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# BULLETIN

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**CONTRIBUTIONS FROM THE HERPETOLOGY CONFERENCE  
INCLUDING THE ALL FLORIDA HERPETOLOGICAL EVENT**

**Amber L. Pitt, Editor**

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# CONTRIBUTIONS FROM THE HERPETOLOGY CONFERENCE INCLUDING THE ALL FLORIDA HERPETOLOGICAL EVENT

Amber L. Pitt, Editor\*

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## INTRODUCTION

The Herpetology Conference including the All Florida Herpetological Event began more than three decades ago as a small gathering of some of Florida's esteemed herpetological forefathers including the late Ray Ashton (1945–2010) and Walter Auffenberg (1928–2004). The gathering became an annual event with all but one meeting (which was held in Miami) taking place in Gainesville, home of the University of Florida and the Florida Museum of Natural History. Perhaps as a testament to the dedication of the conference's forefathers for sharing knowledge and teaching others about herpetofauna, the then dubbed All Florida Herpetological Event evolved from a meeting of established herpetologists to a conference that invited and encouraged student contributors. The conference continued to grow, attracting attendees and speakers from around the world. As a result, the no longer aptly named conference was renamed "The Herpetology Conference including the All Florida Herpetological Event". In its current form, the Herpetology Conference including the All Florida Herpetological Event not only provides a venue for established herpetologists, students, and enthusiasts to meet, present research, and discuss ideas, but all net revenue generated through conference events (e.g., silent and live auctions, registration fees, donations) are contributed to the Reptile and Amphibian Conservation Corps, a 501(c)(3) nonprofit organization that funds reptile and amphibian conservation and research.

The success of the conference has not gone unnoticed by journal editors and the organizers of the event have been approached by several different venues with the intent of publishing a special volume featuring the research presented at each year's conference. Although the conference is not yet in a position to produce an annual publication, this special volume of the Bulletin of the Florida Museum of Natural History is the first to offer a selection of manuscripts based on research presented at the Herpetology Conference including the All Florida Herpetological Event. The Bulletin of the Florida Museum of Natural History was a natural choice of journals for this volume as many

of the conference forefathers and past and present contributors have been affiliated with the Florida Museum of Natural History at some point in their careers (see <http://www.flmnh.ufl.edu/herpetology/about/alumni.htm> for a list of museum alumni and research associates).

It has been my distinct pleasure to serve as the editor<sup>1</sup> of this special volume of the Bulletin of the Florida Museum of Natural History featuring selected contributions from the Herpetology Conference including the All Florida Herpetological Event. The manuscripts included in this special volume offer insights into: 1) the effects of a common environmental pollutant and a commonly used anesthetic on amphibian development, growth, and behavior; 2) population ecology of the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) in the Great Smoky Mountains National Park; 3) population ecology of the Florida snapping turtle (*Chelydra serpentina osceola*) in the Santa Fe River; 4) a historical river turtle population decline in Missouri<sup>1</sup>; and 5) timber rattlesnake (*Crotalus horridus*) movement in the South Carolina mountains. I hope that you enjoy this volume and have the opportunity to attend future meetings of the Herpetology Conference including the All Florida Herpetological Event.

## DEDICATION

The Herpetology Conference special volume of the Bulletin of the Florida Museum of Natural History is dedicated to Dr. Max Nickerson whose devotion to herpetology, conservation, and education has kept the Herpetology Conference including the All Florida Herpetological Event successful for more than a decade.

## ACKNOWLEDGMENTS

Partial funding for this volume was provided by the Reptile and Amphibian Conservation Corps. The Herpetology Conference including the All Florida Herpetological Event is the result of more than three decades of hard work and the dedication

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<sup>1</sup>Richard C. Hulbert Jr., the Editor-in-Chief of the Bulletin of the Florida Museum of Natural History, served as editor for the paper for which I was a co-author in order to avoid a conflict of interest in the review process.

of many individuals. Without the conference committee members, organizers, donors, volunteers, and participants who have made the Herpetology Conference including the All Florida Herpetological Event a successful annual gathering for the past several decades, this volume could not have been made. Thank you to all involved with

both this volume and the Herpetology Conference including the All Florida Herpetological Event. A special thank you to the individuals who contributed to the oral history of the Herpetology Conference including the All Florida Herpetological Event summarized in this volume's introduction.



# EFFECTS OF NITROGEN AMMONIA AND MS-222 ON *XENOPUS LAEVIS* DEVELOPMENT, GROWTH, AND FORAGING BEHAVIOR

J. Kelly Byram<sup>1,2</sup> and Max A. Nickerson<sup>3</sup>

## ABSTRACT

The anesthetic MS-222 (i.e., tricaine, Finquel) is widely used by biologists on amphibians in the field, even though field use of MS-222 on amphibians is not approved by the U.S. Food and Drug Administration (FDA). Previous studies have identified the impact of MS-222 on vision, olfaction, stress, heart, and liver, and have documented its lethality to certain microbes that commonly populate amphibian skin. We examined the potential impacts of “off-label” use of MS-222 on a model aquatic amphibian, the African clawed frog (*Xenopus laevis* Daudin 1802). Animals were exposed to an environmentally relevant concentration of nitrogen ammonia, a pollutant commonly found in U.S. waterways, and unbuffered MS-222 in a manner simulating typical field use of the drug. The animals’ foraging success in the hour post-recovery was observed. MS-222 impacted foraging behavior, with animals exposed to MS-222 eating significantly more food pellets than the control animals ( $P = 0.01$ ). Although an ANOVA revealed no statistically significant difference in the mean weight and length between the animals exposed to nitrogen ammonia and their controls, the group of animals exposed to nitrogen ammonia had an increased variance in weight and length, which may indicate population-level effects.

**Key words:** MS-222, tricaine, *Xenopus laevis*, nitrogen ammonia, ammonia, stress.

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## INTRODUCTION

Naturally occurring ammonia ( $\text{NH}_3$ ) plays an important role in aquatic environments. Aquatic animals excrete ammonia as a byproduct of metabolism and, it is suspected, as a disturbance pheromone powerful enough to elicit an avoidance reaction in tadpoles (Manteifel 2006). Eutrophication results from high levels of nitrogen (e.g., nitrates, nitrogen ammonia) or phosphorous compounds in an ecosystem, and eutrophication resulting from anthropogenic activities has been implicated as one factor in global amphibian decline (Nyström et al. 2007) and pathology (Johnson et al. 2007).

Studies of the effects of ammonia on embryos and larvae have revealed statistically significant findings. Jofre et al. (2000) studied the effects of concentrations of un-ionized  $\text{NH}_3$  up to 2 mg/l on green frogs (*Lithobates clamitans* Latreille 1801) and leopard frogs (*L. pipiens* Schreber 1782). Hatching success declined when leopard frog embryos were exposed to concentrations of  $\text{NH}_3$  greater than 1.5 mg/l, and the frogs were more likely to be deformed. Green frogs experienced similar impacts at a lower concentration of  $\text{NH}_3$  (0.6 mg/l), and the green frogs displayed the additional problem of decreased tadpole survival and growth at this concentration.

MS-222 (i.e., tricaine, Finquel) is widely used by biologists in the laboratory and in the field to euthanize and sedate amphibians for safety and to minimize stress from handling and various procedures, but research indicates MS-222 may actually increase stress (Vethamany-Globus et al. 1977), impair vision (Hoffman & Basinger 1977; Bernstein et al. 1986), and affect the amphibian heart (Bartlett et al. 2004; Cakir & Strauch 2005; Bartlett et al. 2010) and liver (Wayson et al. 1976). Stress has been linked to immunosuppression (Belden & Kiesecker 2005), declines in reproduction potential and survival (Edgington et al. 2003; Barbeau & Guillette 2007), and synergistic toxicity with a pesticide (Relyea & Mills 2001). Additionally, the use of MS-222 can mask the parasitic load and may have affected the results of previous amphibian parasite studies as it anesthetizes parasites as well

(Fedewa & Lindell 2005; Solis et al. 2007a).

Although marketed as a sedative appropriate for use in the field (Argent Chemical Laboratories undated; Western Chemical, Inc. undated), the U.S. Food and Drug Administration (FDA) specifically restricts the use of MS-222 on amphibians to the laboratory, stating “In other fish and cold-blooded animals, the drug should be limited to hatchery or laboratory use” (FDA 1998). Despite this clear restriction, the literature is replete with “off-label” use (i.e., use of a drug in a manner not approved by the FDA) of MS-222 in field studies of amphibian populations (Byram & Nickerson 2009). MS-222 is recommended for use on amphibians in veterinary and zoological publications (Gentz 2007) and for use in the field in government documents (Green 2001). As Crook and Whiteman (2006) stated in their own study of MS-222, “Typically the choice of anesthesia has been based on what other researchers have used rather than a critical evaluation of different methods.”

The off-label use of MS-222 on amphibians in the field is potentially problematic because direct inquiry and anecdotal evidence point to field techniques very different from the suggested usage and safety guidelines, with field biologists often mixing the anesthetic powder with water from a local body of water, as instructed by the package insert provided by Argent Chemical Laboratories (undated) and Western Chemical, Inc. (undated). Additionally, anecdotal evidence indicates the MS-222 bath is often not buffered in the field (Nickerson pers. obs.). Use of unbuffered MS-222 creates the risk of amphibian death, since exposure to a pH below 4-5 can cause death in amphibians (Boutilier et al. 1992). Additionally, as pH decreases, herbicides become more toxic to amphibians (Edgington et al. 2003) and incidence of infection increases (Simon et al. 2002). Quality of the environmental water used for the anesthetic bath is an additional consideration. At the time of this study, the Argent directions for its MS-222 product marketed as “Finquel” read, “Do not use... water containing chlorine, heavy metals (copper, zinc, etc.), or other toxic contaminants” (Argent Chemical Laboratories undated), but with the



decline of water quality in aquatic habitats and the uncertainty of what chemicals and metals are in these habitats, there is the potential risk the anesthetic bath prepared in the field may be unsafe. Because of its use in the aquaculture industry on fish consumed as food for humans, copious research on MS-222's effects on fish is available, but research on its effects on amphibians—especially when it is used off-label—is comparatively scant. This study was designed to determine if MS-222 and nitrogen ammonia impact amphibians, both separately and in a typical field-preparation combination.

### MATERIALS AND METHODS

African clawed frog (*Xenopus laevis* Daudin 1802) tadpoles ( $n = 270$ ) from one clutch were purchased from Xenopus Express (Brooksville, FL) and distributed among four 10-gallon glass aquarium tanks: two control ( $n = 136$ ) and two treatment ( $n = 134$ ) tanks. Each tank was filled with 32 L tap water treated with 1 mL dechlorinator (Top Fin, Pacific Coast Distributing, Inc., Phoenix, AZ) for removal of chlorine, chloramine, and heavy metals. The two treatment tanks were dosed with nitrogen ammonia ( $\text{NH}_3\text{-N}$ ; Ricca Chemical Company, Arlington, TX) to an environmentally relevant concentration of 0.5 mL/L, as indicated by concentrations of total nitrogen (TN) documented in the North Fork of White River (Quinlan & Philips 2007; Solis et al. 2007b) and the Eleven Point River, Missouri (Solis et al. 2007b). We selected to use the nitrogen concentrations found in the North Fork of White River and the Eleven Point River because these rivers provide habitat for declined populations of endangered Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) and documented field use of MS-222 on *C. a. bishopi* has occurred at these sites (Byram & Nickerson 2009). Each 1.00 mL of  $\text{NH}_3\text{-N}$  contained 1.00 mg N and 1.216 mg  $\text{NH}_3$ . The concentration for the treatment environments was calculated based upon the nitrogen content of the  $\text{NH}_3\text{-N}$ . Concentration was verified by testing ammonia ( $\text{NH}_3$ ) levels in the tanks (API, Inc., Chalfont, PA). Temperature, pH (5-in-1 strips, Hach Co., Loveland, CO), nitrite (nitrate/nitrite test strips, Hach Co., Loveland, CO), and nitrate

(nitrate/nitrite test strips, Hach Co., Loveland, CO), were also monitored. The tanks were aerated with 10 cm air stones (Rolf C. Hagen Corporation, Mansfield, MA) run off of one air pump and adjusted to provide the same amount of aeration to each tank (by visual estimation). Tanks were neither heated nor cooled. Air and water temperature were monitored daily. Over the course of the study, the observed water temperature ranged between 16–23°C, but the average observed daily water temperature was usually 20–22°C.

At approximately 55 days of development, animals began to exhibit aggressive behavior and water quality had begun to decline, even though the water in the tanks was changed daily, so the animals were separated into individual habitats. Only animals that had reached a stage where they could eat food pellets (Nieuwkoop-Faber stage 64 or greater) moved on to the next stage of the protocol. These juveniles were given a unique identification number and placed in individual one-gallon bowls marked with the animal's identification number. The remaining animals that had not metamorphosed did not continue in the protocol.

### MEASUREMENTS

Development and growth were monitored throughout the study. Tadpoles were randomly selected from the tanks for measurement and staging at several points of the protocol by scooping or netting in a variable fashion throughout the tank areas. The tadpoles were measured with a ruler to the nearest 1.0 mm and weighed to the nearest 0.1 g using an Ohaus ProScout Scale (Ohaus Corporation, Parsippany, NJ). They were then viewed under a light microscope for staging according to the Nieuwkoop-Faber table (Nieuwkoop & Faber 1994).

Once the animals metamorphosed and were in their individual bowls, they were examined, weighed to the nearest 0.1 g using an Ohaus ProScout Scale (Ohaus Corporation, Parsippany, NJ), and snout-vent length (SVL) was measured to the nearest 1.0 mm using a ruler 3 times (days 55, 96, 112–113, and 130–131) during the water change process to minimize stress. Dimorphism

is not pronounced enough to reliably sex young juveniles without dissection, so data on sex were not collected.

#### ENVIRONMENT

Bowls were housed in numerical order on shelves and unused bench space throughout the laboratory. Water changes, first partial and then full, were performed on a regular basis with room-temperature tap water treated with Top Fin Tap Water Dechlorinator (Pacific Coast Distributing, Inc., Phoenix, AZ) for removal of chlorine, chloramine, and heavy metals.

Temperature was not maintained on an individual basis and was dictated by the ambient temperature of the laboratory space. Laboratory windows provided filtered light for the animals, which was dictated by local weather patterns, and exposure to artificial fluorescent lighting was regular and incidental to periods of human occupation of the laboratory, which could not be regulated, since multiple researchers on variable schedules had access to adjacent laboratory space.

#### DIET

Tadpoles were fed a liquid diet of tadpole powder (Xenopus Express, Brooksville, FL) prepared with filtered tap water (Brita faucet mount filtration system, Brita Products Company, Oakland, CA). The solution was mixed well and shaken as needed to assure the powder remained suspended and distribution of nutrition was equivalent across the tanks. Additionally, food was added to the tanks in a uniform fashion but in a random order determined using a random number generator ([www.random.org](http://www.random.org)), assuring that any variability in food distribution related to suspension of food was distributed evenly over time across the tanks.

After the animals metamorphosed and were separated to individual habitats on day 55, animals were fed 3/32" (2.38 mm) floating frog food pellets (Xenopus Express, Brooksville, FL) on a regimented schedule.

#### MS-222 TREATMENTS

Half of the nitrogen ammonia-exposed

animals and half of the control animals were randomly assigned to the MS-222 treatment group by using a random number generator ([www.random.org](http://www.random.org)) to pick animal numbers. The remaining 50% of the animals were assigned to the control group.

The anesthetic solution of 1 L tap water at 23.3°C dechlorinated with Top Fin Tap Water Dechlorinator (Pacific Coast Distributing, Inc., Phoenix, AZ), 0.50 mg NH<sub>3</sub>-N, and 1 g tricaine powder (Finquel, Argent Labs, Redmond, WA) for the NH<sub>3</sub>-N-exposed animals was prepared without buffer as is often true in the field. The anesthetic solution for the control group (non-nitrogen ammonia) was prepared according to instructions, with 1 L tap water at 23.3°C dechlorinated with Top Fin Tap Water Dechlorinator (Pacific Coast Distributing, Inc., Phoenix, AZ), 1 g tricaine powder, and 1 g baking soda (buffer). Over the course of days 112 and 113, animals were removed from their individual tanks, weighed, measured, and then placed in the anesthetic bath. Animals were kept in the anesthetic bath until they stopped swimming, at which point they were retrieved and gently placed on their backs on the researcher's palm. If the animal attempted to right itself, it was placed back in the anesthetic bath and carefully monitored. When the animals ceased attempting to right themselves in the researcher's hand, they were considered anesthetized. The time it took for the animals to reach the desired anesthetic plane was not recorded, and it varied widely from animal to animal. Typically animals ceased righting themselves within 3–10 minutes.

Then half of the animals (half anesthetized, half not anesthetized) had a small web clip performed. The web clipping control group was handled for a duration and in a fashion mimicking the handling of the web clipping group to control for handling stress.

Anesthetized animals were then placed directly in a recovery bath. For animals in the nitrogen ammonia group, the bath was a mixture of 1 L 23.3°C dechlorinated tap water and 0.50 mg NH<sub>3</sub>-N. This was the same concentration of nitrogen ammonia they had lived in since the beginning of the study. For the control animals, the bath omitted

the  $\text{NH}_3\text{-N}$ . Periodically, water was gently agitated by hand to create a flow of water on the skin of the animals to facilitate recovery. Once the animals recovered gross motor skills and were able to right themselves on the researcher's palm, they were returned to their individual tanks, which had been cleaned and refilled with water in the interim. The tank was then placed in a lighted observation area and the time noted, and 8 pieces of the same brand and size floating food pellets, fed throughout the study, added to the water. Animals were observed intermittently from a distance of approximately 0.3 to 1.3 meters to assure their safety and recovery. At one hour post-treatment, the number of food pellets eaten was noted, uneaten food pellets were removed from the tank, and the animal's tank returned to its assigned place in the laboratory space.

Animals were weighed and measured on days 130 and 131. This ended the protocol, and all study animals were adopted through the University of Florida Animal Care Service's laboratory animal adoption program.

#### STATISTICAL ANALYSES

Stage, total length, SVL, weight, and consumption data were compared for the treatment and control groups. The parametric day 55 weight data were analyzed with a *t*-test, while the other data did not meet the assumptions of parametric analysis and were analyzed with a nonparametric ANOVA (Kruskal-Wallis).

Analysis was complicated by the mortality of treatment subjects due to causes unrelated to the experimental protocol. A graduate student with IACUC certification and years of experience working with animals, who was briefly employed

as a lab assistant, overdosed some of the treatment animals with  $\text{NH}_3\text{-N}$ , presumably by a factor of two, which proved lethal. Therefore, the sample sizes of the groups for the measurement data of days 130–131 were not equal (see Table 1). Post-treatment (MS-222) growth (weight and SVL) were calculated by subtracting day 112 and 113 data from day 130 and 131 data. All statistical analyses were performed with  $\alpha = 0.05$  using SAS software (v. 9.1, SAS Institute, Inc., Cary, NC).

## RESULTS

One variable not being measured for analysis but whose outcome is worthy of note is the pH of the anesthetic baths. The pH of the anesthetic baths were measured as a matter of safety, since Boutilier et al. (1992) report that a pH below 4–5 can cause death in amphibians. While the buffered MS-222 baths were pH 7 (neutral), the pH dropped to 4–5 (acidic) in the unbuffered baths.

#### DEVELOPMENT AND GROWTH

No statistically significant differences were indicated in stage (*P* value range = 0.22 – 0.87,  $n = 20$ ; Fig. 1) or total length (*P* value range = 0.18 – 0.79,  $n = 20$ ; Fig. 2) during metamorphosis between the nitrogen ammonia treatment group and the control group. Nor was there a statistically significant difference between the nitrogen ammonia treatment group and the control group at the end of the study (i.e., days 130–131) in SVL (*P* = 0.17,  $n = 136$ ,  $\chi^2 = 1.88$ ,  $df = 1$ ; Fig. 3) or weight (*P* = 0.24,  $n = 136$ ,  $\chi^2 = 1.40$ ,  $df = 1$ ; Fig. 4).

Animals anesthetized with unbuffered MS-222 exhibited no difference in growth in terms of weight or SVL compared to control subjects 18

**Table 1.** Summary of sample sizes for days 130–131 for the nitrogen ammonia treatment ( $\text{NH}_3\text{-N}$ ) and control groups.

	Web-Clipped		Control	
	$\text{NH}_3\text{-N}$	Control	$\text{NH}_3\text{-N}$	Control
MS-222	17	18	16	19
Control	10	20	16	20

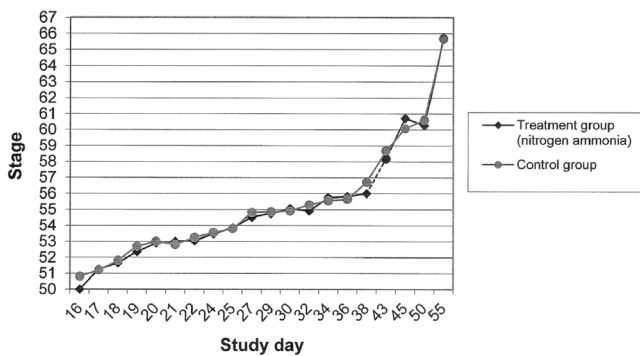
days after treatment ( $P = 0.16$ ,  $n = 136$ ,  $\chi^2 = 1.93$ ,  $df = 1$  and  $P = 0.47$ ,  $n = 136$ ,  $\chi^2 = 0.53$ ,  $df = 1$ , respectively). Weight was likewise unaffected by the web clipping ( $P = 0.23$ ,  $n = 130$ ,  $\chi^2 = 1.41$ ,  $df = 1$ ), but web-clipped animals did show a statistically significant increase in SVL compared to the unclipped animals ( $P = 0.01$ ,  $n = 130$ ,  $\chi^2 = 6.48$ ,  $df = 1$ ).

FORAGING

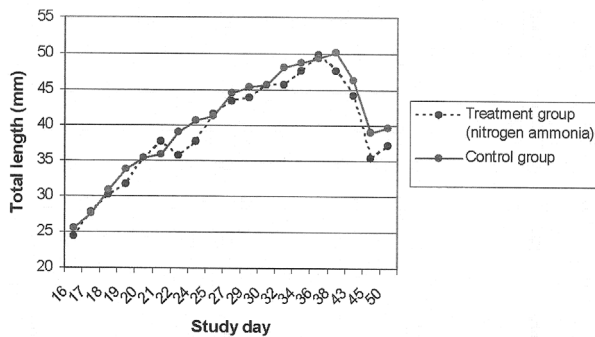
The animals treated with MS-222 ate significantly more food pellets in the recovery hour after treatment than did the control group ( $P = 0.01$ ,  $n = 149$ ,  $\chi^2 = 6.1$ ,  $df = 1$ ). Within-group analysis

of the animals who received MS-222 treatment revealed no difference in food pellet consumption between the animals whose webbing was clipped and their controls ( $P = 0.09$ ,  $n = 149$ ,  $\chi^2 = 2.95$ ,  $df = 1$ ), nor was there any difference between the animals raised in 0.50 mg/L concentration of nitrogen ammonia and their controls ( $P = 0.70$ ,  $n = 149$ ,  $\chi^2 = 0.15$ ,  $df = 1$ ).

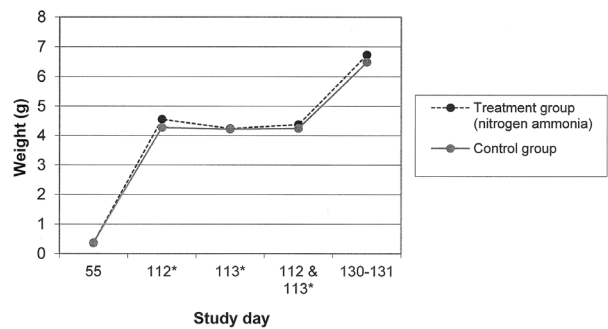
Roughly the same percentage of animals in the MS-222 treatment and control groups ate all of the food pellets offered: 22.9% of the MS-222 group and 19.4% of the control group. However, the percentage of animals that ate nothing varied



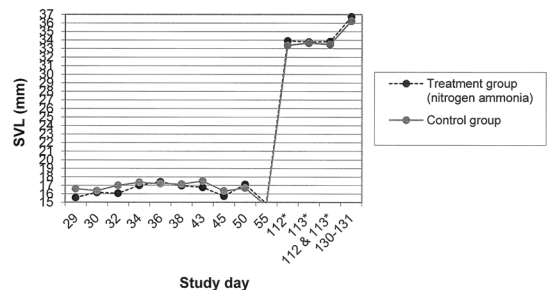
**Figure 1.** Mean Nieuwkoop-Faber developmental stage of African clawed frogs (*Xenopus laevis*) subjected to nitrogen ammonia treatments and no treatment (i.e., control) by study day.



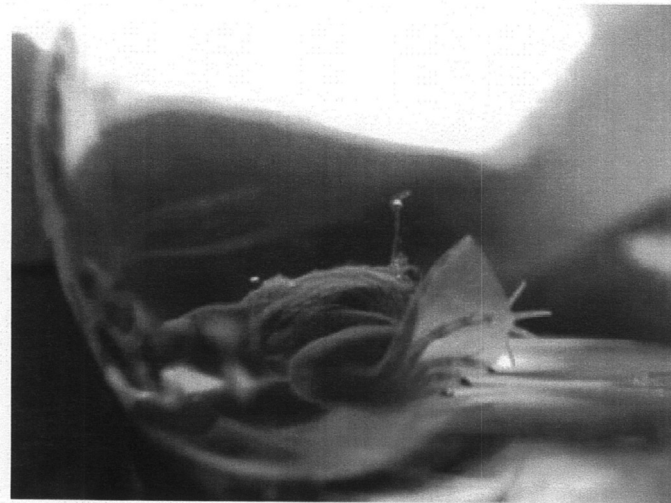
**Figure 2.** Mean total length and SVL of African clawed frogs (*Xenopus laevis*) subjected to nitrogen ammonia treatments and no treatment (i.e., control) by study day. As tadpoles develop into juveniles and their tails begin to disappear, their length decreases. Once the tail is gone, the juveniles continue to grow and their length begins to increase once again.



**Figure 3.** Mean total weight of African clawed frogs (*Xenopus laevis*) subjected to nitrogen ammonia treatments and no treatment (i.e., control) by study day. \*Since half the animals were weighed on day 112 and half were weighed on day 113, the mean weights for days 112 and 113 are reported separately and in combination (112 & 113).



**Figure 4.** Mean snout-vent length (SVL) of African clawed frogs (*Xenopus laevis*) subjected to nitrogen ammonia treatments and no treatment (i.e., control) by study day. \*Since half the animals were measured on day 112 and half were measured on day 113, the mean SVL for days 112 and 113 are reported separately and in combination (112 & 113).



**Figure 5.** Reaction of African clawed frog (*Xenopus laevis*) to treatment with buffered MS-222. This animal had no visible skin problems or defects prior to treatment with MS-222, but exhibited bubbling and sloughing in the hour post-treatment.

greatly between the two groups. Only 10% of the MS-222 animals ate nothing, while nearly one-third (31.9%) of the control animals did not consume any food pellets. Among the unanesthetized animals, web clipping did not impact foraging ( $P = 0.36$ ,  $n = 149$ ,  $\chi^2 = 0.82$ ,  $df = 1$ ).

### DISCUSSION

This study found unbuffered MS-222 dropped to pH 4–5, while the buffered MS-222 bath remained a neutral pH 7. This result should be noted by researchers preparing MS-222 in the field without buffer, since a pH under 4–5 can result in death in amphibians (Boutilier et al. 1992). Additionally herbicides become more toxic to amphibians (Edgington et al. 2003) and incidence of infection increases (Simon et al. 2002) with a decrease of pH. Figure 5 is a photo of one frog taken during the recovery hour after its treatment with *buffered* MS-222 and gentle handling with gloved hands. Even with gentle handling and a buffered, MS-222 bath, the visible white, cottony layer that formed on the frog's skin indicates trauma.

### DEVELOPMENT AND GROWTH

Extrapolating the effects of sublethal, environmentally relevant concentrations of

chemicals from published studies using high concentrations of chemicals can be difficult. Orlando and Guillette (2001) suggest that examining data from pollution-exposed populations in terms of central tendency (e.g., ANOVA) without studying the accompanying variance can result in Type II errors because the variance is indicative of the variation in individual responses to contaminant exposure in the population. This study did not find a statistically significant effect of MS-222 or an environmentally relevant concentration of nitrogen ammonia, either separately or in concert, on the growth and development of *Xenopus laevis* in the laboratory in terms of progression through the stages of development, weight, and length. However, Orlando and Guillette (2001) posit that an early indicator of disruption in a population may be increased phenotypic variance. Therefore, further exploration of the variance of these measures may be a prudent next step in exploring possible impacts of sub-lethal concentrations of nitrogen ammonia.

These results should remain in the context of this research design, i.e., a single dosage with great attention paid to each animal under anesthesia and immediate removal from the bath upon sedation. Dosage and length of exposure are two variables that figure predominantly in the stress response of

animals to MS-222 exposure, and the vast range of susceptibility to MS-222 that seems to vary by species and perhaps be modulated by repeated exposure (Zuccarelli & Ingermann 2005) should be noted. Researchers in the field should realize that, especially when working with some species with limited populations, they are likely working with individuals who have been studied by other researchers and may have previously been exposed to MS-222. This repeated exposure could result in variations in susceptibility to MS-222, so care and individual attention should be given to each animal during the sedation process to avoid possible over-sedation (i.e., death). Additionally, researchers should consider the potential changes in the stress response of animals that are repeatedly exposed to MS-222.

#### FORAGING

There has been some debate about direct biochemical measures of stress. Welker et al. (2007) conclude that hyperglycemia may not be regulated by cortisol alone, although previous research, like Vethamany-Globus et al. (1977) used blood glucose levels to measure the stress response. Therefore, behavioral indicators may inform our interpretation of the research and assist in the development of appropriate measures. For example, some studies have used differences in eating patterns to differentiate between the level of stress and pain. Carr et al. (2002) summarize the research on the relationship between stress and eating patterns across a range of animals, with chronic or severe stress typically inducing anorexia, and relatively minor stress (tail-pinching in rats) causing overeating in response. In our study, therefore, we measured the number of food pellets animals ate in the recovery hour after treatment with MS-222 in order to measure the stress response to the exposure to MS-222. We found that the animals treated with MS-222 ate significantly more food pellets in the recovery hour after treatment than did the control group. A clear difference between the two groups existed in the exhibition of short-term anorexic behavior, with only 10% of the MS-222 exposed animals refusing food compared to food refusal by nearly one-third (31.9%) of the control

animals, but this appeared to be a short-term behavior, since long-term anorexic behavior would have subsequently resulted in significantly smaller weight and length measures in the control group.

The lack of difference in foraging between the web-clipped and unclipped animals indicates that being web-clipped during handling was no more stressful than the handling alone. This is in line with the findings of recent research (e.g., Kinkead et al. 2006; Langkilde & Shine 2006). However, the greater length of web-clipped animals versus their controls indicates that web-clipping did impact the animals, although we noticed no difference in foraging and the web clips healed without incidence. Additionally, since there was no statistically significant difference in weights between the web-clipped animals and their controls, the increase in length without an increase in weight versus the control group may indicate a resulting difference in phenotype (i.e., a frog with a longer, thinner appearance) resulting from the web-clipping experience. This possibility is worth further investigation, as a change in phenotype may be an indicator that populations often subjected to research and clipping are experiencing a measurable impact from this research technique.

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# POPULATION STRUCTURE OF THE HELLBENDER (*CRYPTOBRANCHUS ALLEGANIENSIS*) IN A GREAT SMOKY MOUNTAINS STREAM

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and Phil Colclough<sup>4</sup>

## ABSTRACT

The hellbender (*Cryptobranchus alleganiensis*) is an imperiled salamander that has experienced population declines in many parts of its range. Young hellbenders, particularly larvae, have rarely been found in the wild. In 2000, a short study in Little River in Great Smoky Mountains National Park, Tennessee, discovered a population of *C. alleganiensis* where larvae were regularly encountered and few adults were observed. However, the 2000 study was limited in scope, and additional research was needed to accurately describe the overall hellbender population structure. Three additional studies of *C. alleganiensis* in the same section of Little River occurred from 2004–2010. This paper analyzes the results of all four studies conducted between 2000–2010 to examine trends in the hellbender population structure within Little River, and to provide reference data for future monitoring efforts in the park. From 2000–2010, a total of 533 captures, including 33 recaptures, occurred with larvae representing a quarter of overall captures. Adults were more abundant than suggested by the 2000 study, but individuals representing larger size classes were still relatively rare. Although the structure of the sampled population varied among years, larvae were relatively abundant except following years of extreme stream flow events, suggesting that turbulent current may be an important influence on the population structure of Little River's hellbender population.

**Key Words:** hellbender, *Cryptobranchus alleganiensis*, Great Smoky Mountains, amphibian population, salamanders, population structure, size structure.

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## INTRODUCTION

Size, age, or life stage structures are integral components to understanding population dynamics and can give more insight into population status than population size estimates alone (Alexander 1958; Downing 1980; Gillespie 2010). In species of conservation concern, demographic composition can indicate overall population stability and lead to more accurate predictions regarding future population trends (Crowder et al. 1994). A population composed primarily of older individuals may be at risk of decline or extirpation due to low recruitment (Alexander 1958; Downing 1980). A population with few older individuals, but many young individuals could indicate population growth, high adult mortality, or a failure to recruit young life stage classes into adults (Alexander 1958; Downing 1980). Understanding population structure is also important because demographic rates can vary among different segments of the population (Crowder et al. 1994; Dobson & Oli 2001).

In aquatic environments, organisms often adapt life strategies that can cause differences in demographic rates among age or life stage classes (Duellman & Trueb 1986; Pough et al. 2004). Many species, including fish, aquatic insects, and amphibians, develop complex life cycles or ontogenetic shifts in habitat use and diet, which are believed to be adaptations for increasing survival in a stressful environment (Werner & Gilliam 1984; Foster et al. 1988; Giller & Malmqvist 1998). These types of shifts can serve as a form of refugia, limiting intra-specific competition and predation (Werner & Gilliam 1984; Colley et al. 1989; McGrath et al. 2007). While these adaptations may help reduce individual mortality, they can also make studying population dynamics more complicated. The difficulties associated with studying organisms with complex life cycles or ontogenetic shifts have caused knowledge gaps in the field of amphibian population ecology.

Although many amphibian populations are declining worldwide (Alford & Richards 1999; Vié et al. 2009), population dynamics and demographics of many species remain unstudied

(Duellman & Trueb 1986; Alford & Richards 1999; Swanack et al. 2009; Gillespie 2010). As obtaining amphibian population and life history data that accurately considers all life stage classes can be problematic due to complex life cycles and ontogenetic shifts, data are often lacking for specific size or life stage classes (Swanack et al. 2009; Gillespie 2010). Larval and juvenile classes can be difficult to study because they are generally cryptic, small, and sometimes use different habitats than other life stages (Gillespie 2010). The resulting gaps in population structure data have hindered researchers from fully comprehending the scope of amphibian declines (Lips 2011). The failure to elucidate potential mechanisms affecting individual amphibian populations has limited mitigation efforts (Alford & Richards 1999; Gillespie 2010). Once population declines occur, information is even more difficult to obtain as individuals become rare (Gillespie 2010).

One amphibian species with few studies regarding its basic demographics and population dynamics is the hellbender salamander, *Cryptobranchus alleganiensis* (Daudin 1803). A member of the giant salamander family Cryptobranchidae, this long-lived (at least 29 years), large (740 mm), aquatic species resides primarily in cool, oxygen-rich streams in the eastern United States (Nickerson & Mays 1973a). There are currently two accepted subspecies: the eastern hellbender, *Cryptobranchus alleganiensis alleganiensis* (Daudin 1803) which ranges from Missouri to New York, and the Ozark hellbender, *Cryptobranchus alleganiensis bishopi* (Grobman 1943) found only in Missouri and Arkansas (Nickerson & Mays 1973a). Currently listed as near threatened on the International Union for Conservation of Nature (IUCN) red list (Hammerson & Phillips 2004), hellbender populations appear to be declining in many parts of its range (Trauth et al. 1992; Wheeler et al. 2003; Briggler et al. 2007; Foster et al. 2009; Nickerson et al. 2009; Burgmeier et al. 2011). The exact cause or causes of declines remain difficult to elucidate, but siltation, disease, collection, species introductions, and habitat loss are just some of the cited problems facing this species (Trauth et

al. 1992; Hiler et al. 2005; Briggler et al. 2007; Nickerson & Briggler 2007; Nickerson et al. 2009). Due to these declines, the hellbender is protected at the state-level throughout most of its range, and was recently added to CITES appendix III and the federal endangered species list (Anonymous 2011).

Despite the conservation interest in *Cryptobranchus alleganiensis*, data regarding the population dynamics of this species remain sparse. Many hellbender localities lack data regarding population size, status, and demographics. Population studies have primarily focused on snapshot estimates of population size or adult population structure. Few studies have examined growth rates, fecundity, and survivorship in hellbenders and those that have were restricted to a few localities in Missouri (Taber et al. 1975; Topping & Ingersol 1981; Peterson et al. 1988). Existing examples may not be representative for hellbenders across their range, particularly for the eastern subspecies.

Limited historical data from a few studied drainages in New York and Missouri have given better insight into long-term hellbender population trends and indicated that some populations were declining and shifting in overall structure (Wheeler et al. 2003; Foster et al. 2009). Comparisons of historical and recent data in Missouri populations suggested that in declining hellbender populations, size class distributions shifted towards larger individuals, possibly indicating inadequate recruitment (Wheeler et al. 2003). Foster et al. (2009) noted shifts in the sex ratio towards a male-biased population in the declining hellbender populations of New York's Allegheny River drainage. In both of these studies, few young individuals < 20 cm (i.e. larvae and small subadults) were sampled. It remains uncertain whether these size classes were largely absent from the population or inadequately sampled perhaps due to their association with interstitial spaces in gravel beds (Nickerson & Krysko 2003). Regardless, little is known about larval hellbenders, and few studies include data on larvae.

In 2000, a short survey of the hellbender population in Little River, Tennessee, yielded 33 individuals, of which 48% (n = 16) were larval

sized (< 130 mm) (Nickerson et al. 2002). This percentage was in stark contrast to those recorded for other hellbender populations (e.g., Peterson et al. 1988; Wheeler et al. 2003; Foster et al. 2009). Furthermore, the proportion of adult hellbenders to larvae within Little River was the lowest of any studied river system (Nickerson et al. 2003). However, the findings of Nickerson et al. (2002) were limited by small sample size and reduced search hours. Additional data were needed to confirm the differences in population structure in Little River from those in well-studied streams. We compiled and analyzed data from surveys conducted in Little River from 2004–2010 with the results of Nickerson et al. (2002) in order to investigate the size structure of the hellbender population in Little River, provide reference data for this site, and to investigate long-term trends in population structure.

## MATERIALS AND METHODS

### STUDY SITE

To better elucidate the structure of Little River's hellbender population within Great Smoky Mountains National Park, skin-diving surveys were conducted within the 3 km section investigated by Nickerson et al. (2002). Little River, located in the Blue Ridge Physiographic Province of eastern Tennessee, originates on the north slope of Clingmans Dome, the highest topographical point in both the state and Great Smoky Mountains National Park. Draining ~980 km<sup>2</sup>, Little River flows through the park and several small towns before joining the Tennessee River. Human disturbance, including farming and logging related activities, historically occurred within the present boundary of Great Smoky Mountains National Park (Mast & Turk 1999). Many forests remain in successional stages following the cessation of widespread logging activity in 1939 (Madden et al. 2004). Few large-scale landscape alterations have occurred after 1950 in the park area adjacent to Little River, but human recreational use is common. Spanning 2,108 km<sup>2</sup>, Great Smoky Mountains National Park is the most visited national park in the United States and receives over 9 million visitors each year

(Madden et al. 2004). Little River attracts tourists year-round including a large number of swimmers, snorkelers, and inner tube users during the warmer months, and fishermen throughout the year (pers. obs.). Building temporary rock dams, disturbing rocks, and kayaking are other frequent activities in the stream (pers. obs.).

Little River's exposed bedrock of Late Precambrian Elkmont and Thunderhead metamorphosed sandstone has eroded over time leaving great numbers of dense rounded boulders, cobble, and gravel in the streambed (Mast & Turk 1999). Macroscopic in-stream vegetation was rare during the 2000–2010 survey period. Elevation within the study area ranged from 327–407 m. Surrounding upland habitat was comprised primarily of pine and river cove hardwood forest (Madden et al. 2004). Scenic TN 73, constructed on the site of the former logging railroad that ran along Little River, had several concrete/gravel parking lots and pull-offs providing walking access to Little River. The river was difficult to access near some pull-offs because of steep boulder-covered slopes.

#### FIELD SAMPLING METHODS

Diurnal skin-diving surveys were conducted in Little River between June and October of 2004–2010 in order to locate *Cryptobranchus alleganiensis*. Skin-diving was chosen as the survey method due to its success in locating all size classes of hellbenders (Nickerson & Mays 1973a; Nickerson & Krysko 2003; Nickerson et al. 2003). During 2000, and most occasions in 2008–2010, the amount of time each individual surveyor spent searching for hellbenders was recorded. Surveyors worked upstream, against the current, to prevent visibility issues from displaced sand and silt. Rocks and other potential shelters were mostly hand turned towards the surveyor to limit disturbance to the streambed particles, but studies conducted by Lee University utilized log peaveys to lift large rocks. Rocks were replaced in their original position and orientation. Encountered hellbenders were captured by hand and taken to the river bank for data collection and tagging.

The total length (TL) and snout-vent length

(SVL) of each hellbender was measured in millimeters (mm) with the aid of a ruled, modified PVC pipe. Mass was recorded in grams using an Ohaus® CS2000 compact digital scale (accuracy  $\pm 1.0$  g; Ohaus Corporation, Parsippany, NJ, USA), DYMO® Pelouze SP5 digital scale (accuracy  $\pm 1.0$  g; DYMO, Norwalk, CT, USA), or Pesola® spring scale (accuracy  $\pm 0.3\%$ ; Pesola AG, Baar, Switzerland). Sex was recorded if it could be determined based on the swelling of male cloacal glands in August and September (Nickerson & Mays 1973a). Biomark 9 mm and 12.5 mm Passive Integrated Transponder (PIT) tags (Destron-Fearing, South Saint Paul, MN, USA) were injected dorsal-laterally near the base of the tail in adult and most subadult individuals. Individuals as small as 140 mm TL were tagged, but no standardized minimum hellbender size for injection was used across studies. PIT tag injection needles were disinfected in a 70% ethanol solution between each use. New Skin® liquid bandage (Prestige Brands, Inc., Irvington, NY, USA) was applied at injection sites. From 2008–2010, unique individual combinations of Visible Implant Elastomer (VIE) (Northwest Marine Technology, Inc., Shaw Island, WA, USA) were injected posterior to the limbs on the ventral side of 48 individuals too small for PIT tag injection. New VIE injection needles were used daily, and needles were disinfected with rubbing alcohol wipes between uses. Individuals were returned to their capture site following data collection. GPS localities were recorded using an eTrex® Legend and GPSMAP® 76CSx (Garmin International, Inc., Olathe, KS, USA).

#### DATA ANALYSIS

Mean mass and TL of hellbenders sampled across all years was calculated. Histograms of annual and combined *Cryptobranchus alleganiensis* size class distribution in Little River were constructed based on individual TL. All histograms used 25 mm intervals. Recaptured hellbenders were only represented once in the combined histogram, but we only eliminated individuals recaptured within a single year from the yearly histograms. To determine if the size distribution of Little River's hellbenders

was statistically different from a representative sampled population, our hellbender TL data were compared to data from one of most well-studied hellbender streams, the North Fork of the White River, Missouri (Nickerson & Mays 1973b). Data from the 1969 North Fork of the White River population were used for this comparison because the population has since experienced substantial declines (Wheeler et al. 2003; Nickerson & Briggler 2007), and these data are the best available baseline. To reduce potential bias from unmarked individuals in Little River, data from only the two years with the largest sample sizes that were not directly impacted by flooding (2006 and 2008) were used for analysis. Data were tested against the North Fork of the White River historical data using two-sample boot-strap Kolmogorov-Smirnov tests. The *ks.boot* function, from R Package “Matching” (Sekhon 2011), tested whether probability densities for TL data from the two rivers were the same. The significance level for these tests was set at  $\alpha = 0.05$ .

Size classes may not always correlate with life stage classes, so hellbenders were also divided into life stage classes based on individual total length. Based on previous research, individuals < 125 mm in TL, both gilled and non-gilled, were classified as larvae (Bishop 1941; Nickerson & Mays 1973a). Previous *Cryptobranchus alleganiensis* studies suggested that size at sexual maturity differs among sex and locality, but generally ranges from 300–390 mm TL (Dundee & Dundee 1965; Taber et al. 1975; Peterson et al. 1988). While sex could not be determined for most animals captured during this study period, one small individual of 285 mm TL was verified as sexually mature during late summer because of a swollen cloaca. Due to this capture as well as the general lack of larger adults in Little River, sexual maturity was estimated at 275 mm TL for this analysis. All individuals measuring 125–275 mm TL were considered subadults. Finally, search effort was calculated as the number of person hours required to locate one hellbender. Data analyses were completed using Microsoft Excel for Mac (2008) and R (version 2.12.2; R Development Core Team 2008).

## RESULTS

During 2000–2010, there were 533 total hellbender captures (168 larvae, 159 subadults, and 206 adults) including 33 recaptures of 27 individuals. Three hundred fifty-six individuals were tagged. Sex was determined for 38 individuals (23 males; 15 females). In 2000, search effort to collect one hellbender was 2.54 hrs ( $n = 33$ ; Nickerson et al. 2002). During additional surveys by the University of Florida from 2008–2010, search effort varied annually [2008 = 3.43 hrs/hellbender ( $n = 32$ ); 2009 = 5.01 hrs/hellbender ( $n = 6$ ); 2010 = 2.50 hrs/hellbender ( $n = 80$ )] and was 2.88 hrs/hellbender across all three years ( $n = 118$ ). Mean TL ( $\pm$ SD) for hellbenders across all years in Little River ( $n = 500$ ) was 218.1 mm ( $\pm$ 130.1). Mean mass ( $\pm$ SD) of hellbenders of all size classes ( $n = 494$ ) was 115.1 g ( $\pm$ 142.5), but was influenced by the large number of larval individuals. Mean mass ( $\pm$ SD) of adults ( $n = 183$ ) was 266.6 g ( $\pm$ 128.3). All three life stage classes were well represented over the study period, and 25% of the total captured individuals were classified as larvae. A sharp decline from the 50–75 mm TL size class to the 75–100 mm TL size class was noted, suggesting low survival of hellbenders between the first and second year (Fig. 1). Size class distribution varied among years, but larvae were generally abundant in the population samples (Fig. 2). Hellbender size class distributions from Little River in 2006 ( $n = 113$ ) and 2008 ( $n = 117$ ) were statistically different from the 1969 North Fork of the White River population ( $n = 478$ ; Fig. 3) based on results of Kolmogorov-Smirnov bootstrap tests ( $D = 0.584$ ,  $p < 0.001$ ;  $D = 0.284$ ,  $p < 0.001$ , respectively).

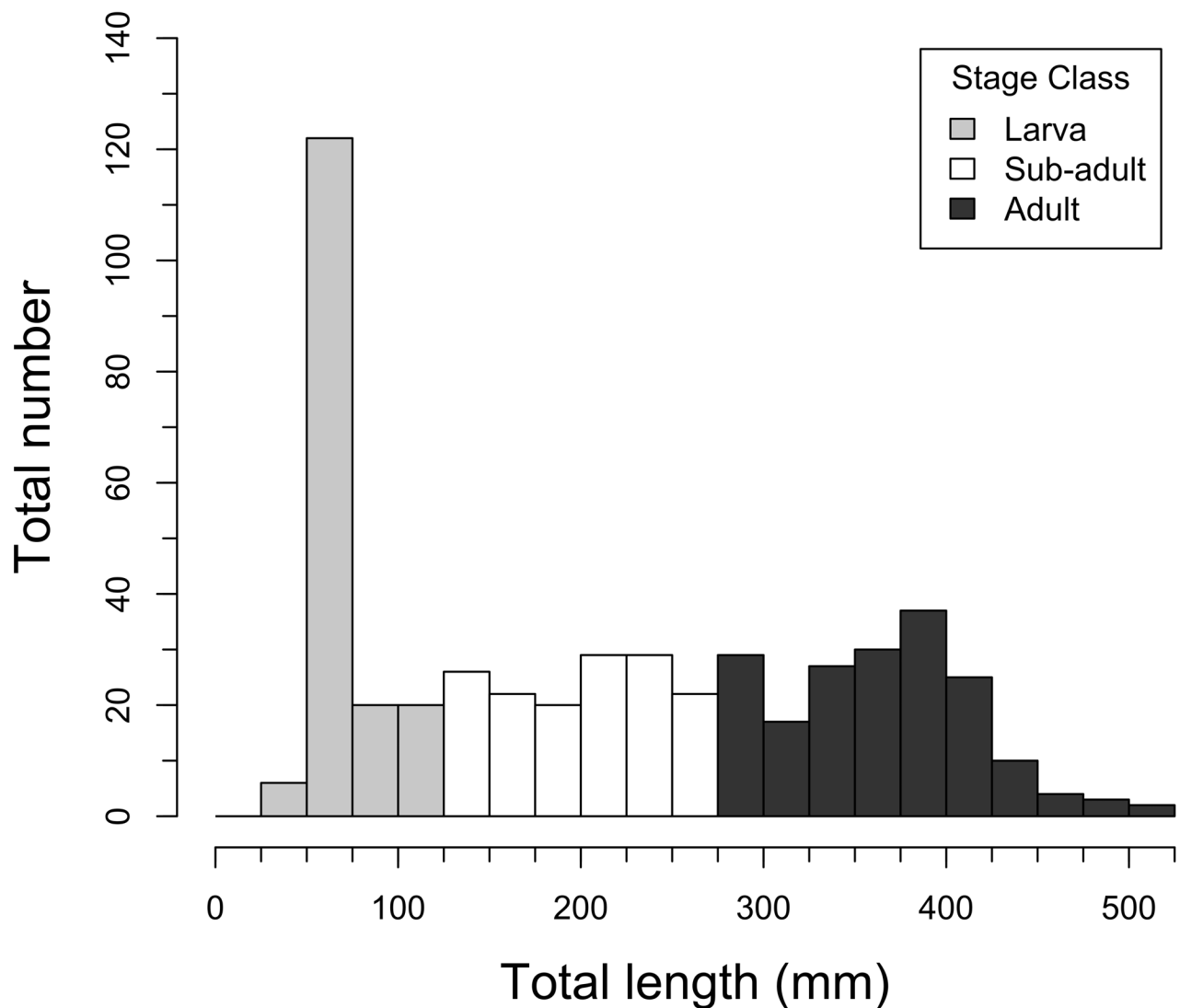
## DISCUSSION

An understanding of the overall population structure, particularly over time, was needed to verify that the Little River population was in fact unique in its larval component from the majority of studied populations. Overall, the population in Little River over the last decade appears stable with regular recruitment of young individuals and representation of all size classes. Our results were consistent with the results of Nickerson

et al. (2002) as larvae represented a significant proportion of the sampled hellbender population both overall and in individual years. Although we captured more adult *Cryptobranchus alleganiensis* since the original study by Nickerson et al. (2002), the general trend of capturing few large adults over 450 mm TL remained. Over the 10 year study period, we captured fewer adults in every size interval, particularly > 475 mm, than were captured in the North Fork of the White River in 1969 (Fig. 4). It remains unclear, however, whether these observations represent true differences in population structure or differences in detectability.

Studies in the Little River suggest that larval-

sized hellbenders primarily utilize cobble and boulders for shelter (Nickerson et al. 2003; Freake & Hecht unpubl. data). Unlike rivers where larvae have been located within gravel beds (Nickerson et al. 2003), larval hellbenders in the Little River can be readily sampled using standard skin-diving methods. Researchers in other localities have not normally used methods to search additional habitats where larval hellbenders might be located (Nickerson & Krysko 2003; Foster et al. 2009). A recent study in the Allegheny River drainage of New York found that despite a decrease in the density of *Cryptobranchus alleganiensis* at study sites within the last 20 years, more individuals

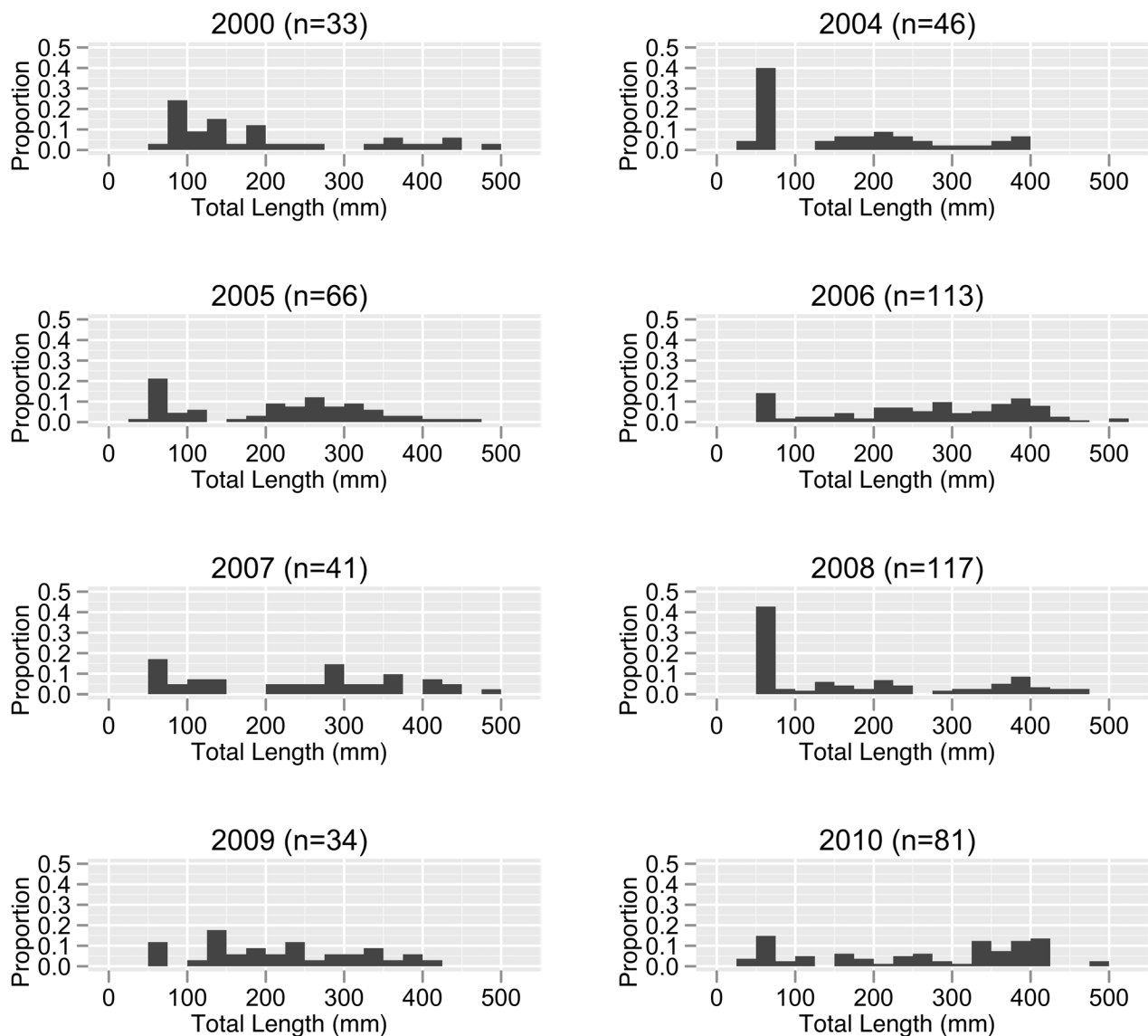


**Figure 1.** Size distribution of captured hellbenders (*Cryptobranchus alleganiensis*) from 2000–2010 in the Little River, Tennessee (n=500).

< 20 mm were captured recently than in the 1980s presumably because of methods specifically targeting these size classes (Foster et al. 2009). It is also unclear how deep larvae may reside within gravel beds in other localities so many larvae may not be accessible even with methods specifically targeting their habitat. Larval hellbenders could potentially be present in some other localities, but not adequately represented in the sample due to low detectability rates. Larger adults may also avoid detection in Little River. Due to the density of rocks and the presence of very large boulders

that could not be lifted, individuals may have been missed during surveys. In addition, deep pools > 3 m in depth, which *C. alleganiensis* sometimes inhabit in other rivers (Green 1933; Nickerson & Mays 1973a), were not surveyed.

Recent studies conducted in other localities within the Blue Ridge Province have also produced young *Cryptobranchus alleganiensis* (Maxwell 2009; Groves & Williams 2011; Burgmeier et al. 2011; Freake unpubl. data). Approximately 21% of hellbenders captured during surveys in the Hiwassee River of the Cherokee National Forest

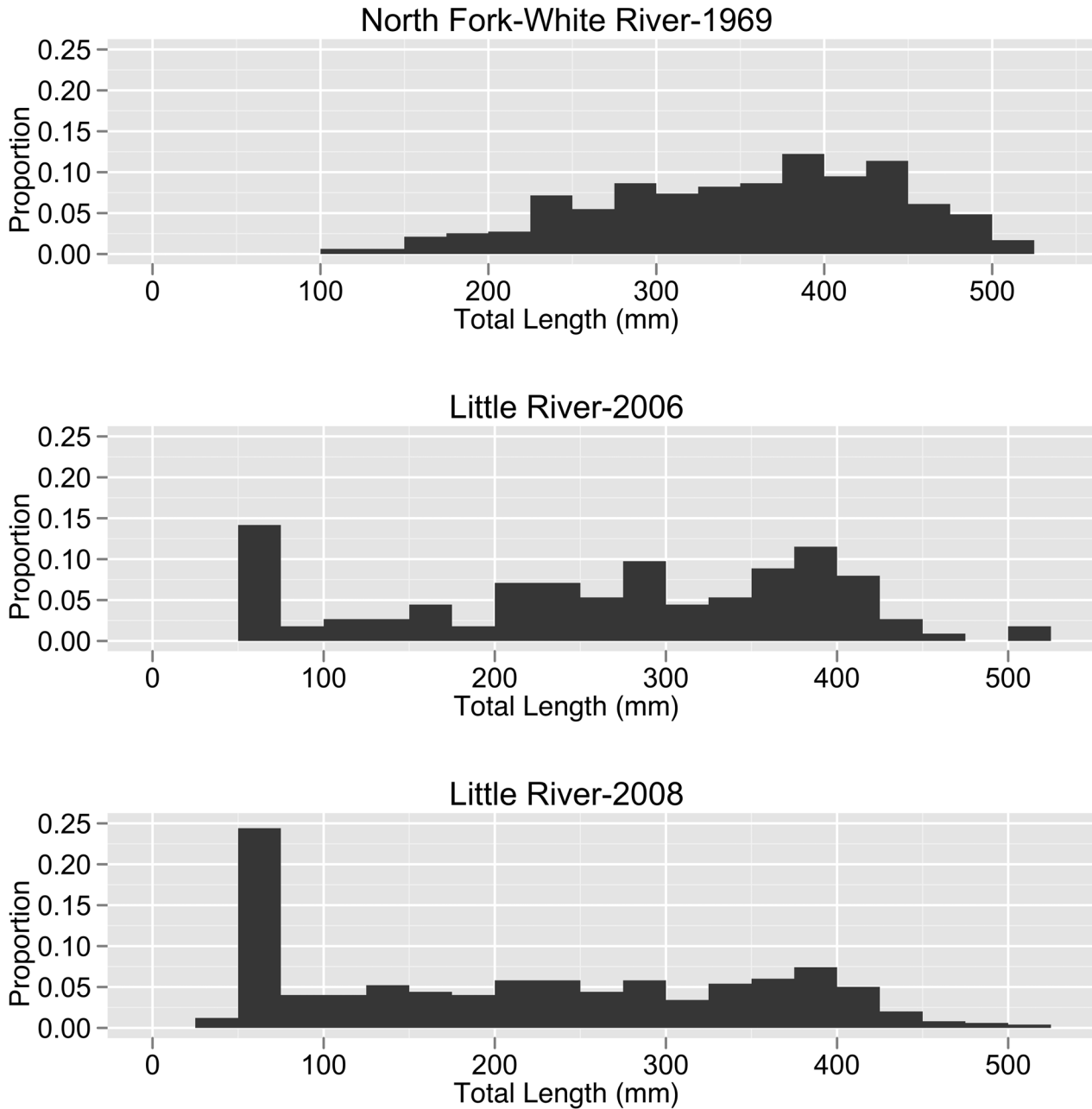


**Figure 2.** Yearly size distribution of captured hellbenders (*Cryptobranchus alleganiensis*) from 2000–2010 in the Little River, Tennessee.

in Tennessee were larval-sized individuals (Freake unpubl. data). Short surveys of the Pigeon River in North Carolina’s Blue Ridge region produced 3 larvae out of only 6 individuals captured (Maxwell 2009). Larvae were located in northern Georgia and other western North Carolina populations (Burgmeier et al. 2011; Groves & Williams 2011). These Blue Ridge populations also do not appear to be impacted by disease and/or serious

abnormalities (Groves & William 2011; Gonynor et al. 2011; Souza et al. 2012) as in other regions (Miller & Miller 2005; Hiler et al. 2005, Nickerson et al. 2009).

Due to geology, topography, and history, the Blue Ridge Province, which has the highest proportion of interior forest habitat in the Southern Appalachian region, remains 80% forested (SAMAB 1996a, 1996b). Relatively

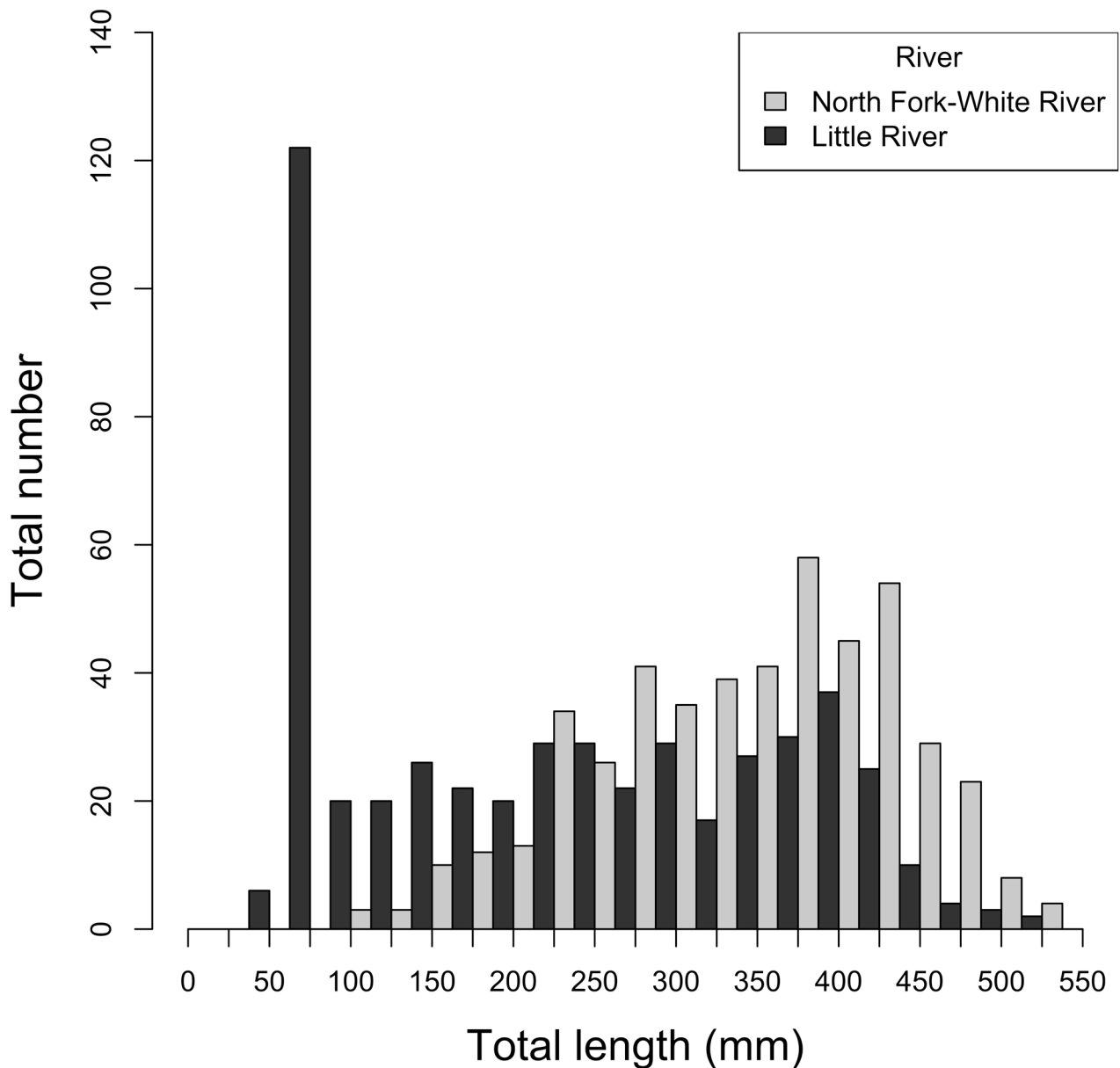


**Figure 3.** Comparison of hellbender (*Cryptobranchus alleganiensis*) size class distributions sampled from the Little River, Tennessee in 2006 (n=113) and 2008 (n=117), with the North Fork of the White River, Missouri in 1969 (n=478).

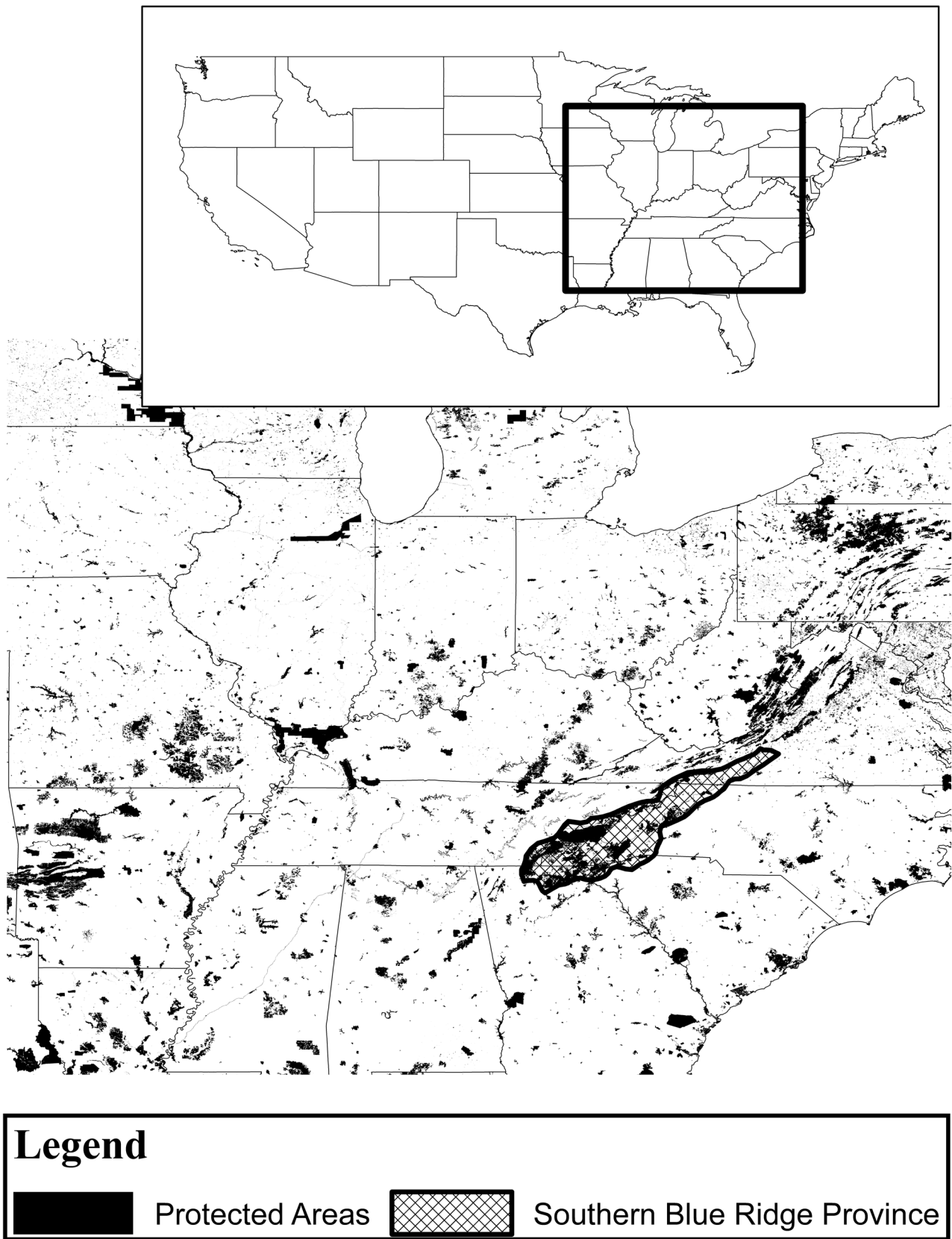


large portions of the Blue Ridge, including the greatest concentration of public lands in the eastern United States, are now protected due to aesthetics and ecological value (SAMAB 1996a, 1996b; Fig. 5). Therefore, the abundance of larvae seen throughout the Blue Ridge Province may be partially due to the decrease in factors which have been suspected in hellbender declines such as siltation, channelization, agriculture, mining, and pollution (Dundee 1971; Nickerson and Mays

1973a; Bury et al. 1980). Recent studies by Groves and Williams (2011) noted a negative correlation between human development and hellbender densities, but the finding was not statistically significant. Many historically studied hellbender populations in West Virginia’s Appalachian Plateau and Valley and Ridge regions appear to be declining, except for some located in the protected Monongahela National Forest (Keitzer 2007). This supports the hypothesis that human disturbance,



**Figure 4.** Size class distribution of hellbenders (*Cryptobranchus alleganiensis*) captured in the Little River, Tennessee from 2000–2010 (n=500) and the North Fork of the White River, Missouri in 1969 (n=478).



**Figure 5.** Map of the eastern United States showing protected areas in the southern Appalachian and Ozark regions (Modified from Fenneman and Johnson 1946; U.S. Geological Survey 2011).

rather than geology alone, may be a major influence on hellbender populations.

Life stage classes were relatively well represented throughout the study period, but many size classes were absent or low in abundance in the individual years. Water regimes can influence the population structure of stream-dwelling amphibians by affecting mortality and recruitment (Metter 1968; Duellman and Trueb 1986). Flooding has been suspected as a source of mortality in hellbenders (Trauth et al. 1992; Humphries 2005; Miller & Miller 2005; Nickerson et al. 2007), but its influence on population dynamics remains unclear. Nickerson et al. (2007) noted that following flooding of the Middle Prong of Little River in 2003, no individuals were captured within the stream the following year despite previously finding four larvae in only eight hours of searching. Second year larvae were also absent from the main portion of Little River in 2004. In 2005, no individuals 125–150 mm TL were captured, and only three individuals measuring 150–200 mm TL were found. Additional small-scale flooding events in 2009 correlated with a missing size class (small subadults from 125–150 mm TL) the following year.

Nickerson et al. (2007) examined the potential impacts of flooding on hellbenders in the Middle Prong of Little River, and cited USGS stream flow readings from station 03497300 beginning in 1997. An examination of peak stream flow data taken at the station within Little River prior to 1997 revealed an extreme flooding event in 1994, where peak stream flow was over 750 m<sup>3</sup>/s (Fig. 6). Unfortunately no data on *Cryptobranchus alleganiensis* populations in Little River are available prior to 2000 to illuminate the effects of this flood on hellbender population structure. However, data from the Great Smoky Mountain National Park's fisheries division found no young of year brown trout (*Salmo trutta*) and few young of year rainbow trout (*Oncorhynchus mykiss*) following the 1994 flooding, suggesting that other taxa were affected by the flooding (Kulp pers. comm.). It is therefore possible that this extreme flooding event also had a substantial impact on the hellbenders in

Little River, potentially contributing to the lack of large individuals seen in the river today.

As individual growth rates of *Cryptobranchus alleganiensis* slow with age (Taber et al. 1975; Peterson et al. 1988) and no growth studies are available for the Little River population, it is difficult to follow cohorts through time based on the available data. However, two size classes (125–150 mm; 300–325 mm), possibly correlating to flooding events in 2003 and 2009 (Fig. 6), were under-represented in Little River's 2010 size class distribution (Fig. 2). Water regimes may be an important influence on hellbender recruitment in Little River, leading to long-term impacts on the population structure. Potential reductions in recruitment following flooding events could be related to larval *C. alleganiensis* habitat use within Little River. Nickerson et al. (2003) hypothesized that larval hellbenders in Little River were forced to use less secure shelters due to the lack of interstitial spaces within the gravel beds.

While turbulent current may influence size structure of the *Cryptobranchus alleganiensis* population in Little River, additional factors could also be affecting this population. Nickerson et al. (2003) suggested that the habitat used by larvae within Little River, in conjunction with relatively small crayfish populations, might explain the hellbender population structure. The relatively unsecure habitat of larval hellbenders in Little River may increase mortality by escalating predation risk and competition with both conspecifics and other organisms, leading to reduced recruitment to the adult stage. In addition, the studied portion of Little River appears to have relatively low densities of crayfish (Nickerson et al. 2003; Hecht & Freake unpubl. data), which could affect the size structure of adults by reducing overall growth potential or increasing mortality. Most adults captured in Little River appeared relatively thin, and the average mass of adult *C. alleganiensis* was less than reported in other localities (Nickerson & Mays 1973a; Burgmeier et al. 2011), but the impacts of this trend remain unclear.

While additional study may be needed to confirm the factors influencing Little River's

hellbender population, the overall population appears to be stable and reproducing. Long-term monitoring of the population structure will help confirm whether the lack of large adults in the last 10 years is a result of the flooding event in 1994 or is instead related to other factors, such as the reduced crayfish population in Little River. Following new cohorts after flooding events in Little River will also increase our understanding of the effects of stream flow on *Cryptobranchus alleganiensis* populations. Predictions of more frequent intense precipitation events due to climate change (Bates et al. 2008) may lead to an increase in flooding events in some hellbender streams. Flooding induced mortality may therefore become an important consideration in future hellbender conservation efforts.

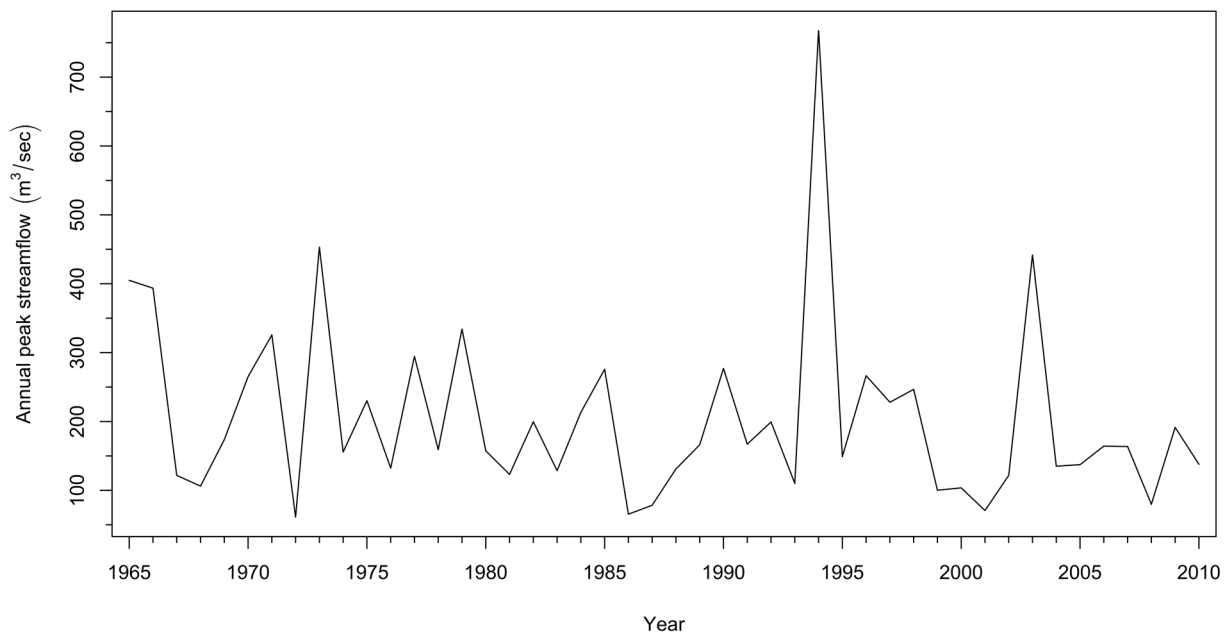
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**Figure 6.** Peak streamflow of water years 1965–2010 at Little River, Tennessee, USGS station within the Great Smoky Mountains National Park (Modified from U.S. Geological Survey 2001).

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# POPULATION ECOLOGY OF THE SNAPPING TURTLE (*CHELYDRA SERPENTINA OSCEOLA*) IN A NORTHERN FLORIDA RIVER

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## ABSTRACT

Snapping turtles (*Chelydra serpentina*) occur in nearly every type of freshwater habitat in North America east of the Rocky Mountains, but little is known about the ecology of populations in the southern part of their range, as well as those in lotic habitats. We conducted a mark-recapture study of the Florida snapping turtle (*C. s. osceola*) in the Santa Fe River in northern Florida between August 2005 and November 2010. Ninety-six percent of all captures occurred within a 9 km section of the river that receives direct input from 21 artesian springs. Within this “high density spring area,” population density and biomass of adults were 2.7 turtles/ha and 24.9 kg/ha, respectively. The total sample (n = 113 turtles) consisted of 14.2% immature individuals, 45.1% adult females, and 40.7% adult males. Adult sex ratio was 1:1. Juveniles occupied spring and spring run habitats disproportionately to the river habitat. Adult males (straight midline carapace length [CL] 243–439 mm, mean = 360 mm) are the largest known in Florida, and adult females (CL 257–380 mm, mean = 325 mm) are similar in size to the largest known conspecifics in Nebraska and South Dakota. The large body sizes in our population are inconsistent with previous studies that indicate a positive relationship between body size and latitude in this species, emphasizing the importance of habitat type in influencing demography. Large body sizes in the Santa Fe River may be related to the thermal/food resources provided by artesian springs, the physical environment of the riverine habitat, or coexistence with alligator snapping turtles (*Macrochelys temminckii*).

**Key Words:** artesian spring, *Chelydra serpentina*, demography, Florida, population, Santa Fe River.

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## INTRODUCTION

Snapping turtles (*Chelydra serpentina*) have one of the broadest geographic distributions of all North American freshwater turtles, occurring in nearly every type of lentic and lotic habitat east of the Rocky Mountains (Buhlmann et al. 2008; Ernst & Lovich 2009). Despite many ecological studies of *C. serpentina* (Steyermark et al. 2008), little is known about populations in the southern part of its range (Aresco & Gunzberger 2007). Less is known about *C. serpentina* populations in lotic habitats, even though this species is well suited for life in rivers. The characteristics of *C. serpentina* fit many of those described for river-adapted turtles (Moll & Moll 2000), including strong swimmers or bottom-walkers, large size, Type 1 reproduction with many small eggs, diverse foraging abilities, streamlined body shape, and body more dense than water. This gap in the lotic habitat literature is substantial, given the fact that demographic and life history parameters vary widely among populations of *C. serpentina* and other turtle species (Tinkle 1961; Gibbons & Tinkle 1969; Galbraith et al. 1989; Brown et al. 1994; Iverson et al. 1997; Litzgus & Mousseau 2004; Moll & Iverson 2008; Iverson & Smith 2010). Furthermore, *C. serpentina* has been harvested historically for food (Clark & Southall 1920; Tucker & Lamer 2004; Aresco et al. 2006), and effective management and conservation of this species requires geographically relevant information.

We conducted a mark-recapture study of *Chelydra serpentina* as part of a broad investigation of the turtle assemblages inhabiting the Santa Fe River in northern Florida (Johnston et al. 2011). Because of the uniqueness of the Santa Fe River population of *C. serpentina* in the southern part of this species' range, we gathered as much information as possible about these turtles. In this paper, we describe spatial distribution, population structure, density, and biomass of *C. serpentina* in the Santa Fe River.

## MATERIALS AND METHODS

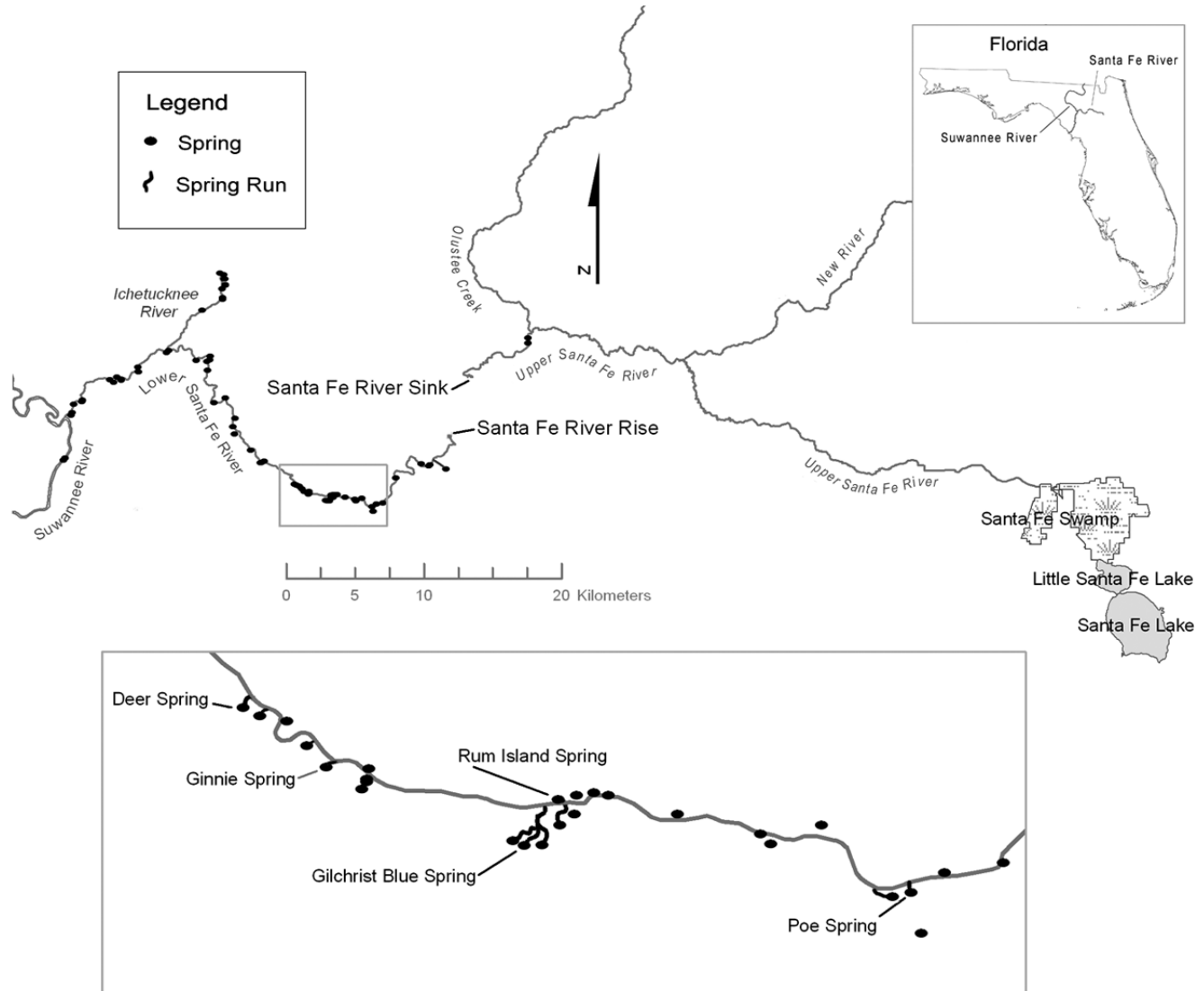
### STUDY SITE

Originating in the Santa Fe Swamp in

northern Florida, the Santa Fe River is classified as a blackwater stream due to areas of highly tannin-stained water (Florida Natural Areas Inventory 2010), but it is a heterogeneous system that becomes increasingly influenced by springs and, as a result, has enhanced water clarity as it flows along its ~115 km course to the Suwannee River (Fig. 1; Hornsby & Ceryak 1998; Scott et al. 2004; Butt et al. 2007). Approximately 60 km downstream from its origin, the upper Santa Fe River disappears underground into a swallet known as the Santa Fe River Sink (Fig. 1). The subterranean river then re-emerges 5 km away at a site known as the Santa Fe River Rise (Fig. 1). From River Rise, the lower Santa Fe River flows approximately 50 km to the Suwannee River, receiving substantial spring input in its final 37 km.

In the upper Santa Fe River, tannin-stained water inhibits growth of submerged aquatic macrophytes. Vegetation in this portion of the river is therefore limited to patches of emergent and floating plants such as spatterdock (*Nuphar advena* Aiton), duckweed (*Lemna* sp.), water spangles (*Salvinia minima* Baker), and water hyacinth (*Eichhornia crassipes* Martius). Coarse woody debris (e.g., partially submerged fallen trees and completely submerged logs) is abundant. The substrate consists of patches of limestone cobble and a sand/organic mix. The upper Santa Fe River varies in width (2–40 m) and depth (< 1–3 m) during average base flow. Water temperatures fluctuate seasonally (10–30°C).

The habitat in the first 13 km of the lower Santa Fe River is similar to the upper Santa Fe River, except that it is consistently wider (20–30 m) and more thermally stable (20–26°C). In the next 9 km downstream, between Poe Spring and Deer Spring (i.e., the “high density spring area”; Fig. 1), the habitat changes substantially due to direct input from 21 artesian springs (two first magnitude, ten second magnitude, nine third magnitude; Scott et al. 2004). Because of the improved water clarity associated with spring input, this “high density spring area” supports large patches of submerged aquatic vegetation including tapegrass (*Vallisneria americana* Michaux), Indian swampweed (*Hygrophila polysperma* Roxburgh), and hydrilla



**Figure 1.** Map of the Santa Fe River drainage in northern Florida. “High density spring area” inset at bottom.

(*Hydrilla verticillata* Linnaeus). Scattered patches of water hyacinth (*Eichhornia crassipes* Martius) are also present. Mid-channel water depth varies between 0.5 and 3.5 m. Although the mid channel substrate is generally hard limestone, a soft sand/organic substrate ( $\leq 1$  m deep) occurs along the edge of the river and in the spring runs feeding the river. Water temperatures in this section of the river vary seasonally between 21 and 24°C, but spring water is consistently 22–23°C. Scott et al. (2004) provided a detailed description of the spring habitats and spring runs that connect springs to the river. In the next 17 km downstream, the habitat is

similar to that of the upstream 9 km river reach but current velocity is proportionately greater and soft sand/organic substrate is rare. In the final 11 km of the river downstream from the confluence with the Ichetucknee River, current velocity increases further, and water depth increases substantially (3–5 m). Aquatic plants, soft sand/organic substrate, and coarse woody debris are rare. The substrate is primarily limestone.

#### SAMPLING

Turtles were sampled throughout the Santa Fe River, except in the section upstream from the confluence with the New River (Fig. 1), between

August 2005 and November 2010 using hoop traps baited with fresh cut fish. Three different trap sizes were used (76 cm diameter, 2.5 cm mesh; 91 cm diameter, 2.5 cm mesh; 122 cm diameter, 6.4 cm mesh) to facilitate placement of traps in sites of varied water depths. Traps were typically placed immediately upstream of snags, submerged logs, undercut banks, and outer bends of the river, with a minimum of 50 m between traps. During each trap session, 8–20 traps were set during late afternoon and checked the next morning. Each trap set overnight constituted one trap-night (TN). Trap captures were supplemented by opportunistic hand captures.

Each captured turtle was measured for straight midline carapace length (CL), maximum carapace length ( $CL_{max}$ ), plastron length (PL), length of posterior lobe of the plastron (PPL), and precloacal tail length (PCL) to the nearest 1 mm using Haglof aluminum tree calipers and weighed to the nearest 1 g using Pesola® spring scales. To individually mark each turtle, we drilled holes in the marginal scutes and peripheral bones using a standard numbering system (Cagle 1939; Froese & Burghardt 1975; Gibbons 1990a) and inserted Passive Integrated Transponder (PIT) tags into the ventrolateral tail muscle (Trauth et al. 1998). Sex was determined by the ratio of PCL to PPL (female < 0.86, male > 0.86; Mosimann & Bider 1960). Females were palpated for eggs, and size at maturity was based on the size of the smallest gravid female. Males were considered mature when they attained a CL at least 55% of the maximum observed in this population (Mosimann & Bider 1960; White & Murphy 1973; Christiansen & Burken 1979). Immature individuals whose sex could not be determined were considered juveniles, whereas immature individuals whose sex could be determined were considered subadults. To quantify the relative difference in body size (CL) between adult females and adult males, we calculated the sexual dimorphism index (SDI) advocated by Lovich & Gibbons (1992): mean size of larger sex divided by mean size of smaller sex with the result arbitrarily defined as positive (minus one) when females are the larger sex and negative (plus one)

in the converse case.

#### POPULATION DENSITY AND BIOMASS ESTIMATION

Because the population was not evenly distributed throughout the Santa Fe River, we estimated population density within the 9 km (area = 34.9 ha) “high density spring area” between Poe Spring and Deer Spring where 94.6% of all 2005–2009 captures occurred. To determine population density in this area during 2010, we thoroughly sampled this section of river and all adjacent springs and spring runs, dividing sampling into two periods (period 1: March–June; period 2: July–November). We used the Lincoln-Petersen model to estimate population size. Although riverine turtle populations generally do not meet the assumption of population closure that is necessary for applying the Lincoln-Petersen estimator, we considered it appropriate for *Chelydra serpentina* at our study site for three reasons. First, a concurrent radio-telemetry study of 10 adult females and 10 adult males in the “high density spring area” provided no evidence of emigration from this area (E. Suarez unpublished data). Second, no individuals trapped in this area were ever trapped outside of this area. Third, only one individual captured outside this area was ever recaptured inside this area. Population size was estimated separately for adult females and adult males. To determine population density, each population size estimate was divided by the area of the “high density spring area” (34.9 ha). Biomass (kg/ha) for each sex was calculated by multiplying population density by mean body mass.

## RESULTS

#### SPATIAL DISTRIBUTION

We captured 113 individual *Chelydra serpentina osceola* (253 total captures), but captures were not evenly distributed throughout the Santa Fe River. No individuals were captured in the upper Santa Fe River (40 trap sessions, 423 TN). In the lower Santa Fe River, capture rates were significantly higher in the “high density spring area” (0.21 captures/TN/session, 59 sessions, 923 TN) than in all other areas combined (0.01 captures/TN/session, 30 sessions, 331 TN; Mann-Whitney Rank Sum Test,  $U = 192.000$ ,  $p < 0.001$ ). Within

the “high density spring area,” capture rates were significantly higher in river habitat (0.27 captures/TN/session, 40 sessions, 637 TN) than in adjacent spring and spring run habitat (0.10 captures/TN/session, 19 sessions, 286 TN; Mann-Whitney Rank Sum Test,  $U = 162.500$ ,  $p < 0.001$ ).

#### BODY SIZE AND POPULATION STRUCTURE

Our sample consisted of 7.1% ( $n = 8$ ) juveniles, 7.1% ( $n = 8$ ) subadults, 45.1% ( $n = 51$ ) adult females, and 40.7% ( $n = 46$ ) adult males (Fig. 2). Immature individuals averaged 179 mm CL (range 40–254;  $SD = 57.8$ ), 187 mm  $CL_{max}$  (range 43–265;  $SD = 60.2$ ), 135 mm PL (range 29–193;  $SD = 44.1$ ), and 1615 g body mass (range 19–3650;  $SD = 1137.5$ ). The smallest turtle was a hatchling (CL = 40 mm,  $CL_{max} = 43$  mm; PL = 29 mm; mass = 19 g) captured 31 October 2009 with a yolk scar and no discernible growth annuli. The adult sex ratio did not differ significantly from 1:1 ( $\chi^2 = 0.36$ ,  $df = 1$ ,  $p = 0.549$ ). Gravid females ( $n = 14$ ) captured between 8 March and 28 May (86% captured in April) were 257 to 367 mm CL (mean = 320 mm). Adult males were significantly larger than adult females: mean male CL = 359.8 mm (243–439), mean female CL = 324.7 mm (257–380), Mann-Whitney Rank Sum Test,  $U = 614.000$ ,  $p < 0.001$ ; mean male  $CL_{max} = 374.2$  mm (256–450), mean female  $CL_{max} = 335.8$  mm (272–390), Mann-Whitney Rank Sum Test,  $U = 543.500$ ,  $p < 0.001$ ; mean male PL = 262.7 mm (175–318), mean female PL = 246.3 mm (187–293), Mann-Whitney Rank Sum Test,  $U = 758.000$ ,  $p = 0.003$ ; mean male mass = 11.46 kg (3.20–20.00), mean female mass = 7.61 kg (3.45–11.75), Mann-Whitney Rank Sum Test,  $U = 572.500$ ,  $p < 0.001$ ). The SDI using CL was -0.11.

Population structure differed between river and adjacent spring and spring run habitat (Table 1). In particular, juveniles comprised a significantly higher proportion of the sample in the spring and spring run habitat than in the river habitat. The five smallest turtles (40–148 mm CL) were captured only in spring and spring run habitat. The smallest turtle in river habitat measured 149 mm CL.

#### POPULATION SIZE AND BIOMASS

In the “high density spring area” during 2010, the estimated numbers of adult females and adult males were 55 (95% CI = 41–69) and 39 (95% CI = 24–54), respectively. Densities were 1.6 adult females/ha and 1.1 adult males/ha. Because adult females averaged 7.6 kg, adult female biomass was estimated to be 12.2 kg/ha. Adult males averaged 11.5 kg resulting in an adult male biomass estimate of 12.7 kg/ha. When data for both sexes were combined, population density and biomass estimates were 2.7 adults/ha and 24.9 kg/ha, respectively.

## DISCUSSION

#### SPATIAL DISTRIBUTION

Prior to this study, *Chelydra serpentina* was known to occur in the Santa Fe River, but little was known about population distribution and structure (Iverson & Etchberger 1989; Aresco et al. 2006). This study demonstrates that the population of *C. serpentina* was not evenly distributed throughout the Santa Fe River. The vast majority of individuals occupied a 9 km reach of the lower Santa Fe River and adjacent springs between Poe Spring and Deer Spring. The restricted distribution of this

**Table 1.** Relative proportions of four size/sex classes of *Chelydra serpentina osceola* in two different habitats in the Santa Fe River drainage. Numbers of individuals in parentheses. Of the 113 individuals captured in this study, 16 were captured at least one time in each habitat.

	River	Spring/Spring Run	z-test
Juvenile	0.031 (3)	0.147 (5)	$Z = 1.999$ ; $p = 0.046$
Subadult	0.053 (5)	0.088 (3)	$Z = 0.311$ ; $p = 0.756$
Adult female	0.474 (45)	0.382 (13)	$Z = 0.725$ ; $p = 0.469$
Adult male	0.442 (42)	0.382 (13)	$Z = 0.405$ ; $p = 0.685$

population is not surprising because the springs and spring runs associated with this river influenced the environment in such a way that provided habitat (soft substrate, abundant aquatic vegetation) that *C. serpentina* is known to prefer (Aresco et al. 2006; Ernst & Lovich 2009). Specifically, greater water clarity enhanced the abundance of aquatic vegetation, and decomposing vegetation helped to create a soft organic substrate. The importance of springs to *C. serpentina* appears more complex, however. Farther downstream, more springs occurred, but abundance of *C. serpentina* was relatively low. The low abundance of *C. serpentina* may be related to less preferred habitat factors such as greater current velocity, greater depths, scarcity of woody debris, or rarity of spring run habitat.

#### POPULATION STRUCTURE

Although adult and subadult *Chelydra serpentina* used both the river habitat and the adjacent spring and spring run habitat, juveniles less than 149 mm CL appeared to prefer the spring and spring run habitat. This observation parallels the ontogenetic shift in habitat use by *C. serpentina* in a Michigan marsh (Congdon et al. 1992). In these two very different locations, juvenile *C. serpentina* appear to behave in similar ways, limiting their activity to shallow and vegetated waters that presumably provide greater foraging success and greater protection from predators. Springs may provide the additional benefit of stable temperatures that maximize growth. If, as suggested by our observations, females in the Santa Fe River nest near springs and, after hatching, juveniles migrate to these springs and spring runs where they spend their first years of life, then these unique habitats could be considered nurseries for *C. serpentina*.

The low number of small turtles in our study does not necessarily indicate low recruitment. These individuals may be common but difficult to sample effectively in spring runs where they can hide in heavy vegetation and bury themselves in soft substrate. They may also be naturally rare in this population due to rapid growth rates that reduce the window of time that this life stage is available for sampling. Germano and Rathbun (2008) reported a similar situation in a western

pond turtle (*Actinemys marmorata*) population that had a population structure caused by relatively fast growth rates rather than a rarity of young turtles. Unfortunately, we know little about juvenile growth rates in our study population. Additional support for the idea that small *C. serpentina* are rare comes from our captures of other small secretive turtle species. We hand captured during day and night 80 striped mud turtles (*Kinosternon baurii*), 73 common musk turtles (*Sternotherus odoratus*), and 391 loggerhead musk turtles (*Sternotherus minor*) in the same areas we found *C. serpentina*, suggesting small *C. serpentina* were not severely under-sampled (Mitchell & Johnston 2012).

The sex ratio between adults in the Santa Fe River population did not differ from 1:1. This is consistent with sex ratios reported from other Florida populations (Aresco et al. 2006; Aresco & Gunzburger 2007; Johnston et al. 2008). Most populations with sex ratios that deviate from 1:1 are in the northern part of the species' range, and they are all male-biased (Kiviat 1980; Galbraith et al. 1988; Steen & Gibbs 2004; Tucker & Lamer 2004; Browne & Hecnar 2007; Iverson & Smith 2010). In northern populations, skewed sex ratios may have been caused by factors such as sampling bias, differences in age at maturity, differential mortality, or nest temperatures that produce mostly males (Gibbons 1990b; Iverson & Smith 2010).

Adults in the Santa Fe River population exhibit a degree of sexual size dimorphism (SDI = -0.11) similar to other reported populations (-0.13 in a Quebec lake, Mosimann & Bider 1960; -0.05 in a South Dakota marsh, Hammer 1969; -0.15 in a Tennessee pond, Froese & Burghardt 1975; -0.03 in a variety of Iowa habitats, Christiansen & Burken 1979; -0.14 and -0.33 in central New York wetlands, Steen & Gibbs 2004; -0.11 in Florida panhandle ponds and a lake, Aresco & Gunzburger 2007; and -0.07 in southern Florida canals, Johnston et al. 2008). However, absolute sizes of females and males are unusually large in the Santa Fe River. In particular, females in the Santa Fe River are larger than any other reported females in Florida (Fig. 2; Bancroft et al. 1983; Aresco et al. 2006; Aresco & Gunzberger 2007; Johnston et al.

2008), and they are equivalent to the largest known females of the species which occur in sandhill lakes in Nebraska (mean CL = 325 mm, size at maturity = 285 mm) and South Dakota (mean CL = 319 mm; size at maturity = 254 mm) (Hammer 1969; Iverson et al. 1997; Moll & Iverson 2008). Fewer comparative data are available for males, but Santa Fe River males are the largest reported in Florida (Fig. 2; Aresco et al. 2006). Large males have been reported from northern populations (429 mm CL<sub>max</sub> in North Carolina [Palmer & Braswell 1995], 415 mm CL in Virginia [Mitchell 1994], 432 mm CL in West Virginia [Green & Pauley 1987], 495 mm CL in Minnesota [Harding 1997], 507 mm CL in Massachusetts [Hunter et al. 1992]), but it is unknown whether these were exceptional individuals or representative of males from their populations. To clarify the apparent uniqueness of Santa Fe River *Chelydra serpentina* adult body sizes, more data are needed regarding male sizes from populations throughout the species' range, as well as data regarding sizes of both sexes throughout Florida.

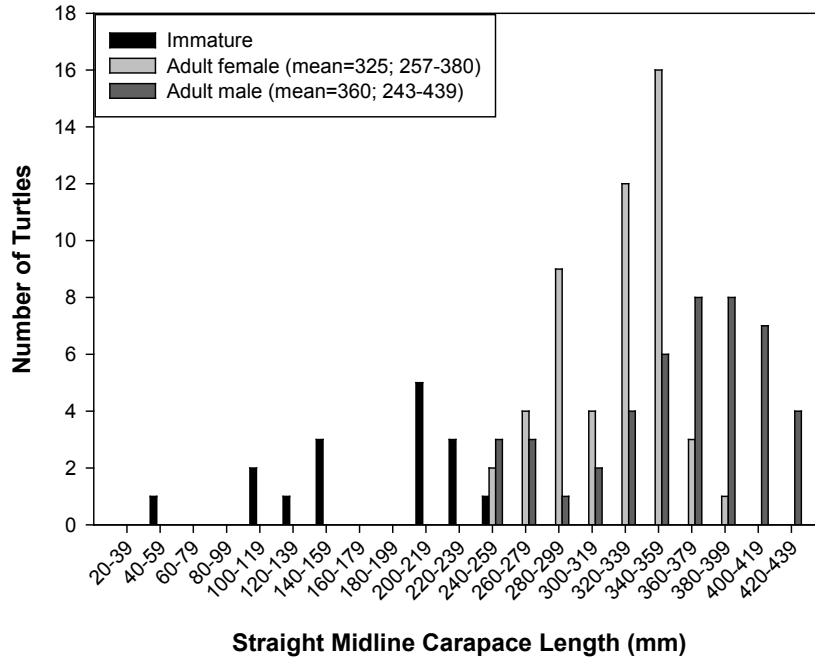
The large body sizes we observed were not anticipated prior to this study. Based on data from populations in lentic habitats throughout eastern North America, Iverson et al. (1997) and Moll and Iverson (2008) reported that average female body size (CL, PL, mass) is positively correlated with latitude and elevation. Our data do not fit that pattern and therefore require explanation. Because published hypotheses explaining large body sizes in populations at high latitudes and high elevations generally involve some aspect of winter survival (Moll & Iverson 2008), we must consider other hypotheses for large body sizes in our northern Florida river where winter temperatures are milder than those typical of northern climates or high elevations. We suspect that some aspect of the habitat is responsible for our observations. Furthermore, the factor(s) responsible for the large sizes of Santa Fe River *Chelydra serpentina* probably affect both sexes equally because relative sizes of males and females in this population are similar to relative sizes in other populations.

Perhaps large body sizes are a phenotypic

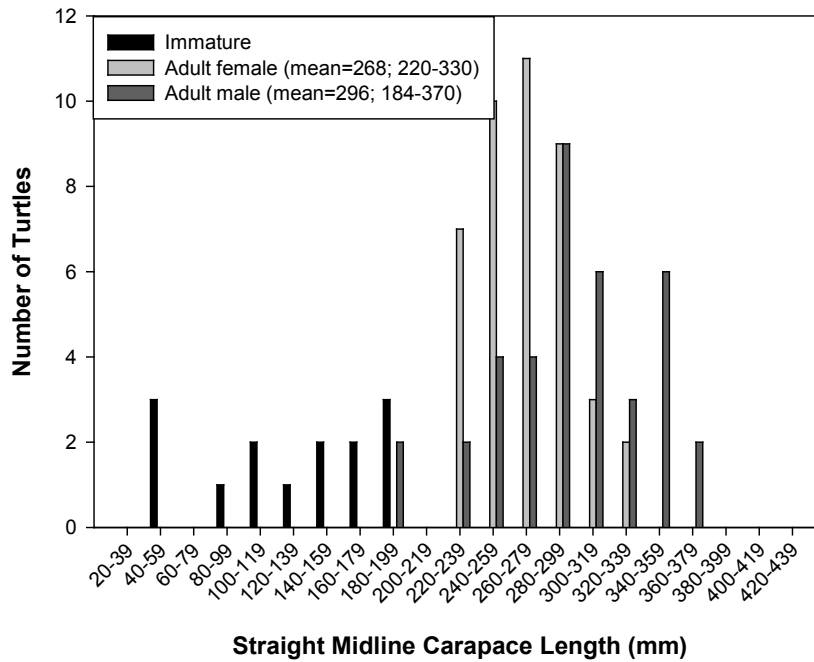
response to habitat that provides optimal conditions for growth. Food abundance, food quality, and temperature are the primary proximate environmental factors affecting turtle growth rates (Gibbons 1967; Moll 1976; Parmenter 1980; Williamson et al. 1989; Avery et al. 1993). Springs may provide the stable water temperatures (i.e., more growing days) and abundant, high quality food sources that maximize growth and body size of *Chelydra serpentina*. Similar effects have been observed in female yellow-bellied sliders (*Trachemys scripta scripta*) inhabiting South Carolina's barrier islands (Gibbons et al. 1979) and a lake that receives thermal effluent from a nuclear reactor (Gibbons et al. 1981). Brown et al. (1994) studied the effect of habitat productivity on growth and body size of adult female *C. serpentina* in Quebec and observed faster growth rates in a eutrophic pond than in an oligotrophic lake (but no difference in body size). Although fast growth does not necessarily result in larger body size, the cooler temperatures in the Quebec habitats compared to our study site may explain why Quebec snapping turtles in the eutrophic habitat did not grow to larger sizes. If the food resources and thermal regime of springs are responsible for the large *C. serpentina* body sizes we observed, then large body sizes should not be limited to the Santa Fe River. Rather, they should occur in the many spring runs throughout northern and central Florida. Future studies of growth and body size in Florida spring runs should provide the information necessary to test this "optimal growth conditions" hypothesis.

It is also possible that large body size is a genotypic response to some aspect of the Santa Fe River environment. For example, large body size may be an adaptation that facilitates more powerful locomotion by this bottom-walking species in fast current. Such an adaptation would be analogous to the more streamlined, hydrodynamic shell morphology of riverine river cooters (*Pseudemys concinna*) in comparison with conspecific populations in lentic habitats (Rivera 2008). Another adaptive explanation could be that *Chelydra serpentina* need to be large to coexist with alligator snapping turtles (*Macrochelys*

A. Santa Fe River (this study)



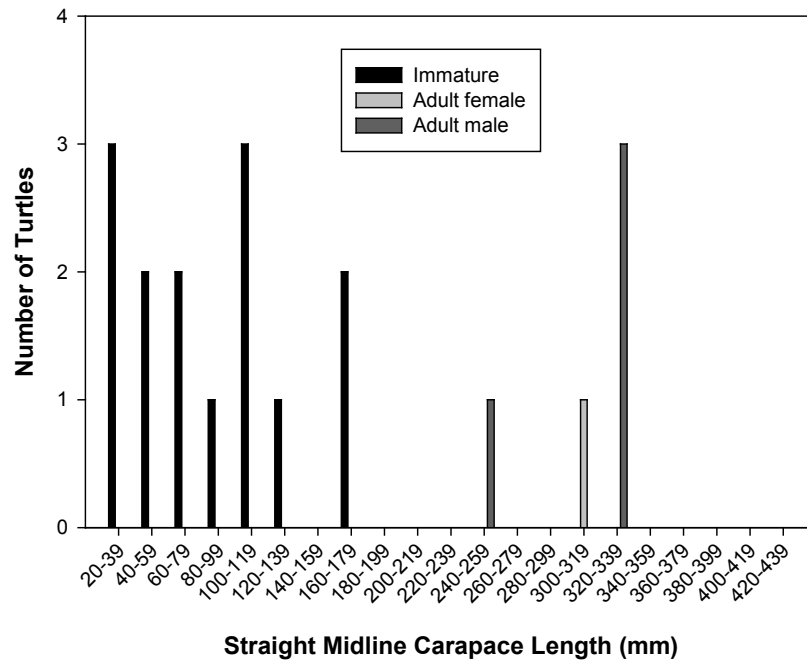
B. Florida panhandle ponds and lake (Aresco & Gunzburger 2007)



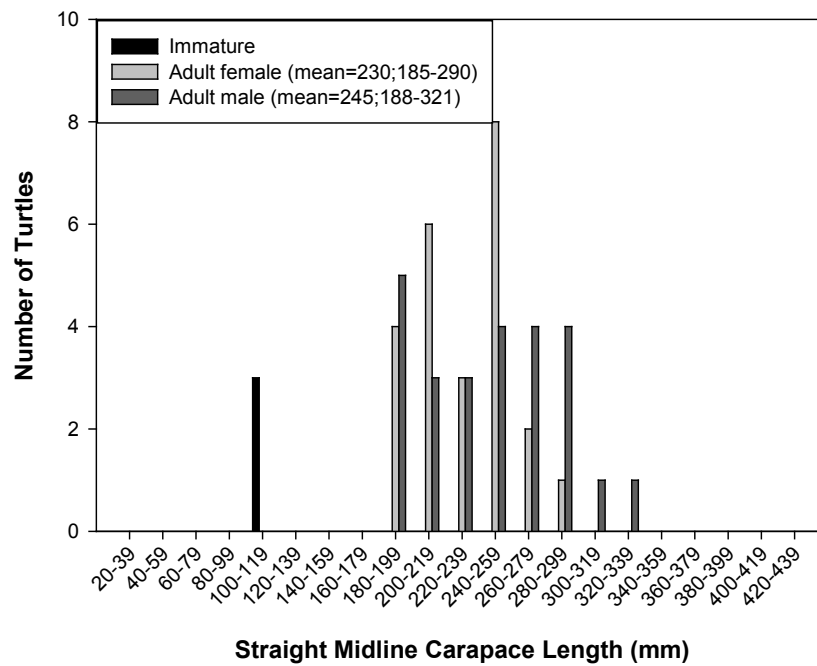
**Figure 2.** Population size structure of snapping turtles (*Chelydra serpentina*) at four different localities in Florida. Each individual turtle is represented once. For turtles captured multiple times, straight midline carapace length (CL) at first capture is used. Midline carapace length is a straight, linear measurement from the anterior edge to the posterior margin at the midline.



C. Central Florida lake (Lake Conway) (Bancroft et al. 1983)



D. Broward County canals (Johnston et al. 2008)



*temminckii*). During this study, we captured 84 *M. temminckii* throughout the Santa Fe River, including 15 adults (399–623 mm CL, 14.0–54.4 kg) in the “high density spring area” of the lower Santa Fe River, and found both snapping turtle species in the same trap three times. Larger *M. temminckii* have been reported to attack and kill smaller *C. serpentina* in captivity, as well as in the wild (Shipman et al. 1994; M. Nickerson in Pritchard 2006). Given the challenges of sharing habitat with *M. temminckii*, *C. serpentina* in the Santa Fe River may have adapted to grow larger to survive agonistic encounters with *M. temminckii*. Large body size in females may also result in increased fecundity to compensate for *M. temminckii* predation on juvenile *C. serpentina*. Despite their co-occurrence in the Santa Fe River and many other rivers (Moll & Moll 2004; Buhlmann et al. 2008; Sterrett et al. 2010), surprisingly little is known about the ecological interactions between *C. serpentina* and *M. temminckii*.

Throughout North America, larger female *Chelydra serpentina* produce larger eggs and larger clutch sizes. We have no data on egg size or clutch size for the Santa Fe River population, but the size of the hatchling we captured is consistent with this pattern regarding egg size. This hatchling (CL = 40 mm; no sign of posthatching CL or PL growth) is larger than any other reported wild-hatched hatchling *C. serpentina* (CL range 16.4–38.1 mm) (Congdon et al. 1999; Ernst & Lovich 2009). Given the strong correlation between hatchling size and egg size (Ewert 1985), this large hatchling probably came from a large egg. Our sample size ( $n = 1$ ) is obviously small, but this observation suggests that future studies of body size/egg size/clutch size relationships in this population may be consistent with the currently known pattern and warrants further investigation.

Tropical snapping turtles (*Chelydra acutirostris* and *C. rossignonii*), which may achieve body sizes similar to *C. serpentina* in the Santa Fe River, deviate slightly from the *C. serpentina* body size/egg size/clutch size pattern (Iverson et al. 1997; Moll & Iverson 2008). Specifically, with increasing body size tropical snapping turtles

produce relatively smaller clutch sizes but probably more clutches. It is therefore reasonable to speculate whether *C. serpentina* in the Santa Fe River exhibit a similar pattern. Our limited data suggest they do not. The fact that 86% of the gravid females in our study were captured in April suggests only one clutch is produced per year, which is consistent with all but the southernmost (Miami-Dade County, Florida) *C. serpentina* populations that have been studied (Ewert 2000; Moll & Iverson 2008).

#### POPULATION SIZE AND BIOMASS

Our population density and biomass estimates are difficult to place in context because there are no published data from other lotic habitats with which to compare. In lentic habitats, population density and biomass vary widely from site to site (0.029–67.5 turtles/ha and 0.1–341.3 kg/ha) (Lagler 1943; Hammer 1969; Froese & Burghardt 1975; Major 1975; Kiviat 1980; Petokas 1981; Iverson 1982; Congdon et al. 1986; Galbraith et al. 1988; Congdon & Gibbons 1989; Brown 1992; Iverson et al. 2000; Tucker & Lamer 2004; Dreslik et al. 2005; Stone et al. 2005; Aresco et al. 2006; Reehl et al. 2006; Smith et al. 2006; Johnston et al. 2008; Iverson & Smith 2010). Our data fall within these ranges, but they are comparatively low, especially if all *Chelydra serpentina* throughout the entire lower Santa Fe River are considered. It is more relevant to consider the possible factors that limit *C. serpentina* abundance in the Santa Fe River and its adjacent spring systems. Although ten other native turtle species share the river with *C. serpentina* (Iverson & Etchberger 1989; Meylan 2006; Johnston et al. 2011), competition for food is not a likely limiting factor. Three major components of *C. serpentina* diet at this site are vegetation, crayfish, and snails, and these prey are all abundant (G. Johnston personal observation). Competition for space may be a factor. Both *C. serpentina* and *Macrochelys temminckii* use beaver (*Castor canadensis*) burrows along the riverbank (E. Suarez, G. Johnston personal observations), and it is possible that agonistic interactions with this larger snapping turtle species limit the availability of this source of shelter. Another form of shelter may be even more limiting, however. In the Santa

Fe River, *C. serpentina* is not present where soft substrate is rare. All life stages in our population use this substrate for concealment, but it is only available in springs, spring runs, and a relatively narrow (typically  $\leq 5$  m) strip along the river shoreline in the vicinity of springs.

#### ECOLOGICAL STATUS

Our data indicate collectively that there is a small but healthy population of *Chelydra serpentina* centered in and around the springs and spring runs in the lower Santa Fe River. A key sign of population health is the large body sizes of both sexes. This is especially important given the past history of harvest that occurred in our study area. According to Mark Wray (owner of Ginnie Springs Outdoors since 1971), “turtles of all kinds were hunted by a group of local residents during the 1960s and 1970s to the point they were almost wiped out ... all the turtles we see today, especially the big ones, weren’t here 40 years ago.” If these anecdotal observations are accurate regarding *C. serpentina*, then our data describe a population that has recovered from harvest within a relatively short period of time, perhaps because of ideal conditions for growth that may be unique to this habitat. Looking to the future, this population should not experience such a decline again. In July 2009, the Florida Fish and Wildlife Conservation Commission approved a law (Rule 68A-25.002(6)) that prohibited collection of any wild snapping turtles (*C. serpentina* and *Macrochelys temminckii*) in the State of Florida. Enforcement of this law (with the help of vigilant local residents, recreational paddlers, divers, and owners of private parks such as Ginnie Springs Outdoors and Gilchrist Blue Springs) will ensure that this unique population of large snapping turtles persists as part of a healthy riverine ecosystem.

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# HISTORICAL TURTLE POPULATION DECLINE AND COMMUNITY CHANGES IN AN OZARK RIVER

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## ABSTRACT

Turtle populations are declining worldwide yet few long term studies exist to confirm this trend. Mark-recapture data collected in 1969 and 1980 exist for the turtle community inhabiting a 4.6 km section of the North Fork of White River, Ozark County, Missouri. Using the available data, we compared the turtle communities and common map turtle (*Graptemys geographica*) populations observed in the research section in 1969 and 1980. Community composition changes indicated that red-eared sliders (*Trachemys scripta elegans*), a native species that was not observed in the research section in 1969, became established in the research section by 1980. Population estimates for *G. geographica* indicated that the population declined significantly ( $N_{1969} = 274$ ,  $N_{1980} = 139$ ,  $z = 3.39$ ,  $P < 0.001$ ) between 1969 and 1980 and the decline was associated with a marked decrease in the number of large adult females. The loss of large adult female *G. geographica* suggested the decline may have been a result of targeted harvest for the food trade as females of this species attain much larger body sizes and would therefore be preferred for the food trade. The results of this study elucidate changes that occurred in a turtle community and provide a historical baseline for comparison with future studies of this community.

**Key Words:** turtles, Missouri, *Graptemys geographica*, *Trachemys scripta elegans*, community, population decline, harvest.

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## INTRODUCTION

River turtle populations are threatened by many factors (e.g., habitat degradation and destruction, exploitation, pollution, disease) and are believed to be declining globally (Ernst et al. 1994; Buhlmann & Gibbons 1997; Jacobson 1997; van Dijk et al. 2000; Moll & Moll 2004). Because turtles are long-lived, long-term studies on the scale of decades are required to accurately assess changes in population size, yet few long-term studies exist to substantiate claims of widespread turtle population declines (Congdon et al. 1993, 1994; Foscariini & Brooks 1997; Moll & Moll 2004). Documenting population estimates and trends is essential for identifying and conserving imperiled populations (Gibbons et al. 2000) and every attempt should be made to use and publish historical data in order to evaluate changes in turtle populations and minimize the shifting baseline effect that often occurs with studies of declining populations (Pauly 1995; Zeller et al. 2005).

Extensive herpetological data dating back to 1968 exist for the North Fork of White River (NFWR), Ozark County, Missouri (Nickerson & Mays 1973; Nickerson unpubl. data). Included in the NFWR data set are data resulting from two intensive mark-recapture surveys of turtles conducted in 1969 and 1980. We revisited the available turtle data from the NFWR in an effort to 1) characterize the turtle community in the NFWR as it was during the time of the studies, 2) estimate and compare the historical population size of the predominant turtle species, and 3) provide a baseline for comparison with future studies.

## MATERIALS AND METHODS

### STUDY SITE

The NFWR is a third order river of the White River system (Nickerson et al. 2007) located in a region typified by dolomite and sandstone geology with prominent karst features (Miller & Wilkerson 2001). The NFWR receives a large volume of water from springs including Double (i.e., Rainbow) and North Fork Springs – the two largest of the 283 springs located within the North Fork Watershed (Nickerson & Mays 1973; Miller & Wilkerson

2001). Nickerson and Mays (1973) documented many of the abiotic and biotic characteristics of the NFWR and the surrounding landscape as it was from 1968–1971. During the 1968–1971 time period, the NFWR was characterized by low turbidity and minimal siltation (Nickerson & Mays 1973). Shallow riffles were interspersed among deeper pools and the substrate of the NFWR varied between dolomite or limestone bedrock and gravel beds (Nickerson & Mays 1973). The landscape surrounding the NFWR was predominantly forested with oak-hickory and oak-pine dominated stands (Nickerson & Mays 1973).

A 4.6 km section of the NFWR was selected for intensive surveys based on ease of accessibility. The 4.6 km research section was divided into fifty 92 m-long stations (for map and detailed site description, see Nickerson & Mays 1973). Stream width was measured at each station marker using a measuring tape. Area surveyed was estimated as the product of the research section length and the mean stream width.

### SURVEY METHODS

Diurnal (0900–1900 h) turtle surveys were conducted between 1968 and 1980 with surveying dates occurring in every calendar month. Sampling effort was temporally concentrated with the most intensive surveys occurring between 12 June–7 August 1969 [33 survey days; 344 person hours; surveys targeted turtles and hellbenders (*Cryptobranchus alleganiensis*)] and 26 August–15 October 1980 (15 survey days; 129 person hours; surveys targeted turtles only). Surveys conducted between 12 June–7 August 1969 were spatially concentrated within the 4.6 km research section. Surveys conducted between 26 August–15 October 1980 were spatially concentrated within a 4.0 km subsection of the 4.6 km research section. We surveyed areas upstream and downstream of the 4.6 km research section on 13 separate occasions during the months of June–October to assess the extent to which turtles moved outside of the 4.6 km research section, including seasonal migrations, during the months relevant to this study (i.e., data collected outside of the 4.6 km research section allowed us to assess whether the populations located within



the 4.6 km research section studied in 1969 and 4.0 km research section studied in 1980 were closed). Downstream surveys encompassed the area up to 8.0 km downstream from the downstream terminus of the research section. Upstream surveys encompassed the area up to 16.0 km upstream from the upstream boundary of the research section.

Surveys were conducted by hand capturing turtles while snorkeling. This method proved effective for capturing turtles within the NFWR due to high water clarity and the prevalence of basking turtle species. Basking turtle species, especially the common map turtle (*Graptemys geographica*), are often wary of traps (Pluto & Bellis 1986) and may be unresponsive to bait (Lagler 1943). To assess movement of individual turtles, we noted the station in which each turtle was captured for each capture/recapture event.

We uniquely marked each turtle using carapace notching or Turtox mammalian ear tags (General Biological Supply House, Inc., Chicago, IL), depending on size. We measured the midline plastron length (PL) to the nearest 0.1 cm using a flexible measuring tape. Sex was visually determined when possible based on morphological diagnostic characteristics including tail length and thickness, foreclaw characteristics, and relative position of the anal opening, depending on species (Ernst et al. 1994). We released turtles at their capture site.

#### COMMUNITY AND POPULATION ANALYSES

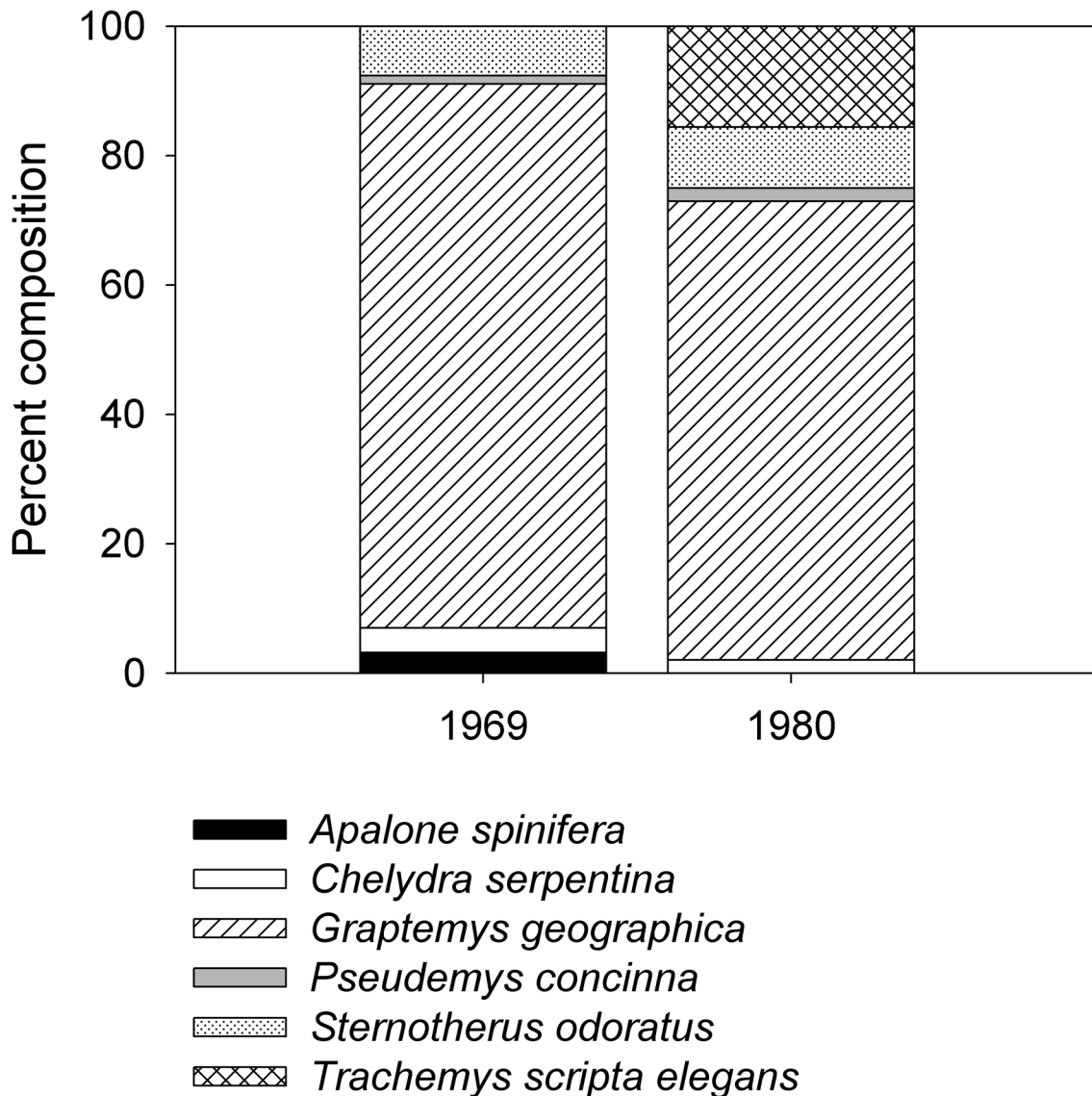
We used the data collected from the 4.6 km research section between 12 June–7 August 1969 and from the 4.0 km subsection of the

4.6 km research section between 26 August–15 October 1980 for turtle community and population estimates as concentrated sampling efforts yielded data conducive for community-level analyses and population size estimation. We measured species richness of the turtle community observed in 1969 and 1980 using the rarefaction method which allows comparison of communities from which unequal sample sizes were collected (Krebs 1989). We assessed heterogeneity, which accounts for species richness and evenness (Krebs 1989), using Hurlbert's Probability of an Interspecific Encounter (PIE) index. We used EcoSim v7.72 (Gotelli & Entsminger 2011) to complete the community analyses. Model parameters were set at 1000 iterations and a random number seed of 0.

Population-level analyses were limited to *Graptemys geographica* due to greater sample sizes. We calculated population size estimates and 95% confidence intervals for *G. geographica* using the Schumacher-Eschmeyer method, which assumes a closed population (Krebs 1989). We compared population estimates using the Chapman and Overton (1966) method to identify significant differences ( $P \leq 0.05$ ) between sampling years. To ensure that uneven sampling area between the 1969 and 1980 survey seasons did not significantly affect the population and density estimates and comparisons, we calculated and compared population estimates based on *G. geographica* found in both the 4.6 km and 4.0 km study areas in 1969. We calculated standardized density estimates using the Schumacher-Eschmeyer estimated population sizes and the areas calculated from the products of the mean stream width and research section length

**Table 1.** Total number of individual turtles captured from the North Fork of White River, Ozark County, Missouri in 1969 and 1980.

Turtle species	1969	1980
<i>Apalone spinifera</i>	5	0
<i>Chelydra serpentina</i>	6	2
<i>Graptemys geographica</i>	132	68
<i>Pseudemys concinna concinna</i>	2	2
<i>Sternotherus odoratus</i>	12	9
<i>Trachemys scripta elegans</i>	0	15



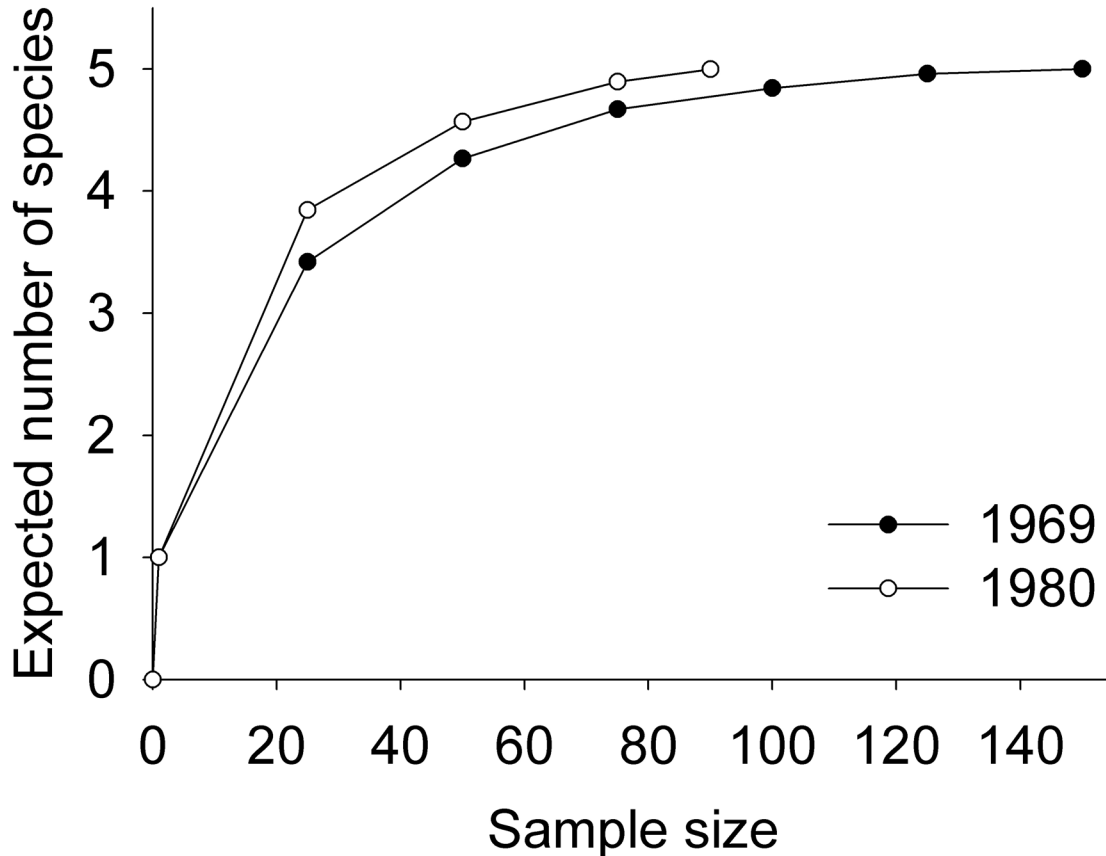
**Figure 1.** Turtle community composition in the North Fork of White River, Ozark County, Missouri in the years 1969 and 1980.

from 1969 and 1980. We compared the mean plastron length of *G. geographica*, partitioned by sex, using independent samples *t*-tests. We used a chi-square ( $\chi^2$ ) test of independence to identify if sex ratios remained the same between sample years. Statistical analyses were performed using SPSS version 11.5 (2002, SPSS Inc., Chicago, IL).

## RESULTS

The mean stream width ( $\pm$  SD) of the NFWR was  $41.6 \pm 9.9$  m. Area estimates for the survey sections for 1969 and 1980 were 191,360 m<sup>2</sup> and 166,400 m<sup>2</sup>, respectively.

*Graptemys geographica* was the most abundant turtle species within the research section in both sampling years (Table 1; Fig. 1). Common musk turtles (*Sternotherus odoratus*), snapping turtles (*Chelydra serpentina*), and eastern river cooters (*Pseudemys concinna concinna*) were present in low numbers in both years (Table 1). Red-eared sliders (*Trachemys scripta elegans*) were observed within the 92 m section above the 4.6 km research section in 1969, but none were found within the research section (Table 1). In 1980, we observed *T. s. elegans* within the research section (Table 1). Spiny softshells (*Apalone spinifera*) were



**Figure 2.** Rarefaction curves indicating species richness of the turtle community in the North Fork of White River, Ozark County, Missouri in the years 1969 and 1980.

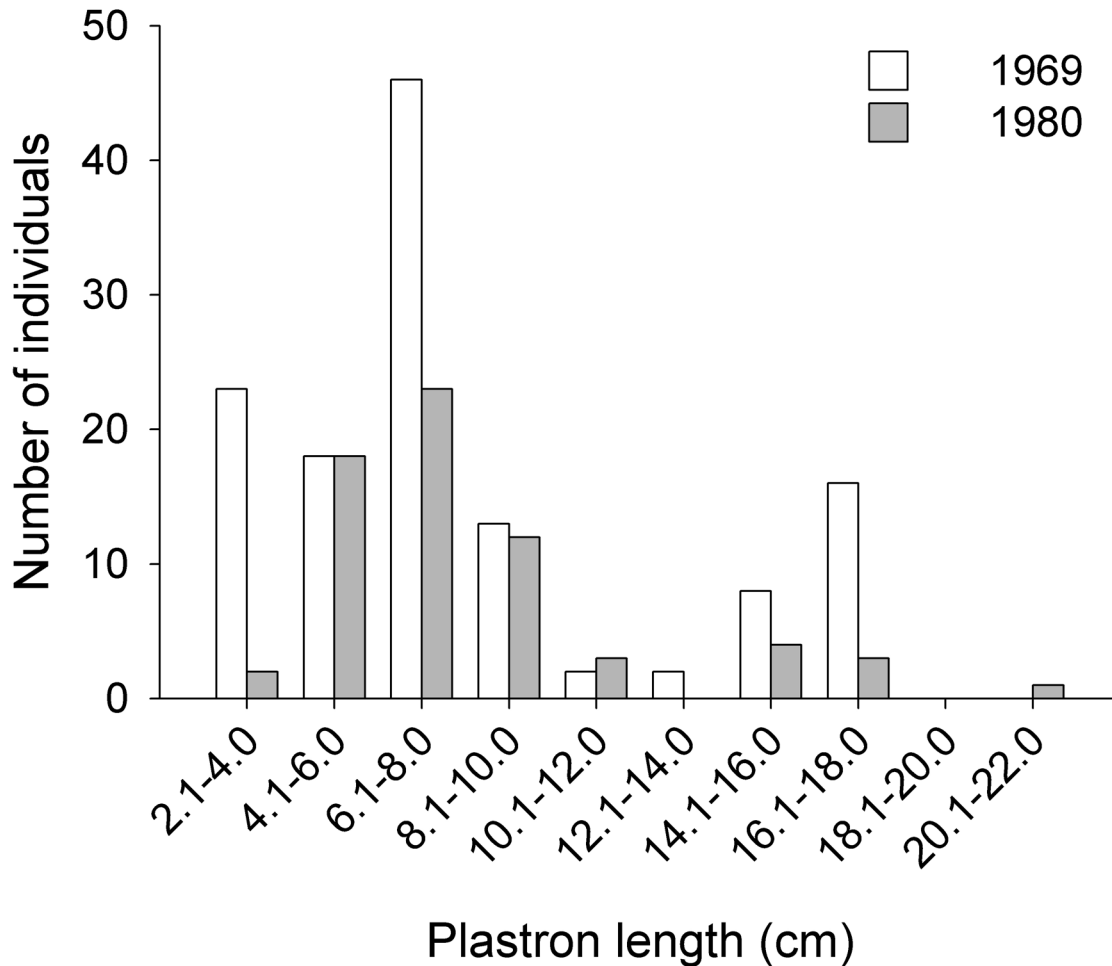
**Table 2.** Schumacher-Eschmeyer estimations of population size (N), 95% confidence interval (CI), and density of common map turtles (*Graptemys geographica*) in the North Fork of White River, Ozark County, Missouri.

Year	Area Sampled (m <sup>2</sup> )	N	95% CI	Estimated Density
1969	191,360	274	237–324	1 turtle/ 698 m <sup>2</sup> 14 turtles/ ha
1969	166,400	261	225–311	1 turtle/ 638 m <sup>2</sup> 16 turtles/ ha
1980	166,400	139	94–264	1 turtle/ 1197 m <sup>2</sup> 8 turtles/ ha

present in 1969, but were not observed in 1980. Rarefaction curves indicated that species richness was slightly higher in 1980 than in 1969 (Fig. 2). Similarly, heterogeneity was higher in 1980 ( $PIE_{1980} = 0.469$ ) than in 1969 ( $PIE_{1969} = 0.286$ ).

The *Graptemys geographica* population declined significantly between 1969 and 1980

( $z = 3.39$ ,  $P < 0.001$ ; Table 2). The exclusion of the stations that were not sampled in 1980 did not significantly affect the 1969 population estimate ( $z = 0.335$ ,  $P = 0.738$ ; Table 2). Mean female PL was significantly larger in 1969 (mean  $\pm$  SD = 13.4  $\pm$  4.7 cm,  $n = 33$ ) than in 1980 (mean  $\pm$  SD = 9.9  $\pm$  5.0 cm,  $n = 26$ ;  $t = 2.76$ ,  $P = 0.008$ ,  $df = 52$ ; Fig. 3).



**Figure 3.** Size distribution of the population of common map turtles (*Graptemys geographica*) in the North Fork of White River, Ozark County, Missouri in the years 1969 and 1980 (1969:  $n = 128$ ; 1980:  $n = 66$ ). Individuals with plastron length  $> 10$  cm are all females.

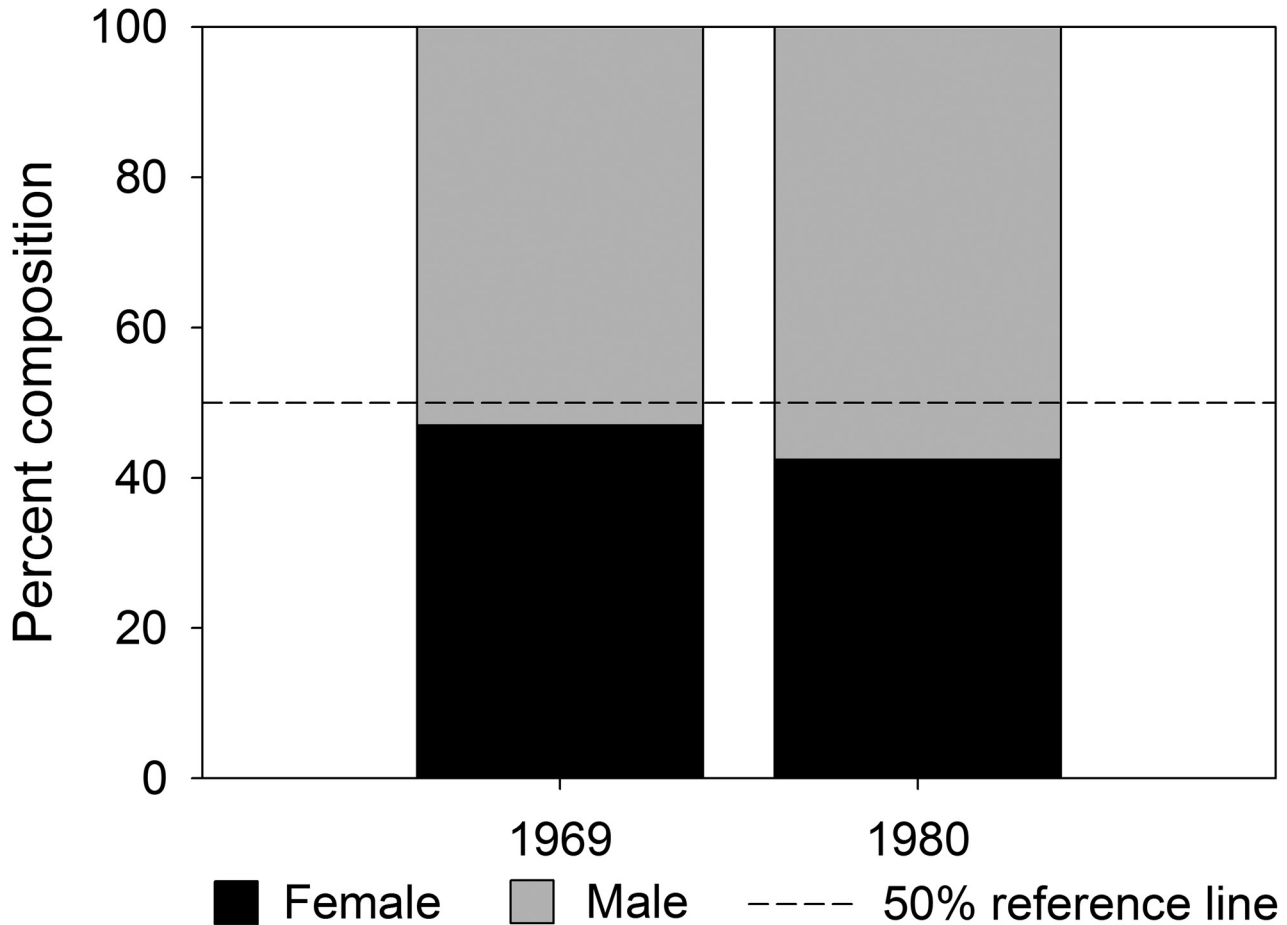
Mean male PL did not differ significantly between sample years (mean<sub>1969</sub>  $\pm$  SD =  $7.9 \pm 2.4$  cm,  $n_{1969} = 39$ ; mean<sub>1980</sub>  $\pm$  SD =  $7.1 \pm 1.4$  cm,  $n_{1980} = 38$ ;  $t = 1.88$ ,  $P = 0.064$ ,  $df = 61$ ; Fig. 3). Sex ratios of mature turtles were not significantly different between sampling years ( $\chi^2 = 0.309$ ,  $df = 1$ ,  $P = 0.578$ ; Fig. 4).

In 1969, 75% of the recaptured *Graptemys geographica* were found within 460 m of their original capture site; 52% of all recaptured *G. geographica* were found within 92 m of their original capture site. The longest documented distance moved by an individual was that of a small female which traveled 3,725 m upstream between 14 June and 8 July 1969. In 1980, 85% of the recaptured *G. geographica* were found within 460 m of their original capture site; 53% of

all recaptured *G. geographica* were found within 92 m of their original capture site. The longest documented distance moved by an individual was 1,255 m. During our 13 surveys conducted outside of the research section, we recaptured five *G. geographica* that had been originally captured and marked in our research area. All five recaptures were within 200 m of the downstream terminus of the research section. In 1980, we recaptured two *Trachemys scripta elegans* within 50 m of their original capture sites with one recaptured 27 days after the original capture.

## DISCUSSION

The NFWR turtle community exhibited diversity patterns typical of a North American river turtle assemblage in that it had low species richness and



**Figure 4.** Sex ratios of mature common map turtles (*Graptemys geographica*) in the North Fork of White River, Ozark County, Missouri in the years 1969 and 1980 (1969:  $n = 83$ ; 1980:  $n = 66$ ).

the community was dominated by a single species of emydid turtle (Moll & Moll 2004). Species richness and heterogeneity of the NFWR turtle community were higher in 1980 than in 1969 due to the addition of *Trachemys scripta elegans* to the community and increased community evenness, respectively. The population of *Graptemys geographica* had significantly declined by 1980. Changes in the size class distributions and mean PL values for *G. geographica* indicated that there were fewer large adult females and hatchlings in 1980, suggesting the population decline was due to stressors that were disproportionately affecting large adult female *G. geographica*. Adult female turtles can suffer disproportionately high death rates during nesting migrations if they must cross roads or are subjected to high populations of terrestrial predators (Cochran 1987; Moll & Moll

2004). In such cases, all adult female size classes would be reduced, unlike the pattern observed in the NFWR population where the mean PL of female *G. geographica* was significantly smaller in 1980 than in 1969. Close and Seigel (1997) found that harvesting could result in smaller mean body size for populations of *T. s. elegans* in other locations. *Graptemys geographica* are highly sexually dimorphic with females achieving much larger body size than males (Gordon & MacCulloch 1980) and larger turtles would be selectively harvested for the food trade (Close & Seigel 1997). *Graptemys geographica* has been and continues to be a popular species in the food trade, both domestically and internationally (Arndt & Potter 1973; Roche 2002; Moll & Moll 2004). The reduced mean PL observed for female *G. geographica* in 1980 relative to 1969 are consistent with what would be

expected if humans were harvesting this species for the food trade. Anecdotal evidence supporting this hypothesis was provided by a local merchant who observed and conversed with two wetsuit-wearing men who were hand-catching turtles to sell to St. Louis restaurants (M. Tole pers. comm.). Our ability to efficiently and effectively hand-capture *G. geographica* while snorkeling suggests that individuals using this technique could greatly affect the population structure of *G. geographica* in the NFWR research section in one or two days time. Additionally, illegal harvest of Ozark hellbenders (*Cryptobranchus alleganiensis bishopi*) in this section of the NFWR during this time period is one of the best documented examples of herpetofauna harvest in the U.S. (Nickerson & Briggler 2007) and it is reasonable to suspect that other species in this area may have been subjected to harvest. The decrease in hatchling numbers may be attributed to the reduction of mature females and accordingly, reproductive output. Turtle reproductive output is positively correlated with body size (Congdon & Gibbons 1985) so the reduced number of reproductive female *G. geographica*, especially those of larger body size, is a plausible explanation for the observed corresponding reduction in number of hatchlings in 1980.

Because our sampling efforts were during different seasons, we reviewed the movement data we collected to determine if we could legitimately compare our population estimates. Movement data suggest that *Graptemys geographica* retain a small home range in the studied area of the NFWR at least during the months relevant to this study, but likely year-round. In general, movement is related to acquisition of resources (e.g., food, mates, shelter) and movement patterns correspond with the spatiotemporal distribution of necessary resources (Pough et al. 2004). Turtles may undergo seasonal migrations to locate food, mates, nesting habitat, and hibernaculum (Gordon & MacCulloch 1980; Pluto & Bellis 1988; Ryan et al. 2008; Carrière et al. 2009). White and Moll (1992) found that *G. geographica* in the Niangua River, Missouri were dietary specialists that fed primarily on the snail *Elimia potosiensis*. Evidence

suggests that *G. geographica* in the NFWR may have had a similar diet as that documented by White and Moll (1992). During this study, we regularly observed *G. geographica* of all size classes selectively feeding on snails (Nickerson unpubl. data). Additionally, the stomach contents of a small number of *G. geographica* collected from an area within the NFWR but outside of the research section revealed a specialized diet of snails (Nickerson unpubl. data). These snails were abundant and provided a seasonally stable food source for *G. geographica* throughout the research section (Nickerson & Mays 1973; Cooper 1975) therefore we do not suspect that *G. geographica* would have needed to move outside of the research section to acquire sufficient food. Courtship and mating of *G. geographica* likely occur from late March through May in Missouri (Johnson 2000) so any movement related to courtship and mating would not have taken place during the months included in our study periods. Nesting migrations have been reported for female *G. geographica* in other locations (Gordon & MacCulloch 1980; Carrière et al. 2009) and may occur to some extent in the NFWR. However, recapture data coupled with our direct observations of nesting in two cleared areas that bordered the research section near stations 15–17 and 50 suggests that nesting migrations were minimal as nesting habitats were available adjacent to the research section. Seasonal movements into and out of hibernacula have also been reported for *G. geographica* (Pluto & Bellis 1988; Graham et al. 2000). Graham et al. (2000) believed that hibernacula were deeper areas with slow-moving water and ample structural components (e.g., ledges, boulders, tree trunks) that could provide some form of security or shelter for overwintering turtles. Three areas within the 4.6 km research section of the NFWR fit this physical description: an approximately 3 m deep pool in station 3; a very large 2–3 m deep pool that encompassed portions of stations 18–24; and a 2–2.5 m deep pool at the end of station 50. Winter surveys confirmed the presence of *G. geographica* in the pool between stations 18–24 (Nickerson unpubl. data). The majority of studies that address

overwintering of *G. geographica* have occurred in the northern portion of the species' range where turtles must deal with extreme cold, ice, and anoxic conditions (Ultsch 2006). Turtles inhabiting the NFWR face far less extreme winter temperatures than turtles located in states and provinces within the northern distribution of *G. geographica* and these milder conditions allow at least occasional winter basking of *G. geographica* in Missouri (Johnson 2000). Within stream characteristics are also less extreme as the NFWR receives a large volume of water from springs which effectively buffers the water temperature and inhibits ice formation (Mundt & Turner 1926; Nickerson & Mays 1973). The NFWR's dissolved oxygen content and pH remain relatively stable year-round (Nickerson & Mays 1973). The availability of deep water pools and the less extreme conditions likely allow *G. geographica* to avoid seasonal migrations to hibernaculum outside of our research section.

Movement data suggest that *Trachemys scripta elegans* was established within the study area by 1980. We hypothesize that the significant reduction of large *Graptemys geographica* made available some component of the total chelonian niche for *T. s. elegans* to exploit. The sudden removal of adult female *G. geographica* would create the availability of suitable unoccupied basking sites and may be one factor enabling *T. s. elegans* to successfully establish within this section of the river. In other sections of the NFWR, *T. s. elegans* were often encountered basking on sites similar to those utilized by *G. geographica*, including small rocks jutting from the water. These rocky basking sites were scarce in many portions of the research area and large female *G. geographica* occupied them almost immediately at daybreak.

This study provides information regarding the population status of *Graptemys geographica* in a southerly portion of its range and illustrates community changes that may be precipitated by the population decline of just one species. The data presented in this study are no less relevant or necessary today as they were during the collection period in that they provide evidence of a turtle population decline and serve as a reference

for future comparative turtle population and community studies in the NFWR. Indirect evidence suggests that river turtle populations are declining worldwide (Moll and Moll 2004) but few long-term population studies are available to assess this hypothesis. Publishing historical studies, such as this one, will provide the direct evidence necessary for determining whether river turtle populations have declined, as well as provide baseline data to compare with current and future data.

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# MOVEMENTS OF THE TIMBER RATTLESNAKE (*CROTALUS HORRIDUS*) IN THE SOUTH CAROLINA MOUNTAINS

Jeffrey R. Mohr<sup>1</sup>

## ABSTRACT

Although the timber rattlesnake (*Crotalus horridus*) is the most common rattlesnake in the eastern United States, populations have declined and only scattered metapopulations remain in what was once a large and extensive North American range. Whereas some *C. horridus* populations in forest communities of the northeastern and western US have been studied, information on those occurring along the southern part of its range is virtually non-existent. In South Carolina there has been relatively little research done on this species and there has been no formal study on *C. horridus* in the mountainous regions of the state. From 2006 to 2009, I radio-tracked several *C. horridus* in Table Rock State Park, South Carolina and documented their movement patterns. For the duration of the study, males moved a mean annual distance ( $\pm$  SE) of  $3,047 \pm 488$  m, non-gravid females moved a mean ( $\pm$  SE) of  $1,688 \pm 517$  m, and gravid females moved a mean ( $\pm$  SE) of  $2,248 \pm 597$  m. Although mean distances moved were not statistically significant among groups in this study, mean distances travelled for all sexes were much shorter than observed in other populations. I hypothesize that forest management involving natural regeneration and fire suppression, and prey availability may influence *C. horridus* movements in Table Rock State Park.

**Key Words:** *Crotalus horridus*, timber rattlesnake, movement, fire suppression, forest management.

## INTRODUCTION

The timber rattlesnake (*Crotalus horridus*) is a large, heavy-bodied pit viper found in the eastern half of the United States (Fig. 1). Throughout what was once a large and extensive North American range, *C. horridus* populations have severely declined due to purposeful eradication (Klauber 1972; Dodd 1987; Fritsch 1992), hunting, collecting (Galligan & Dunson 1979; Dodd 1987; Reinert 1990; Keyler & Oldfield 1992), and habitat destruction and fragmentation (Martin 1982; Brown 1993; Martin et al. 2008), and only a few scattered metapopulations remain (Martin 1992a). *Crotalus horridus* has been extirpated from Canada, Maine, and Rhode Island (Breisch 1992; Brown 1993),

and experts consider it to be vulnerable, imperiled, or critically imperiled in 20 of the 30 states in which it still occurs (Brown 1993; Ernst & Ernst 2003). A proposal submitted to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) by the U.S. Fish and Wildlife Service (2000) indicated *C. horridus* was state-listed as threatened in 4 states (Illinois, New York, Minnesota, and Texas) and endangered in 7 states (Connecticut, Indiana, Massachusetts, New Hampshire, New Jersey, Ohio, and Vermont).

Although *Crotalus horridus* has been relatively well studied in the northern part of its range (Brown 1982, 1991, 1995; Brown et al. 1982; Reinert 1984a, 1984b; Reinert & Zappalorti

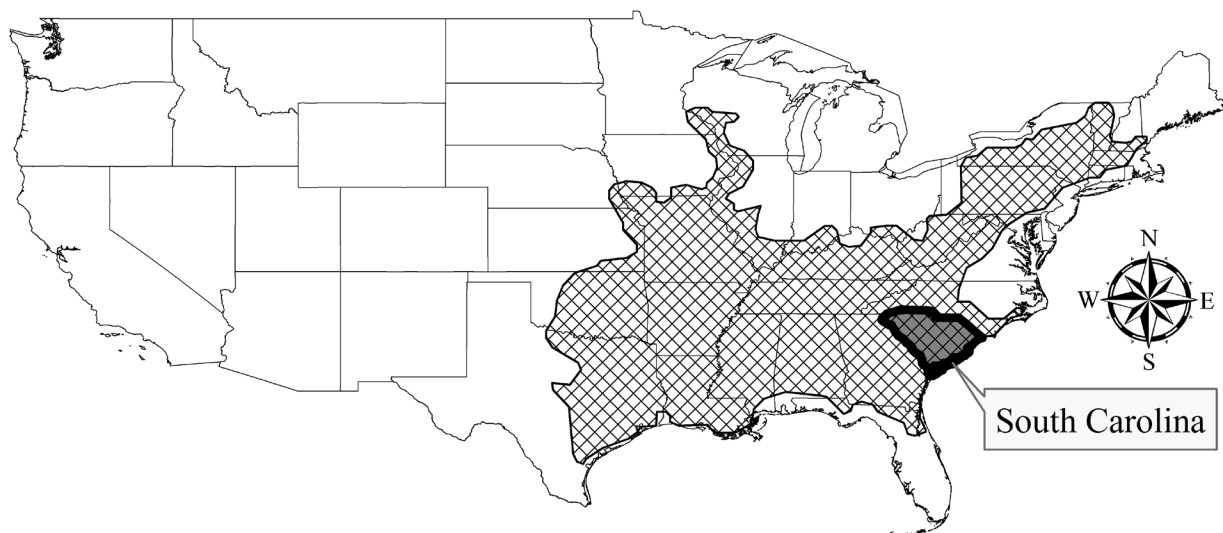
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1988; Reinert & Rupert 1999; Aldridge & Brown 1995; Bushar et al. 1998) few studies have focused on populations in the southern part of its range (Gibbons 1972; Sealy 1997; Waldron et al. 2006a, 2006b), despite being listed as a species of concern in some southern states (e.g., South Carolina; SCDNR 2005). Furthermore, with the exception of a study by Sealy (1997), investigations of southeastern populations of *C. horridus* have been limited to those inhabiting the coastal plain ecoregion and very little is known about populations located in the mountains. Although historical records indicate *C. horridus* was common throughout South Carolina (Logan 1859), recent accounts indicate that *C. horridus* is most common in the mountains (located in the northwestern portion of the state) and coastal plain (located along the eastern portion of the state) and tend to be uncommon or absent in much of the intervening landscape (SCDNR 2005; Fig. 2).

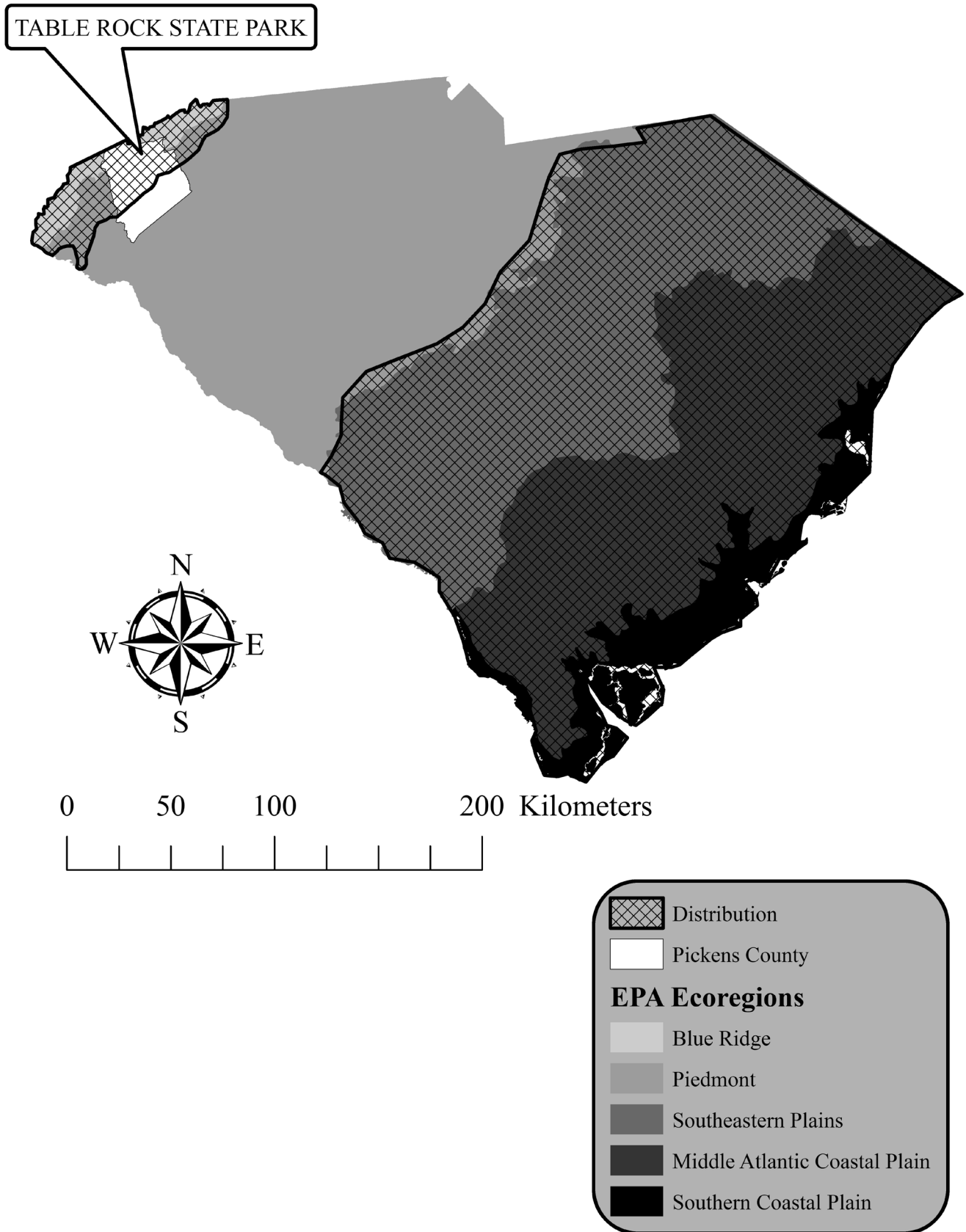
Table Rock State Park, located at the edge of the mountainous Blue Ridge ecoregion in Pickens County, South Carolina (Fig. 2), has characteristic upland habitat typical of that used by *Crotalus horridus* (Brown 1993), and sightings of this species

on park property have been well documented (S. Stegenga, pers. comm.). At the time of the study, the property consisted of approximately 1,247 ha of upland deciduous forest and had more than 20 km of managed trails (South Carolina Department of Parks, Recreation & Tourism 2010). With the creation of the park in the 1930's, logging and other uses of the land that were previously extensive throughout this locale were curtailed (S. Stegenga, pers. comm.). With the exception of the construction and maintenance of trails, roads, and campground/public use areas, the area that encompasses the park remained in a state of deciduous forest regeneration with suppression of all fires (S. Stegenga, pers. comm.).

In an effort to build a better species portfolio for *Crotalus horridus* in the southern mountainous region of its range, I employed a radio telemetry study of *C. horridus* in the foothills of South Carolina. Specifically, I examined movement patterns of *C. horridus* as these patterns can elicit a multitude of information including habitat association (Brown 1982), foraging areas (Reinert et al. 2011), and egress/ingress routes (Brown et al. 1982).



**Figure 1.** The distribution of the timber rattlesnake, *Crotalus horridus*, in the United States based on Conant and Collins (1998).



**Figure 2.** EPA ecoregions of South Carolina and current distribution of the timber rattlesnake, *Crotalus horridus*, based on Martin (1992a:Fig. 1) and SCDNR (2005).

## MATERIALS AND METHODS

Natural history observations (e.g., behavior, predation) and general habitat characteristics including tree species dominance, coarse woody debris (e.g., downed trees), leaf litter, rock outcrops, hiking trail proximity, and presence of water in the area in which *Crotalus horridus* were known to occur were documented in field notes. Canopy closure—and subcanopy closure when present—were estimated with a Spherical Crown Densimeter (Forestry Suppliers, Jackson, MS) using random locations ( $n = 50$ ) within the area where *C. horridus* were known to frequent. Micro-habitat was determined in the field when the rattlesnakes were visually observed. Six micro-habitat categories were employed: less than 1 m from: 1) log/fallen branch, 2) rock, 3) live tree; or in/under: 4) vines/forbs/shrubs, 5) leaf litter; or 6) in the open. If the observation satisfied more than one category, the closest micro-habitat variable to the individual was recorded as primary micro-habitat.

Radio telemetry was used to determine movement patterns from 2006 to 2009 with locating events occurring in March–November of each sample year. During this period, 18 adult *Crotalus horridus* (8 male, 10 female) were fitted with temperature-sensitive radio transmitters (SI-2T and AI-2T transmitter for snakes, Holohil Systems, Ltd., Ontario, Canada). The technique used to implant the transmitter into *C. horridus* closely followed that described by Reinert and Cundall (1982) and Reinert (1992). The mass of the transmitter implanted into each snake did not exceed 5% of the snake's body mass. All surgical procedures were performed at the field laboratory of Table Rock State Park. *Crotalus horridus* locations were fixed using Telonics TR-2 (2006–2007) and TR-4 (2007–2009) receivers and hand-held "H" antennas (Mesa, AZ). Visual verification of *C. horridus* was attempted each time the snake was located using radio telemetry. During the active season (March–November), individual *C. horridus* were located a minimum of twice weekly. Locating events were performed on nonconsecutive days to prevent possible avoidance behavior and locations were recorded in universal transverse mercator

(UTM) coordinates using a handheld Global Positioning System (GPS) instrument (Garmin Inc., Olathe, KS) with accuracies  $< \pm 4\text{m}$ .

Movements were calculated for *Crotalus horridus* that were successfully tracked for more than one year and/or had greater than 25 unique movement data points ( $n = 8$ ;  $n_{\text{male}} = 3$ ,  $n_{\text{female}} = 5$ ). I plotted rattlesnake locations using ArcGIS (version 9.3, Environmental Systems Research Institute, Redlands, CA). Due to the fact that male, non-gravid female, and gravid female *Crotalus* spp. can differ in movements (Brown 1982; Reinert & Zappalorti 1988; Timmerman 1995; Marshall et al. 2006; Durbian et al. 2008), snakes were sorted into three categories for analysis: male, non-gravid female, and gravid female. For years in which a female was gravid, movements were treated as unique annual movements. To eliminate outliers and since patterns were often very similar year to year for these sex categories, I calculated the mean annual distance traveled for males and non-gravid females that had multiple year movements. Total annual distance moved was determined by summing the straight line distance between consecutive locations from initial capture (or emergence from hibernacula) until the rattlesnake returned to a winter refuge at the end of the active season (November). Although this method may underestimate true total distance moved by not accounting for deviation from straight line movement paths (Secor 1994); it does provide a standardized measure for comparing movements between individuals and multi-year observations.

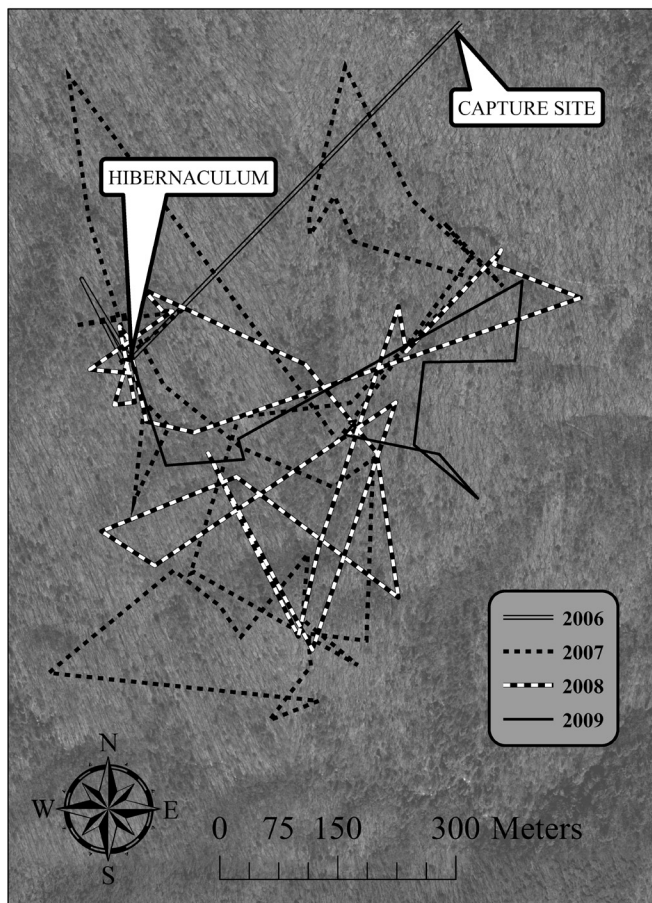
One-way analysis of variance (ANOVA) was used to compare the mean movements (total distance and mean distance) of males, non-gravid females, and gravid females and a Tukey-Kramer test was used to determine the significance of the separation of group means. Logistic regression was used to compare percentages of habitat use among the study categories of sex and year as well as for individual *Crotalus horridus*. All statistical analyses were performed using JMP (Version 8.0, SAS Institute, Inc., Cary, NC).

## RESULTS

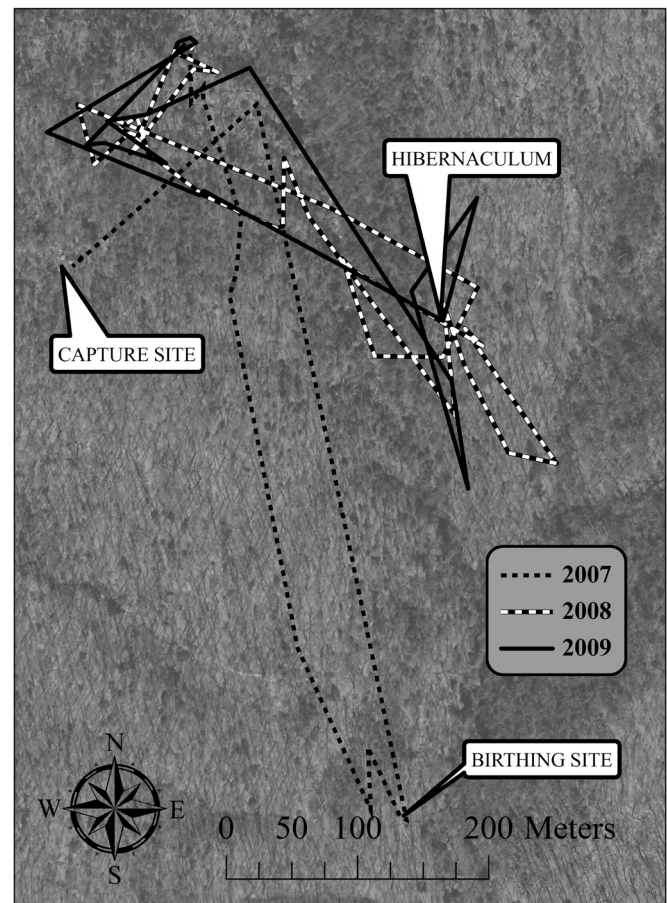
The study area was homogenous oak-hickory deciduous forest with greater than 85% canopy

closure except for a few localized small rock outcrops and the major exposed bald rock outcrops. Sub-canopy was sparse throughout the study area except for areas of tree falls or extreme changes in elevation or along infrequent, seasonal riparian areas. Logs and downed trees were available throughout the habitat and leaf litter was uniform throughout much of the habitat likely due to fire suppression in the area for many years. Timber rattlesnakes were associated significantly more with fallen logs/branches than any other micro-habitat type ( $F = 22.17$ ,  $df = 5$ ,  $136$ ,  $p < 0.0001$ ). There were no significant differences between sexes ( $F = 0.548$ ,  $df = 2$ ,  $136$ ,  $p = 0.58$ ) or years ( $F = 1.36$ ,  $df = 2$ ,  $127$ ,  $p = 0.26$ ).

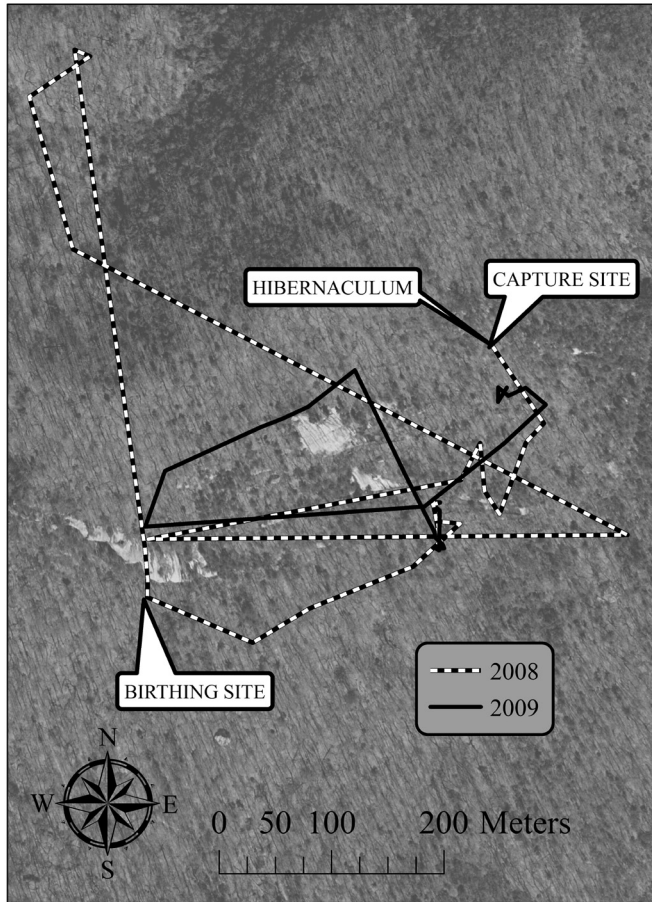
Radio telemetry conducted during 2006–2009 yielded 844 encounters with *Crotalus horridus*. In successive years, individual *C. horridus* utilized the same general areas (i.e., individuals exhibited site fidelity) as illustrated by the examples mapped in Figures 3, 4, and 5. Total distance moved by individual *C. horridus* from egress in the spring (March) to ingress in the fall (November) was not significantly related to sorted categories of sex nor were there significant differences among years ( $F = 1.85$ ,  $df = 2$ ,  $p = 0.183$ ). For the duration of the study, males moved a mean annual distance ( $\pm$  SE) of  $3,047 \pm 488$  m, non-gravid females moved a mean ( $\pm$  SE) of  $1,688 \pm 517$  m, and gravid females moved a mean ( $\pm$  SE) of  $2,248 \pm 597$  m (Table 1).



**Figure 3.** Successive yearly movements of a male timber rattlesnake, *Crotalus horridus*, in Table Rock State Park, Pickens County, South Carolina in 2006 ( $n = 9$ ), 2007 ( $n = 58$ ), 2008 ( $n = 46$ ), and 2009 ( $n = 23$ ).



**Figure 4.** Successive yearly movements of a female timber rattlesnake, *Crotalus horridus*, that was gravid in 2007, in Table Rock State Park, Pickens County, South Carolina in 2007 ( $n = 30$ ), 2008 ( $n = 51$ ), and 2009 ( $n = 33$ ).



**Figure 5.** Successive yearly movements of a female timber rattlesnake, *Crotalus horridus*, that was gravid in 2008, in Table Rock State Park, Pickens County, South Carolina in 2008 (n = 38), and 2009 (n = 30).

**Table 1.** Mean movements ( $\pm$  SE) for all radio-tracked timber rattlesnakes (*Crotalus horridus*) in Table Rock State Park, Pickens County, South Carolina by year and sex.

	Sample Size	Total Distance (m)	Average Distance per movement (m)
Male	8	3047.0 $\pm$ 488.1	141.1 $\pm$ 10.7*
Female (non-gravid)	8	1687.6 $\pm$ 517.7	73.0 $\pm$ 11.3
Female (gravid)	6	2248.3 $\pm$ 597.8	86.2 $\pm$ 13.1
2007	5	3615.6 $\pm$ 738.1	120.4 $\pm$ 16.9
2008	4	2655.8 $\pm$ 825.2	91.0 $\pm$ 18.9
2009	6	1691.5 $\pm$ 673.8	83.5 $\pm$ 15.4

\* denotes significance (ANOVA  $F = 10.73$ ,  $df = 2$ ,  $p < 0.0007$ )



Mean distance ( $\pm$  SE) moved per movement was significantly greater for males ( $141 \pm 11$  m) than non-gravid ( $73 \pm 11$  m) or gravid females ( $86 \pm 13$  m;  $F = 10.73$ ,  $df = 2, 19$ ,  $p < 0.0007$ ;  $q = 5.8$ ,  $df = 19$ ,  $p < 0.05$ ).

## DISCUSSION

Snakes change locations for a variety of reasons. They will move to a specific habitat to forage (Duvall et al. 1990), to find different microhabitats for thermoregulation (Huey & Peterson 1989), or in search of mates (Duvall & Schuett 1997). In addition to exhibiting inter-annual den site fidelity, other studies found *Crotalus horridus* utilize similar egress routes in the spring and ingress routes in the fall (Brown et al. 1982). Although this pattern of similar egress and ingress routes was not clearly seen in this study, many of the *C. horridus* spent the active season in the same area in consecutive years (Figs. 3–5). This occupation of similar active season home ranges year after year has been observed in other populations of *C. horridus* and *Crotalus* spp. as well (Landreth 1973; Reinert & Zappalorti 1988; Timmerman 1995).

Studies from other populations indicate that male *Crotalus* spp. move farther than females (Timmerman 1995; Reed & Douglas 2002) and *Crotalus horridus* is no exception (Brown 1982; Reinert & Zappalorti 1988; Walker 2000; Gibson 2003). Even though I did not find a significant difference in the mean distance moved among males, non-gravid females, and gravid females, this result is likely an artifact of small sample size. Although not significantly different, but nevertheless in contrast with the existing literature on *C. horridus*, it appeared that gravid females in this study may have had longer movements than non-gravid females (Figs. 4 & 5). As gravid *C. horridus* have been observed to prefer open sites (Keenlyne 1972; Reinert 1984b; Reinert & Zappalorti 1988; Martin 1992b; Fogell et al. 2002; Sealy 2002; Gibson et al. 2008) and open sites are uncommon within the study area, the perceived longer movements of gravid females may be due to the need of these females to travel to find these open areas for enhanced thermoregulation or other

physiological requirements. It is also possible, however, gravid females may be dispersing due to other factors such as competition and predation but this has not been investigated in this study. Furthermore, all gravid females gave birth in the year of their surgery and this may have also contributed to abnormal movements.

Most studies involving *Crotalus horridus* indicate two to three times longer total movements for all sexes than was observed in this study (Reinert & Zappalorti 1988; Reinert & Rupert 1999; Gibson 2003; Adams 2005). Possible reasons for the results observed in this study could be the abundance of leaf litter and fallen logs and branches due to a management regime of unmanaged forest regeneration and fire suppression as well as the availability of a large, continuous tract of land. I hypothesize that high levels of leaf litter and debris in the study area, as well as a large tract of undeveloped land, may offer preferred habitat for small mammals—the chief prey item of *C. horridus*. In this study, observed predation events indicated *C. horridus* preyed on white-footed mice (*Peromyscus leucopus*), an eastern chipmunk (*Tamias striatus*), and a gray squirrel (*Sciurus carolinensis*), and these species are common prey throughout *C. horridus* range (Clark 2002; Fitch et al. 2004; Reinert et al. 2011). Studies involving *P. leucopus* have indicated decreased numbers in burned areas or areas of little or no woody ground litter (Planz & Kirkland 1992; Kirkland et al. 1996). Rosenblatt et al. (1999) found *T. striatus* and *S. carolinensis* had a preference for large, continuous sites and avoided areas of high habitat fragmentation. Based on personal observation, it appears small mammal density may be high but trapping was not conducted and consequently any comparisons involving small mammal populations and *C. horridus* movement patterns need to be made carefully. Future research in Table Rock State Park will include a study to estimate the densities of potential prey species.

Movement patterns of the different sexes of *C. horridus* observed in this study were similar to those of other populations; however, total movement observed was much less than reported in

other studies (Reinert & Zappalorti 1988; Reinert & Rupert 1999; Gibson 2003; Adams 2005). Further research of this and other populations in the southeastern United States would provide a better understanding of *C. horridus* in the southern part of its range. Considering populations of *C. horridus* are declining throughout the species' range (Brown 1993), basic ecological information, such as that presented in this study, is key to creating a viable conservation plan.

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