fellers & Drost 1994, Reading 1997). During our study, coverboard arrays (N = 5) consisted of nine 120 × 120 cm plywood sheets placed flat on the ground and arranged in an array formation. Arrays were established in dry areas and checked once a week from early-June to mid-August 2006.

Secondary sampling techniques

We performed standardized road searches on the four roads at BNS with the clearest ground visibility. Each route was 2 km long and was surveyed using an all-terrain vehicle (ATV) traveling at 17 to 24 kilometer/hour. We conducted six searches per route each field season: three 1-hour before and three 1-hour after sunset during May - June 2005 and June - August 2006.

We established PVC piping grids (N = 6) to sample treefrogs. Grids were randomly distributed, but distanced at least 30 m from other capture techniques. Grids consisted of 12 PVC pipes 3.75 cm in diameter cut into lengths of 1 m. Pipes were driven 5 to 7 cm into the ground in a grid pattern consisting of rows of 4 pipes and columns of 3 pipes and each pipe was spaced 2 m apart. Piping grids were checked weekly from June to August 2006.

We established line transects (N = 4) of 0.8 km in length on four roads or sections of roads not surveyed by standardized road searches. Two observers walked transects, checking opposite sides of the road. Individuals that could be identified to species were counted and their distance from the center of the road determined. Observers conducted two searches on each transect, one in the morning and one in the afternoon, from July to August 2006.

We randomly distributed auditory survey sites (N = 5) on roads without regard to distance from other techniques. We conducted two surveys for 20 minutes at each site with number of individuals, species and estimated distance recorded.

Tertiary sampling techniques

We distributed six aquatic funnel traps, constructed of aluminum window screening or hardware cloth, in canals and ditches throughout BNS. We checked traps each morning from June to August 2005 and May to August 2006. Similarly, two pyramid crayfish traps (Lee Fisher International, Inc., Tampa Bay, Florida) were placed in canals and ditches around BNS. We checked pyramid traps daily from May to August 2006.

One basking trap (Memphis Net and Twine, Memphis, Tennessee) was deployed at several sites to capture turtles. The basking trap could only be placed in wide canals with easy access to banks. We checked the basking trap daily from May to August 2006. Opportunistic encounters consisted of species captured at any time, while walking, checking traps, or driving through our study area.

Marking

Captured individuals from all techniques were identified to species, measured, weighed and marked. We marked snakes ($\geq$300 mm snout-vent length (SVL)), turtles ($\geq$120 mm carapace length), lizards ($\geq$150 mm SVL) and large amphibians (i.e. two-toed amphiumas *Amphiuma means* and American bullfrogs *Rana catesbeiana*) with passive integrated transponder (PIT) tags. We employed visible implant fluorescent elastomer (VIE) to mark all other amphibians and double-mark snakes (Hutchens et al. 2008).

Statistical analyses

To evaluate capture efficacy among capture techniques and categories (i.e. primary, secondary and tertiary), we compared observed species richness (S_{obs}) and species richness estimates (S) for data collected during May - August 2005 and 2006. Also, we incorporated unique species captured, cost and cost-per-species-captured. We calculated richness estimates for primary and secondary techniques from X-matrices of abundance data using the classic Chao2 formula in EstimateS 8.0 (Colwell 2005). Estimates for the tertiary techniques and total richness were obtained using X-matrices of incidence data (Colwell et al. 2004). Sample-based rarefaction curves of computed species observations (i.e. Mao Tau) were employed to determine efficacy by comparing asymptotic richness across categories. Sampling units for rarefaction curves were defined as individual sampling sites for each capture technique. We employed individual-based curves, derived from Coleman estimates in EstimateS 8.0, whenever sample-based curves failed to reach an asymptote and rescaling of curves was required (Gotelli & Colwell 2001).

We evaluated unique species (i.e. species captured by only one technique) among categories. Set-up, labour and operations costs for all techniques were compared to determine cost-per-species-captured. Costs for fuel consumption were calculated based on an estimated 17 km/liter for an ATV on dirt roads at US$ 0.74/liter. Additionally, labour costs were derived as the cost of paying US$ 8.00 an hour for one field technician.

Results

During May - August in 2005 and 2006, 1,576 individuals were captured representing 33 species (Table 1). Primary techniques detected 13 species (S_{obs}) for an estimated species richness (S) of 14. In contrast, secondary techniques detected 18 species (S = 29) and tertiary techniques detected 24 species (S = 25; Table 2). The number of individuals captured by primary techniques were four times those of secondary and tertiary methodologies. All 11 techniques detected 33 total species for an estimated species richness of 34 (see Table 2).

Sample-based rarefaction curves of the computed number of species illustrated the accumulation of species for all categories (Fig. 2). Primary techniques captured several individuals of only a few species, requiring 84% of sampling units to reach an asymptote. Conversely, secondary and tertiary methodologies captured more species with fewer individuals but failed to reach a clear asymptote (see Fig. 2). Rescaled individual-based curves of secondary and tertiary categories allowed easier comparison and demonstrated a sharper slope of accumulation for tertiary methodologies despite both categories failing to reach an asymptote (Fig. 3). Consecutively adding categories augmented primary and secondary sampling techniques, which resulted in large differences in rarefaction curves and species richness (Fig. 4).

Unique species were captured by techniques in all categories (see Tables 2 and 3). Interestingly, seven of the 10 unique species captured by secondary and tertiary techniques were detected within seven sampling occasions (see Table 3). Moreover, secondary and tertiary capture techniques efficiently sampled many of the same species captured by primary techniques (Table 4).

Table 1. Species and numbers of individuals detected by all capture techniques at Bull Neck Swamp, Washington County, North Carolina from May to August 2005 and 2006.

Common name	Scientific Name	Primary	Secondary	Tertiary
Atlantic Coast Slimy Salamander	*Plethodon chlorobryonis*	5	0	0
Two-toed Amphiuma	*Amphiuma means*	0	0	8
Green Treefrog	*Hyla cinerea*	0	13	0
Gray Treefrog	*Hyla versicolor*	0	4	0
Pine Woods Treefrog	*Hyla femoralis*	0	6	0
Southern Cricket Frog	*Acris gryllus*	59	36	1
Green Frog	*Rana clamitans*	882	81	22
Southern Leopard Frog	*Rana sphenocephala*	43	37	2
American Bullfrog	*Rana catesbeiana*	3	8	2
Southern Toad	*Bufo terrestris*	50	54	0
Striped Mud Turtle	*Kinosternon baurii*	0	0	5
Eastern Mud Trutle	*Kinosternon subrubum*	0	0	2
Stinkpot Turtle	*Sternotherus odoratus*	0	0	6
Spotted Turtle	*Clemmys guttata*	1	0	5
Yellow-bellied slider	*Trachemys scripta*	0	0	44
Eastern Box Turtle	*Terrapene carolina*	0	1	4
Painted Turtle	*Chrysemys picta*	0	0	8
River Cooter	*Pseudemys concinna*	0	0	1
Coastal Plain Cooter	*Pseudemys c. floridana*	0	0	8
Snapping Turtle	*Chelydra serpentina*	0	1	12
Little Brown Skink	*Scincella lateralis*	1	6	0
Southeastern Five-lined Skink	*Eumeces inexpectatus*	3	2	0
Green Anole	*Anolis carolinensis*	0	1	0
Common Kingsnake	*Lampropeltis getula*	0	0	32
Eastern Ratsnake	*Elaphe obsoleta*	0	0	19
Southern Watersnake	*Nerodia fasciata*	6	4	24
Plain-bellied Watersnake	*Nerodia erythrogaster*	3	1	9
Rough Greensnake	*Opheodrys aestivus*	0	0	4
Eastern Racer	*Coluber constrictor*	3	1	9
Eastern Ribbonsnake	*Thamnophis sauritus*	0	1	8
DeKay's Brownsnake	*Storeria dekayi*	1	0	0
Rainbow Snake	*Farancia erytrogramma*	0	0	1
Cottonmouth	*Agkistrodon piscivorus*	0	3	20

Table 2. Total number of individuals and unique species captured, observed species richness, and species richness estimates for all techniques at Bull Neck Swamp, Washington County, North Carolina from May to August 2005 and 2006.

Category	Capture technique	Total individuals captured	S_{obs}	S	Unique species
Primary	Drift fence arrays	953	10	11	1
	Pitfall traps	489	5		0
	Funnel traps	464	9		1
	Visual encounter surveys	91	7	7	1
	Coverboard arrays	16	4	4	0
	Category totals	1060		14 ± 4	2
Secondary	Road searches	31	10	22	0
	PVC piping grids	5	1	1	0
	Line transects	164	10	10	1
	Auditory surveys	60	7	7	2
	Category totals	260		29 ± 11	3
Tertiary	Opportunistic encounters	189	17	17	4
	Aquatic funnel traps	43	17	26	3
	Crayfish traps	24	7	7	0
	Basking trap	0	0	0	0
	Category totals	256		25 ± 5	7
Totals		1576	33	34 ± 5	12

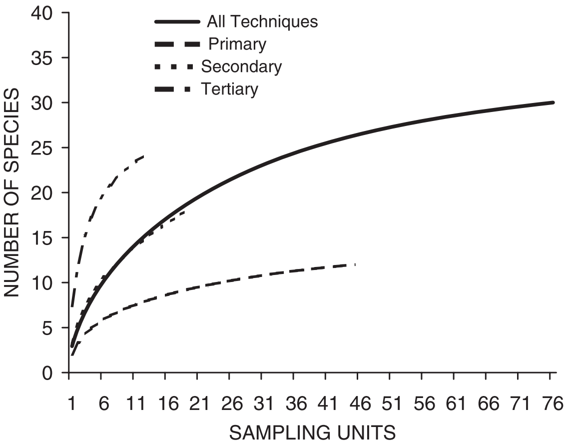

Figure 2. Sample-based rarefaction curve of computed species observations for each category and all capture methodologies for data collected from May to August 2005 and 2006. Sampling units were defined as individual sampling sites for all capture techniques.

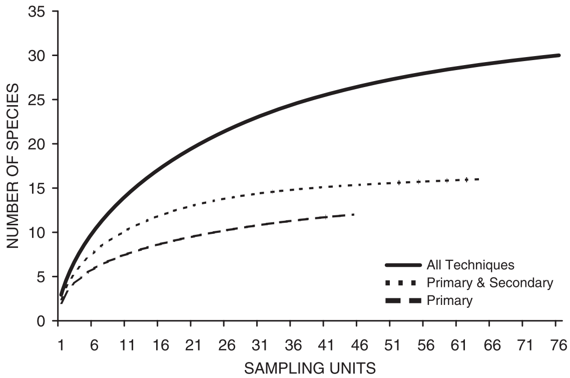

Figure 4. Additive sample-based rarefaction curves of computed number of species. The addition of secondary and tertiary categories to primary methodologies added more accumulated species. The addition of secondary and tertiary categories to the estimated richness of primary methodologies added 44% and 15% more species to the total richness estimate of reptile and amphibian species, respectively.

Materials, set-up and labour costs were high for our study (Table 5). Primary methodologies had the highest costs followed by tertiary and secondary techniques. Primary capture techniques accounted for 67% of total costs. Set-up costs and labour for operation drove costs up for PVC piping grids and road searches. Costs for tertiary techniques were mainly due to fuel consumption. However, the number of species detected by secondary and tertiary techniques lowered costs-per-species-captured for these categories (see Table 5).

Discussion

The evasive nature of reptiles and amphibians makes the taxa difficult to detect and requires using several capture techniques to sample all species present in a community (Williams & Berkson 2004, Flint & Harris 2005). Moreover, capture techniques vary in success of species detection (Yoccoz et al. 2001, Bailey et al. 2004). In our study, if only primary capture techniques (i.e. drift fence arrays with pitfall and funnel traps, VES and coverboard arrays) were used, species richness would have been underestimated by 59% (see Table 2). The addition of secondary capture techniques more than doubled the estimated species richness and incorporating tertiary capture techniques further increased species richness (see Table 2).

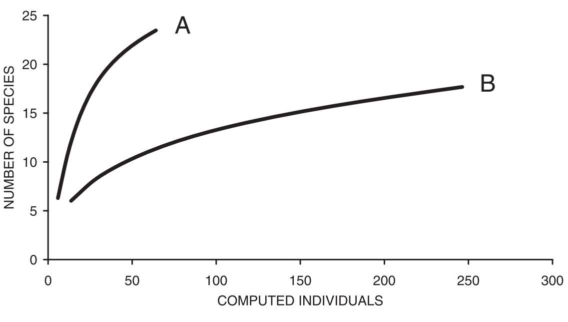

Figure 3. Individual-based rarefaction curves of species richness for tertiary techniques (A), and secondary techniques (B). Because the curves for secondary and tertiary capture methodologies did not reach clear asymptotes, curves were rescaled to the computed number of individuals captured for comparison (Gotelli & Colwell 2001).

Similar to other studies, primary techniques captured some, but not all species present (Gibbons & Semlitsch 1981, Bury & Corn 1987, 1988, Mitchell et al. 1993, Kjoss & Litvaitis 2001). Unfortunately, most studies rely on only primary methodologies (Gibbons & Semlitsch 1981, Vogt & Hine 1982, Mitchell et al. 1993, Flint & Harris 2005). During our study, primary capture techniques had low initial capture success and required 33 sampling units to detect only 13 species (see Fig. 2). Low numbers of species captured indicated that primary sampling techniques did not provide an accurate estimate of species richness. However, primary methodologies successfully captured the most common species (see Table 4), which implied their usefulness for scientific objectives or studies deriving detection probabilities using mark-recapture or removal designs (Yoccoz et al. 2001, Pollock et al. 2002). Also, two unique species were captured with primary techniques, indicating their value to species richness studies. How-

Table 3. Duration until capture of unique species for each category and technique at Bull Neck Swamp, Washington County, North Carolina from May to August 2005 and 2006.

Common Name	Scientific Name	Group	Capture Technique	Sampling sessions until capture
Atlantic Coast Slimy Salamander	*Plethodon chlorobryonis*	Primary	VES (visual encounter survey)	15
Grey Treefrog	*Hyla versicolor*	Secondary	Auditory survey	1
Pine Woods Treefrog	*Hyla femoralis*	Secondary	Auditory survey	1
Eastern Mud Turtle	*Kinosternon subrubrum*	Tertiary	Opportunistic encounter	50
Eastern Musk Turtle	*Stenothorus odoratus*	Tertiary	Aquatic funnel trap	3
River Cooter	*Pseudemys concinna*	Tertiary	Aquatic funnel trap	24
Green Anole	*Anolis carolinensis*	Secondary	Line transect	6
DeKay's Brownsnake	*Storeria dekayi*	Primary	Drift fence array - funnel trap	42
Eastern Kingsnake	*Lampropeltis getula*	Tertiary	Opportunistic encounter	4
Rat Snake	*Elaphe obsoleta*	Tertiary	Opportunistic encounter	5
Rough Greensnake	*Opheodrys aestivus*	Tertiary	Opportunistic encounter	7
Rainbow Snake	*Farancia erytrogramma*	Tertiary	Aquatic funnel trap	24

Table 4. Duration until capture of species detected by more than one capture technique at Bull Neck Swamp, Washington County, North Carolina from May to August 2005 and 2006. Zeros indicate no captures were recorded for that species in that technique category.

		Days until capture		
Common name	Scientific name	Primary	Secondary	Tertiary
Southern Cricket Frog	*Acris gryllus*	1	1	44
Green Frog	*Rana clamitans*	1	1	6
Southern Leopard Frog	*Rana sphenocephala*	1	1	41
American Bullfrog	*Rana catesbaeiana*	15	11	136
Southern Toad	*Bufo terrestris*	1	1	0
Ground Skink	*Scincella lateralis*	6	2	0
Southeastern Five-lined Skink	*Eumeces inexpectatus*	6	7	0
Spotted Turtle	*Clemmys guttata*	74	0	98
Eastern Box Turtle	*Terrapene carolina*	0	11	52
Common Snapping Turtle	*Chelydra serpentine*	0	3	1
Banded Watersnake	*Nerodia fasciata*	35	13	7
Red-bellied Watersnake	*Nerodia erythrogastor*	39	15	7
Black Racer	*Coluber constrictor*	42	2	27
Eastern Ribbonsnake	*Thamnophis sauritus*	0	11	28
Cottonmouth	*Agkistrodon piscivorous*	0	7	28

Table 5. Material and labour costs (given in US$) for set-up and operation of all capture techniques at Bull Neck Swamp, Washington County, North Carolina from May to August 2005 and 2006.

Group	Capture technique	Cost (set-up)	Labour-hours (set-up)	Cost (operation)	Labour-hours (operation)	Monthly costs/labour	Total cost	S_{obs}	Unique species	Total cost/S_{obs}
Primary	Pitfall traps	226	304	33	1137	671	1700	5	0	340
	Funnel traps	149	1872	21	711	594	2752	9	1	306
	VES (Visual encounter survey)	0	240	42	480	141	762	7	1	109
	Coverboard arrays	298	80	7	400	501	785	4	0	196
	Group total	672	2496	103	2728	1906	5999		2	
Secondary	Road searches	0	0	85	176	195	262	10	0	26
	PVC piping grids	111	48	7	240	330	406	1	0	406
	Line transects	0	0	2	80	164	82	10	1	8
	Auditory surveys	0	0	2	53	31	55	7	2	8
	Group total	111	48	97	549	720	806		3	
Tertiary	Opportunistic encounters	0	0	317	201	63	518	17	4	30
	Aquatic funnel traps	23	288	159	201	230	670	17	3	39
	Crayfish traps	90	0	159	201	153	449	7	0	64
	Basking trap	100	0	159	201	163	459	0	0	0
	Group total	212	288	793	803	608	2097		7	
Totals						3235	8901		12	

ever, the extra time and costs required to sample these species implies the effectiveness of primary methodologies would be limited if sampling periods were short or with limited budgets.

Secondary techniques demonstrated low initial captures for observed species (see Fig. 2), but surpassed primary techniques after 53% of sampling units. Although the richness curve did not reach a clear asymptote, 18 species were accumulated in less than half the sampling units needed by primary techniques, which justified the use of secondary methodologies. Gotelli & Colwell (2001) determined that curves having not yet reached an asymptote could be compared after appropriate rescaling to individual-based curves, which demonstrated a sharp slope for species accumulation by this category. Secondary methodologies augmented species accumulation to 16 when combined with primary capture techniques (see Fig. 4). Moreover, secondary techniques were remarkably versatile and resulted in the capture of three unique species, and efficiently detected all but one of the most common species detected by primary techniques (see Tables 3 and 4). Greater success was likely due to the active nature of secondary capture techniques, such as standardized road searches and line transects, compared to the predominantly passive primary techniques.

Tertiary techniques sampled more species than other techniques (see Fig. 2). After rescaling rarefaction curves to the individual, species richness for tertiary methodologies (S = 25) was similar to secondary techniques (S = 29; see Fig. 3). However, species accumulation was faster with a much sharper slope for tertiary techniques, which was due to tertiary methodologies capturing many more species per individual (see Table 2 and Fig. 3). Adding tertiary capture techniques to primary and secondary rarefaction curves demonstrated the importance of employing multiple methodologies (see Fig. 4). It is likely that active trapping was important for the effectiveness of opportunistic encounters. For instance, Fair & Henke (1997) determined that opportunistic encounters provided more captures-per-unit-effort than standardized methodologies. Similarly, in our study, opportunistic encounters captured more species than primary techniques.

Unique species, defined as species detected by only one capture technique, were detected by methodologies from each category with varying success (see Table 3) and were important in accurately indicating 'presence' when deriving species richness estimates (MacKenzie et al. 2002, Colwell 2005). Primary capture techniques sampled fewer unique species than secondary and tertiary methodologies, which reiterated the aptitude of primary techniques at capturing the most common species present in an area. Secondary and tertiary capture techniques detected the most unique species while reliably catching common species, which supported the implementation of multiple sampling methodologies. Also, it should be noted that all unique species detected by secondary and tertiary capture techniques, except for hylid frogs, were capable of being detected by primary methodologies. Importantly, detection probabilities for some species can be low to zero (MacKenzie et al. 2002), which reinforces the need for several sampling techniques, and marking or distance sampling, to reduce detection errors (Yoccoz et al. 2001).

Total costs were much higher for primary techniques compared to other technique categories and comprised 67% of total sampling costs (see Table 5). Implementing primary capture techniques required high costs for materials, labour and time. For example, drift fence arrays were expensive to construct, maintain and operate (Gibbons & Semlitsch 1981, Bury & Corn 1987). Manufacturing and materials for funnel traps (six per array) contributed a considerable portion of the total cost for establishing arrays, while fuel consumption contributed to high costs for VES, and materials to high costs of coverboard arrays. Conversely, secondary and tertiary capture techniques had low costs-per-species-captured (see Table 5) due to fewer materials and less labour needed for maintenance and operation. The low cost and great success of secondary and tertiary techniques suggest their application for short- or long-term studies.

When combined, employing all 11 capture techniques provided a comprehensive estimate of species richness (S = 34). Using all 11 sampling techniques reduced the likelihood of bias in our estimate, a result useful to scientific and management objectives. We acknowledge that our sampling techniques did not detect all species and that non-detection does not discount a species' presence (MacKenzie et al. 2002, Pollock et al. 2002). However, the upper limit of our estimate's analytical standard deviation (S = 34 + 5) indicated the precision of our estimate, and we believe the estimated species richness closely resembled the reptile and amphibian community at BNS. Further, the high precision of our estimate implied the efficacy of using multiple technique sampling in scientific or management objectives.

After evaluating observed species richness, estimated species richness, unique species captured, cost and cost-per-species-captured data for primary (i.e. drift fence arrays with pitfall and funnel traps, VES and coverboard arrays), secondary (i.e. road searches, PVC piping grids, line transects and auditory surveys), and tertiary (i.e. opportunistic encounters, aquatic funnel traps, crayfish traps and basking traps) sampling techniques, we recommend the use of as many techniques as possible to obtain a better representation of the studied community. Our results determined that 1) primary capture techniques alone do not capture enough species for an accurate estimate of species richness, that 2) secondary and tertiary techniques added enough species to justify their time and cost, and that 3) secondary and tertiary techniques would be useful for short- or long-term studies. Consideration of an inventory study's objective (i.e. scientific or management) is important when choosing sampling techniques. We recommend inventories of biodiversity arrange sampling techniques in a hierarchical design to minimize undetected species, and we believe that our success with multiple technique sampling can be generalized to all habitat types and taxa. Moreover, standardization of effort and observer effects, and inclusion of detectability would reduce heterogeneity across techniques (Yoccoz et al. 2001). Future research should implement several sampling techniques for a variety of habitat types and taxa to compare efficacy, thus enhancing our understanding of species richness and diversity.

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