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## Sampling Assemblages of Turtles in Central Illinois: A Case Study of Capture Efficiency and Species Coverage

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#### ABSTRACT

Low and variable rates of capture are chronic problems in chelonian studies. We conducted a pilot study to evaluate protocols for future inventories of turtles in Illinois by comparing capture efficiency and species coverage for 2 devices (hoop net and cage trap), baits (fresh and day-old fish), habitats (lentic and lotic) and time periods . We accrued 402 captures of 378 individuals representing 7 species. At Sanganois State Fish and Wildlife Area (Sanganois), hoop nets produced more captures of more species (n = 231; 6 species) than cage traps (n = 119; 4 species). Statistical tests were equivocal for a reach of the Sangamon River, where both devices had 26 captures but hoop nets detected more species (n = 6) than cage traps (n = 3). At Sanganois, catch per unit effort varied with sampling session (time) and freshness of baits; one measure of species coverage varied with session. Results helped us make informed decisions about protocols for future inventories.

#### INTRODUCTION

Many ecological studies rely on captures of animals to describe demographic traits of a population or assemblage. Valid results hinge on capturing adequate and representative samples (McDonald 2004). Doing so can be especially challenging during studies of turtles (Cagle 1942, Plummer 1979). One reason is that processes affecting dynamics of a population or assemblage often operate at geographic scales larger than those which can be sampled effectively (Burke et al. 1995). Heterogeneous rates of capture or detection pose another common problem (Boulinier et al. 1998, Koper and Brooks 1998, Link 2003, Royle 2006). For example, inferences about sex ratios are misleading if one group is captured preferentially (Ream and Ream 1966). Such findings make it difficult to assess broad paradigms or risks with confidence (Gibbons 1970, Dodd 1997, Holmes 2001).

Efficiency and bias are difficult to evaluate quantitatively. The most informative approach requires direct comparisons of metrics derived from sampling to those of

known populations (Bayless 1975). Circumstances that allow such comparisons are rare (Burgin et al. 1999). Therefore, most evaluations are based on relative comparisons of rates of capture for 2 or more methods of collection, sites, time periods or other potential sources of variability (e.g., Ream and Ream 1966, Frazer et al. 1990, Jensen 1998, Thomas et al. 1999, McKenna 2001, Browne and Hecnar 2005, Gamble 2006, Wallace et al. 2007, Thomas et al. 2008, Nall and Thomas 2009, Sterrett et al. 2010). These comparisons do not speak to representativeness of a sample, but they allow researchers to test hypotheses about equal probabilities of capture that are useful for evaluating efficiency and, depending on the design of a study, finding violations of assumptions to infer the presence of bias (e.g., Lindeman 1990, Olivier et al. 2010).

Pilot studies are a good way to evaluate protocols so shortcomings can be identified and addressed proactively in the design of full-fledged investigations (Green 1979, Frazer et al. 1990, Mazerolle et al. 2007). We used this approach to improve protocols for inventories of turtles in Illinois (e.g., Major et al. 2009). We did so by evaluating capture efficiency and species coverage for 2 devices (hoop net and cage trap), baits (fresh and dayold fish), habitats (lentic and lotic) and time periods.

#### STUDY AREA

The study was conducted at 2 sites in central Illinois. Both occur in the Lower Sangamon River Valley, which drains approximately 11,849 km<sup>2</sup> (Illinois Department of Natural Resources 2003). Sanganois State Fish and Wildlife Area (Sanganois) encompasses 3,835 ha located at the confluence of the Illinois and Sangamon rivers in Cass and Mason counties. Major habitats include forested wetland (2,159 ha), lake (665 ha) and scrub-shrub (354 ha; Yetter et al. 1999). We chose Barkhausen Waterfowl Refuge as a focal area within Sanganois, and, more specifically, that part of the refuge that lies on the tail (west) end of Wilcox Lake (first station 40.06638°N, 090.26012°W; WGS 84). We measured water temperatures at the surface with a digital thermometer; temperatures varied from 25.9-29.0°C. River stages on the Illinois River (as reported by the State Journal Register for Beardstown, IL) were greater during the first session (3.149-3.286 m) than second (2.963-2.987 m). Substrates varied from fine silt to sand. Past sampling (in an area that included but was not limited to our study site; R. D. Bluett, unpublished data) indicated that turtles were abundant with a relatively diverse assemblage composed of Red-eared Slider (Trachemys scripta; later referred to as slider for brevity), Spiny Softshell (Apalone spinifera), Painted (Chrysemys picta), Snapping (Chelydra serpentina) and Eastern Musk (Sternotherus odoratus) turtles. A single Ouachita Map Turtle (Graptemys ouachitensis) was captured in the study area on 1 occasion during past sampling.

Our other study area was a reach of the Sangamon River located southeast of Petersburg in Menard County. Most of the area we sampled (first station 39.98006°N, 089.83537°W; WGS 84) was bordered by Lincoln's New Salem State Historic Site (New Salem). The river is incised deeply (e.g., 2-4 m), with steep banks in most places. Substrates include clay, fine silt, sand, and sand mixed with gravel. As at Sanganois, basking sites, mainly downed trees and logjams, were common in the study area. River stages on the Sangamon River (at Oakford, IL) were similar during both sessions (1.003-1.143 m). Water temperatures varied from 24.7-27.7°C. Past sampling (in an area that included but was not limited to our study site; R. D. Bluett, unpublished data) indicated that turtles were less abundant at New Salem than Sanganois and included a slightly different assemblage composed of *T. scripta*, *A. spinifera*, *C. picta*, *C. serpentina*, *G. ouachitensis* and Smooth Softshell (*Apalone mutica*).

#### METHODS

We compared capture efficiency and species sampling by a wire-mesh (2.54 cm x 2.54 cm, 14-gauge) cage trap (Pied Piper Animal Traps, 445 Garner-Adell Rd., Weatherford, Texas, USA) to that of a hoop net. The cage trap measured 43.18 cm x 43.18 cm x 91.44 cm, had a single wire throat at the front, and a holding compartment at the back accessed by turtles through 2 one-way doors. Hoop nets were made locally by a commercial fisherman. They had 3 hoops (60.96 cm in diameter), 3.81-cm-mesh, and a single flat (sometimes referred to as "Arkansas style") throat.

We conducted 2 trapping sessions at each site. A session consisted of 4 consecutive trapnights, each of which was approximately 24 hours in duration. Sessions at Sanganois started on 25 June and 16 July 2007; those at New Salem began on 30 July and 20 August 2007. We used a cross-over design to reduce potential sources of bias. The first station (i.e., trap location) at each site was located a random distance (range 0-99 m) from a predetermined starting point. At the beginning of each session, we flipped a coin to determine which type of device to place at the first station. Thereafter, we alternated device types at 130-m intervals along the shoreline, deploying 10 of each type at Sanganois and 6 of each at New Salem.

Distances were determined with a hand-held Global Positioning System (Garmin International, Inc., Olathe, Kansas, USA). Execution was straightforward at Sanganois, where water depths along the shoreline were somewhat uniform, allowing us to place either device with at least two thirds of the throat underwater and the back of the net or cage at least 7.6 cm above the waterline to allow turtles to breathe. Depths were more variable at New Salem, so we chose 1 bank or the other depending on suitability of conditions at locations 130 meters apart. After 2 trap-nights, we switched devices so that each station accrued equal amounts of effort with each device during each session. We censored data from devices that had been tampered with and were no longer fully operational when checked.

We baited devices with 400-600 g of fresh frozen fish (*Cyprinus carpio*, *Ctenopharyngo-don idella*, *Hypothalmichthys molitrix*, *H. nobilis*) suspended underwater from the back of the cage or third hoop in a nylon-mesh bag made from drain-tile sleeve (DrainKnit Filtration Barrier Fabric, Dickson Industries, Inc., Des Moines, Iowa, USA). To avoid bias, we mixed bagged baits in a cooler and drew them as sets were made. We replaced baits with fresh ones after 2 trap-nights. Based on past experience, we anticipated that some baits would be consumed entirely by turtles during the first trap-night. Recognizing that freshness can affect capture success for some species (Lagler 1943, Ernst 1965), we cached extra baits underwater near the study area and replaced missing day-old baits with these extra baits when we checked devices after the first and third trap-nights. Captured turtles were marked by shell notching (Cagle 1939), measured with a "bump board" commonly used for studies of fishes (carapace length to nearest 0.5 cm), and sexed based on secondary sex characteristics (Ernst et al. 1994).

Statistical tests followed McCulloch and Searle (2001). Data for analyses of catch per unit effort (CPUE) and species coverage included recaptures. We used a mixed Poisson model to test for differences in CPUE between devices, bait ages (1- vs. 2-day-old), and sessions (categorical fixed effects, including pairwise interactions), treating stations as independent experimental subjects (random effects). The response variable was the total number of turtles (of all species) captured at a given day and station. Because CPUE was clearly lower at New Salem than Sanganois, we analyzed data from each site separately. Poisson regression assumes a linear relationship between the log number captured and explanatory variables, so back-transformed estimates provide multiplicative effect sizes. Interactions were dropped from the model if P > 0.1, in order of highest P-value.

We used 2 complementary analyses to test for differences in species coverage between devices (i.e., the ability of different devices to effectively sample the range of species present). Because sliders were clearly dominant in the captured sample, we first simply tested for differences in the proportion of captures composed of other species (i.e., not sliders), using mixed-model logistic regression. For this analysis, session, bait, and device (and their pairwise interactions) were included as categorical fixed effects, and stations were treated as independent subjects. Effect size estimates from logistic regression were converted to odds-ratios.

The second analysis of species coverage examined the relationship between sample size (no. turtles captured) and the number of species observed, known as the rarefaction curve (Gotelli and Colwell 2001). The steeper this relationship, the greater species coverage is provided by a given number of captured turtles. For this analysis, we used mixed-model Poisson regression with the number of species captured as the response variable. Because instances of 0 or 1 captures were uninformative (i.e., only 0 or 1 species can be observed, respectively), we only included instances where  $\geq 2$  turtles were captured. The model included the number of captures minus 1 as the primary explanatory variable, and intercepts were fixed at 0 [i.e., log(# species) = 0 if no. captures -1 = 0]. The slope versus captures -1 was allowed to vary randomly among stations, and we tested whether slope differed with session, bait age, and device.

#### RESULTS

We censored 6 trap-nights because of tampering. At Sanganois, each device was deployed for 40 trap-nights during the first session and 38 during the second, accruing total efforts of 78 trap-nights for cage traps (1836 hr) and the same number of trap-nights for hoop nets (1842 hr). At New Salem, each device was deployed for 23 trap-nights during the first session and 24 during the second, accruing total efforts of 47 trap-nights for cage traps (1113 hr) and the same for hoop nets (1109 hr). Day-old baits were replaced with the same at 6 sets because no bait remained when devices were tended after the first and third trap-nights of a session; 4 of these devices contained  $\geq 1$  turtle when sets were checked.

We recorded 402 captures of 378 individuals (Table 1). Original captures of males and females at all sites and for all combinations of gear and bait were similar for *T. scripta* and *C. picta* but not *A. spinifera* (Table 2); we deemed sample sizes too small for

comparisons of other species. Median carapace lengths were similar for sexes of *C. picta* and *A. spinifera* but not *T. scripta*.

Catch per unit effort was greater at Sanganois (2.24) than New Salem (0.55) for all species and both devices combined. Cage traps failed to detect 1 species captured in hoop nets at Sanganois (*C. serpentina*) and 3 at New Salem (*C. serpentina*, *G. ouachitensis*, *A. mutica*). Recapture rates for all species and both devices combined were greater at New Salem (17.3%) than Sanganois (4.3%). Most recaptures occurred in hoop nets at Sanganois (86.7%) and New Salem (77.8%). Only *T. scripta* was recaptured in cage traps; 4 species were recaptured in hoop nets.

**CPUE.** The final model for Sanganois included only main effects of session, bait, and device (all  $F_{1,19} > 12.4$ , P < 0.002). At Sanganois, fresh bait increased CPUE nearly 3-fold relative to 2-day-old bait (2.94 vs. 1.05 turtles/day, respectively), and CPUE was approximately 2-fold higher for hoop nets than cage traps (2.38 vs. 1.30 turtles/day, respectively) and for the second than the first session (2.58 vs. 1.20 turtles/day, respectively). For New Salem, interactions with session were dropped from the model and no remaining variables yielded P < 0.05. Confidence intervals for the main effects at New Salem were wide, however, and generally overlapped the estimated bait and device effects for Sanganois (Fig. 1).

**Species coverage.** The final model for Sanganois included no interactions, and the odds of a captured turtle being a non-slider were estimated to be 8.80-fold higher (95% CI: 2.78-27.87) for hoop nets than for cage traps ( $F_{1,18} = 15.75$ , P < 0.001). Bait age had no apparent effect on species coverage ( $F_{1,16} = 0.21$ , P = 0.65), and there was marginal evidence for a session effect ( $F_{1,16} = 3.89$ , P = 0.066) at Sanganois. For New Salem, no main effect or interaction was significant (all P > 0.17), but the estimated odds-ratio between devices (2.10-fold) had a wide 95% confidence interval (0.37-11.80) that included the Sanganois estimate.

For Sanganois, the slope of log (no. species) vs. captures -1 was greater for hoop nets than cage traps (0.097 vs. 0.011) and greater in the early session (both  $F_{1,49} > 10$ , P < 0.002), but we detected no effect of bait age ( $F_{1,49} = 1.7$ , P = 0.20) on the slope.

#### DISCUSSION

We do not know the true composition of assemblages on our study areas, but can make some reasonable comparisons to museum records and other sampling efforts. Twelve of 15 species of aquatic turtles that occur in Illinois have been documented in the region (Phillips et al. 1999). The lone record for 1 species (*Macrochelys temminckii*) is suspect (Moll 1988). Three others (*G. geographica, G. pseudogeographica, Kinosternon flavescens*) are not likely to occur in habitats we sampled (Ernst et al. 1994), but their presence cannot be discounted completely. At worst, hoop nets detected 64% of species known to occur in the region. At best, they detected 87% of species in the region (i.e., excluding 3 species that prefer habitats other than those we sampled). At Sanganois, hoop nets were almost 9 times as likely to capture a non-slider as cage traps. No significant differences occurred at New Salem, but samples were small and trends were similar [e.g., hoop nets captured more species than cage traps during both the first (4 vs. 3) and second

(5 vs. 3) sessions]. We concluded that hoop nets were more effective than cage traps for detecting species that occur in central Illinois and recommended dropping cage traps from consideration as a sampling device for future inventories.

Anecdotal reports suggest that fresh baits are better than putrid ones (e.g., Lagler 1943, Legler 1960, Plummer 1979). Captures at Sanganois supported these observations, with fresh baits outperforming day-old baits by nearly 3:1. Implications for future inventories were unclear because we did not observe any effects of baits on species coverage. To be on the safe side, we chose to standardize protocols for future inventories by requiring daily changes of baits. We recommend the same conservative approach for other studies that use baited traps and methods of analyses that are sensitive to heterogeneous rates of capture. For example, waning attractiveness of baits could cause the appearance of time or behavioral effects (i.e., trap shyness) in analyses of data from capture-recapture and occupancy studies.

We did not recapture enough turtles of any species to compare robust estimates of abundance to catch per unit effort. Use of CPUE requires narrow limits on its application, including assumptions of closure and equal catchability (Lancia et al. 1996, Maunder et al. 2006). Differences between sessions at Sanganois implied violation of 1 or both of these assumptions. We speculated that high water levels during the first session at Sanganois contributed to differences in CPUE by allowing turtles to exploit abundant food sources in flooded margins of the lake, thereby making our baits less attractive than during the second session when these conditions did not exist. We concluded that water level is a good candidate for monitoring as a covariate during studies of the demography of turtles in or near lotic systems. Although CPUE has been used as an index of abundance in chelonian studies (e.g., Aresco 2009), we decided that data we planned to collect during future inventories would not suit this purpose because of the likelihood of violations of assumptions caused by protocols (i.e., multiple observers targeting multiple species during multiple years and long periods of time within years) and varying environmental conditions.

Few of our findings can be applied directly to other research because our study was tailored to specific needs, study areas were chosen opportunistically and assemblages differ in other areas. However, some aspects of our study are relevant in a broader context. Waning effectiveness of baits could be a widespread problem in chelonian studies because few researchers change baits daily. Unlike most studies of efficiency and bias, we used an efficient design to evaluate multiple variables and estimated the magnitude of effects (i.e., odds ratios) to interpret outcomes readily (Hayek 1994). Mostly, our findings demonstrate the value of *a priori* studies for choosing effective survey methods, reducing biases caused by protocols, and identifying covariates or other statistical tools that help to cope with variable rates of capture (e.g., Amstrup et al. 2005, MacKenzie et al. 2006, Olivier et al. 2010).

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	<u>Sanganois</u>				New Salem			
	Session 1		Session 2		Session 1		Session 2	
Species	Cage	Net	Cage	Net	Cage	Net	Cage	Net
Trachemys scripta	41	50	74	129	14	8	4	4
Apalone spinifera	1	13	0	13	1	0	2	3
Chrysemys picta	0	5	2	12	2	2	3	1
Chelydra serpentina	0	1	0	2	0	4	0	1
Sternotherus odoratus	0	4	1	2	0	0	0	0
Graptemys ouachitensis	0	0	0	0	0	1	0	0
Apalone mutica	0	0	0	0	0	0	0	2
Combined	42	73	77	158	17	15	9	11

Table 1. Captures (including recaptures) of freshwater turtles in cage traps and hoop nets at two study areas in central Illinois, 2007.

Table 2. Sexes and sizes of three species of turtles captured at two study areas in central Illinois, 2007.

				Carapace length (measured to nearest 0.5 cm)							
	Sex (no. individuals)			N	<u>lale</u>	Female					
	Male	Female	Unknown	Median	Range	Median	Range				
Chrysemys picta	13	13	0	14.0	10.5-16.5	14.25	12.5-17.5				
Trachemys scripta	156	147	3	16.5	9.5-23.0	18.50	7.0-26.0				
Apalone spinifera	17	7	0	18.5	14.0-43.0	18.25	16.5-25.0				

Figure 1. Estimated multiplicative effects, presented as estimated ratios (y:1) with 95% confidence intervals, of bait age (1- vs. 2-days old), capture device, and session on catch per unit effort (CPUE) in capturing aquatic turtles in two Illinois study areas, June-August 2007. The dotted horizontal line represents a 1:1 ratio, indicating no effect.

