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COTTONMOUTH (*Agkistrodon piscivorus*) SPATIAL ECOLOGY

A Dissertation APPROVED FOR THE
DEPARTMENT OF ZOOLOGY

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Table of Contents

Chapter 1 – Spatial Ecology of a Cottonmouth (*Agkistrodon piscivorus*) Population in East Texas

Chapter 2 – Buffer Zone Applications in Snake Ecology: A Case Study Using Cottonmouths (*Agkistrodon piscivorus*)

Chapter 3 – Sex Differences in Spatial Dispersion Patterns within a Cottonmouth (*Agkistrodon piscivorus*) Population in East Texas

Chapter 4 – Size-based Variation in Antipredator Behavior within a Snake (*Agkistrodon piscivorus*) Population

Chapter 5 – Relative Medial and Dorsal Cortex Volume in Relation to Sex Differences in Spatial Ecology of a Snake Population

Preface

Chapter 1 is in press (E.D. Roth. 2005. *Journal of Herpetology*.)

Chapter 2 is in press: (E.D. Roth. 2005. *Copeia*.)

Chapter 3 is formatted for *Behavioral Ecology*.

Chapter 4 is coauthored by Joel A. Johnson: (E.D. Roth, J.A. Johnson. 2004. *Behavioral Ecology* 15:365-370.)

Chapter 5 is co-authored by William I Lutterschmidt and Donald A Wilson. This chapter is formatted for *Brain, Behavior and Evolution.*

Chapter 1

Spatial Ecology of a Cottonmouth (*Agkistrodon piscivorus*) Population in East Texas

Abstract

Radio-telemetry was used to study spatial ecology of a Cottonmouth (*Agkistrodon piscivorus*) population in eastern Texas. I examined effects of sex, reproductive state, and body size on spatial use within a riparian habitat. Snakes inhabited a relatively linear environment, restricting movements to the vicinity of the stream. Males occupied larger home ranges than both gravid and non-gravid females. Gravid females exhibited marginally larger home ranges than non-gravid females, but any effects attributable to reproductive state were relatively small when compared to sex differences in spatial use. Body size was positively correlated with home range size but did not account for observed home range differences among population subunits.

Introduction

To further understand the spatial ecology of snakes, it is important to first define general patterns of spatial use and then determine what factors are influencing spatial patterns. Within snakes, spatial use differences in relation to sex and reproductive state are commonly reported (e.g., Macartney et al., 1988; Webb and Shine, 1997; Whitaker and Shine, 2003). However, these relationships are often variable and mechanisms driving divergent spatial patterns in relation to sex and reproductive state remain unclear.

Another factor influencing spatial use is body size. An animal with greater energetic requirements may require a larger area to satisfy its energetic needs (McNab, 1963). One theoretical model defines minimum home range as, "the minimum area that can sustain the individual's energetic requirements" (Harestad and Bunnell, 1979). While this model may oversimplify some factors (e.g., factors relating to reproductive success) influencing home range area, it suggests that body size and other measures of metabolic requirements can likely explain some variance in home range size (e.g., Mace and Harvey, 1983; Kelt and Van Vuren, 1999; Perry and Garland, 2002). In snakes, some intraspecific studies are suggestive of a relationship between body size and home range (e.g., Clark, 1974; Shine, 1987; Whitaker and Shine, 2003), but data are yet too limited for detailed interspecific analyses. Furthermore, it is evident that spatial patterns often vary within and between individuals, populations, species, seasons, and years (e.g., Shine, 1987; Tiebout and Cary, 1987; Slip and Shine 1988). Attaining a greater understanding of snake spatial ecology will require additional comparative studies that consider the influence of sex, reproductive state, body size, and other ecological interactions.

I explored patterns of spatial use within a population of *A. piscivorus* in east Texas. *A. piscivorus* is a semi-aquatic snake that occurs throughout much of the southeastern United States. Aspects of natural history and ecology have been examined (Burkett, 1966; Gloyd and Conant, 1990; Ford, 2002), but very little is known about their spatial ecology. Previous studies on spatial use in *A. piscivorus* (Tinkle, 1959; Wharton, 1969; Martin, 1982) will provide a comparative template for this investigation, which examines patterns of spatial use in relation to sex, reproductive state, and body size.

Materials and Methods

Study Site.—I conducted this study from May 2000 to January 2002 at Sam Houston State University's Center for Biological Field Studies (CBFS). This site was adjacent to the Sam Houston National Forest and consisted of a stream system within a pine woodlands habitat. See Dent and Lutterschmidt (2001) for a detailed description of the study site. *A. piscivorus* were collected from the study site at the beginning (April-June) of each radio-tracking season. Over the duration of the study, I monitored 17 adult snakes using radio-telemetry. Each snake weighed greater than 160 g, and each 5.5 g transmitter comprised less than 5% of total body weight. During the first summer (May to August 2000), I radio-tracked six females, and one male. During the second summer (May to August 2001), I radio-tracked six females and four males.

In the summer of 2000, one female died 15 days after being released, and another female dislodged her transmitter from the body cavity. Observations and home range calculations on these individuals were discarded. Thus, results are reported for 15 adult *A. piscivorus* (5 gravid females, 5 non-gravid females, 5 males).

Surgical Procedures.—Holohil Systems Inc. SB-2 transmitters were coated with a 1:1 mixture of paraffin and beeswax and surgically implanted into the body cavity using methods described by Reinert and Cundall (1982). Prior to surgery, I administered isoflurane (inhalation gas) until subjects were unresponsive to touch. After surgery, I maintained snakes in the laboratory for approximately one week. Water was provided *ad libitum* during this observational period until snakes were released at original capture locations.

Data Collection and Analysis.—Radio-tagged snakes were usually located every other day during the early morning and afternoon hours (0500-1500). Upon location of each snake, geographic coordinates were recorded with a global positioning system receiver (Magellan GPS 320). Individual snakes were never relocated twice on a single day and at least 15 hours elapsed between each observation. Geographic coordinates were imported into ArcView 3.3 (Environmental Systems Research Institute, Inc., 1999) and home range sizes were calculated with the Animal Movement Analysis Extension (Hooge and Eichenlaub, 1997) using 95% minimum convex polygons (MCPs) (Mohr, 1947) and 95% fixed kernel methods (Worton, 1989) with least-squares cross-validation to determine smoothing parameters (Seaman and Powell, 1996).

Before comparing population subunits (i.e., males, non-gravid females, and gravid females), the effects of body size were first examined. A simple regression was used to explore the relationship between body size or snout-vent length (SVL) and home range size. Home ranges of population subunits were then compared using an ANCOVA with SVL as the covariate.

To address potential biases due to variation in the duration of monitoring period and number of observations per individual, incremental area analyses (IAA) were used to graphically examine increases in home range area over time and number of observations for each individual. Simple regressions were used to explore relationships between home range area, number of observations, and monitoring duration. Finally, Kruskal-Wallis tests were used to compare average duration of monitoring periods and number of observations among population subunits.

Results and Discussion

Fixed kernel methods often produced larger home range estimates than MCPs. Inflated fixed kernel estimates may be related to the compact linear nature of the observed spatial use patterns, as fixed kernels often included additional terrestrial areas around the stream that most subjects never inhabited. Nonetheless, general conclusions and all statistical inferences remained constant regardless of which home range estimator was used in the analyses. Thus, both home range estimates are reported in Table 1, but further descriptive and inferential statistics are only reported for comparisons of MCPs.

Similar to observations from laboratory studies (Savitzsky, 1992), *A. piscivorus* were generally located near the water/shore interface, exhibiting linear home ranges oriented around the stream (Fig. 1). These linear home ranges varied in size from 0.033 to 3.337 ha. To address home range size variation body size was first considered. As body size increased, home range area increased ($r^2 = 0.429$, $F_{1,13} = 9.749$, $P < 0.01$). An ANCOVA, which compared the home ranges of population subunits with body size (SVL) as the covariate, showed no significant interaction ($F_{2,9} = 1.611$, $P = 0.252$). After the interaction term was removed from the model, the main effects of both body size ($F_{1,11} = 6.924$, $P < 0.05$) and population subunit groupings ($F_{2,11} = 5.584$, $P < 0.05$) were significant. Thus, after controlling for body size, significant differences in home range size between population subunits remained. These results suggest that although factors related to body size may influence spatial patterns, these factors alone do not account for differences observed in home range area among population subunits.

Mean MCP home range sizes (Fig. 2) were 1.86 ha ± 0.407 ($\overline{X}$ ± SEM) for males, 0.963 ± 0.264 for gravid females, and 0.372 ± 0.081 for non-gravid females. In contrast to prior studies on *A. piscivorus* (Tinkle, 1959; Wharton, 1969; Martin, 1982), males exhibited significantly larger home ranges than gravid (Post Hoc: Fisher's Protected Least Significant Difference (FPLSD): $P < 0.05$) and non-gravid ($P < 0.001$) females. Although larger home ranges in males of many snake species have been reported (e.g., Reinert and Zappalorti, 1988; Slip and Shine, 1988; Fitzgerald et al., 2002; Whitaker and Shine, 2003), causal mechanisms remain unclear. It has been suggested that similar spatial patterns in other taxa may be related to breeding system and reproductive strategies (e.g., Morreale et al., 1984; Reinert and Zappalorti, 1988; Ostfeld, 1990). Since territoriality has not yet been demonstrated in snakes, this hypothesis suggests that sex differences in spatial use will primarily occur due to male competition for females during the breeding season. It is generally assumed that breeding among *A. piscivorus* occurs in the early spring, (Gloyd and Conant, 1990). This suggests that influences related to breeding activities were likely minimized for the duration of this study (May-August) and may not account for the differences in home range size between sexes. These results combined with other studies that report larger home ranges for females (e.g., Madsen, 1984; Johnson, 2000) highlight a need to further explore alternative explanations, such as foraging strategies, predation pressures, and/or social interactions that may be driving divergent spatial patterns between the sexes.

Reproductive state may also affect resource requirements and physiological needs, thus influencing spatial use (e.g., Shine, 1980; Reinert, 1993; Madsen and Shine, 2000). Although statistical differences were marginal (FPLSD: $P < 0.1$), gravid females tended to exhibit larger average home range areas than non-gravid females (Fig. 2.). This contradicts results from many studies which report significantly smaller home ranges for gravid females (e.g., Reinert and Zappalorti, 1988; Johnson, 2000; Whitaker and Shine, 2003). Future research exploring the effects of reproductive state on spatial use in *A. piscivorus* would be of interest.

Average home range sizes, which ranged from 0.372 ha in non-gravid females to1.86 ha in males, in this study were larger than those reported in previous studies on *A. piscivorus*. Wharton (1969) and Tinkle (1959) report average home range sizes of 0.14 to 0.17 ha. Martin (1982) reported average home range values of 0.93 ha for females and 0.88 ha for males. When comparing *A. piscivorus* to other snake species of similar body size (e.g., *Agkistrodon contortrix*: 9.9 ha home range, Fitch 1960; *Crotalus cerastes*: 23.2 ha, Secor, 1994; *Heterodon platirhinos*: 50.2 ha, Plummer and Mills, 2000), it is evident that average home ranges of *A. piscivorus* are relatively small. One direction of future research exploring the relatively small home ranges of *A. piscivorus* may focus on resource distribution. Inhabiting a relatively linear environment surrounding the stream allows movements to and from patchy resources, such as prey, retreat sites, and potential mates, to be directionally focused either upstream or downstream. Location of patchy resources within a simplified linear stream environment may be more efficient and reduce home range size requirements compared to other species inhabiting a more complex two dimensional area with resources scattered in many directions.

The timing and duration of this study may have also influenced home range estimates. Individuals were radio-tracked for 50.6 ± 3.4 ($\overline{X}$ ± SE) days with 26.4 ± 0.97 ($\overline{X}$ ± SE) observations per individual. Duration of monitoring periods varied between individuals ranging from 26 to 81 days, while observations per individual ranged from 20 to 33. Asymptotic curves from IAA suggest that observations were sufficient to produce reliable estimates of home range size for the time period studied. Home range size was not related to duration of monitoring period ($r^2 = 0.048$, $F_{1,13} = 0.656$, $P = 0.433$) or number of observations ($r^2 = 0.105$, $F_{1,13} = 1.525$, $P = 0.239$). Variation in duration of monitoring periods (Kruskal-Wallis, $H = 1.683$, $P = 0 .431$) and number of observations (Kruskal-Wallis, $H = 3.296$, $P = 0 .192$) appeared to be randomized across population subunits. Thus, any bias resulting from random variation in length of tracking periods and number of observations appears to be minimal and does not account for significant differences in home range size observed between population subunits. Nonetheless, monitoring periods were restricted to the summer months, thus limiting interpretations. Although anecdotal recapture observations (Roth, unpub. data) are not suggestive of movements outside the summer home range in the early spring or late winter, it is plausible that larger home ranges may have been obtained if radio-telemetry studies had begun earlier in the year. This is especially true of males, if indeed breeding is occurring in the early spring, and males move more to increase mating opportunities.

This study has explored the spatial ecology of a snake population with consideration of factors that may influence spatial use. The spatial patterns of population subunits were compared by examining the effects of sex, reproductive state, and body size within an ecological context. Such comparisons enhance the general understanding of snake spatial ecology and provide the foundation for future comparative studies.

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Table 1. For each of the 15 radio-tracked *A. piscivorus*, 95% MCP and 95% fixed kernel home range estimations are displayed. The snake identification number (ID) corresponds to labeled home ranges in Fig. 1. Population subunit (gravid female: GF, nongravid female: NGF, male: M), snout-vent length (SVL), year studied (year), number of days monitored (days), and total number of observations (Obs.) are also provided.

ID	Subunit	SVL (cm)	Year	Days	Obs.	MCP 95%	Kernel 95%
1	GF	64.0	2000	72	27	0.997	2.373
2	GF	58.0	2000	72	33	0.033	0.006
3	GF	55.5	2001	52	29	1.480	2.124
4	GF	55.5	2001	49	27	1.462	3.224
5	GF	54.0	2001	48	28	0.842	1.150
6	NGF	55.0	2000	59	22	0.405	0.912
7	NGF	60.0	2000	55	25	0.624	0.625
8	NGF	50.0	2001	48	28	0.369	0.760
9	NGF	53.0	2001	39	25	0.341	0.381
10	NGF	48.0	2001	40	26	0.119	0.409
11	M	53.0	2000	81	31	2.034	5.770
12	M	73.5	2001	27	20	3.337	14.18
13	M	61.5	2001	49	25	1.638	7.607
14	M	50.5	2001	49	30	0.999	1.274
15	M	56	2001	26	20	1.303	4.862

Figure Legends

Figure 1. Home range (95% MCP) maps for radio-tagged *A. piscivorus* categorized by population subunit. Identification numbers labeling each home range correspond with the individual identification numbers listed in Table 1. Inset boxes depict enlarged views of congested or overlapping areas.

Figure 2. Mean home range size (95% MCP) and standard errors are depicted for each population subunit.

Figure 1

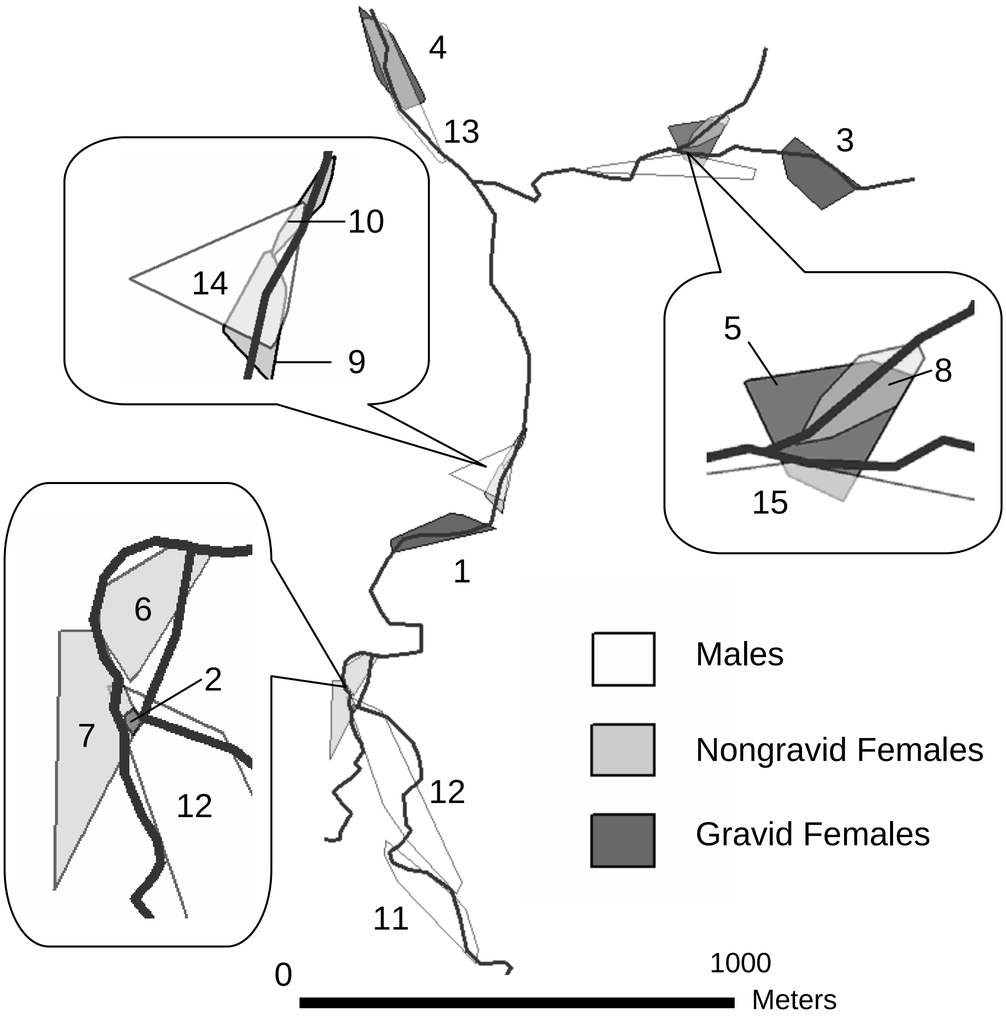
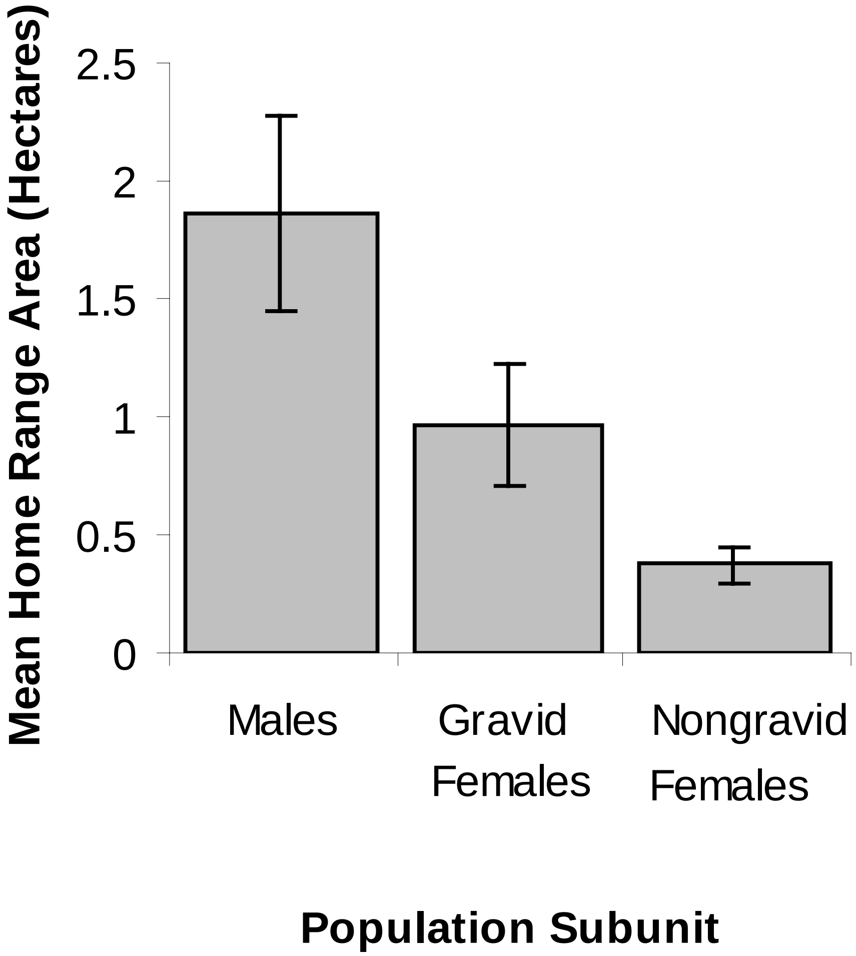

4
13
3
10
14
9
5
8
15
1
6
2
7
12
12
11
Males
Nongravid Females
Gravid Females
0
1000
Meters

Figure 2


Mean Home Range Area (Hectares)
2.5
2
1.5
1
0.5
0
Males
Gravid Females
Nongravid Females
Population Subunit

Chapter 2

Buffer Zone Applications in Snake Ecology: A Case Study Using Cottonmouths (*Agkistrodon piscivorus*)

Abstract

Riparian areas alone are often insufficient for preservation of riparian taxa. Case studies on many vertebrate taxa have addressed the importance of establishing buffer zones around riparian habitats. The goal of this investigation was to build upon previous studies and assess the relative importance of buffer zones to riparian snakes. A case study was conducted on a Cottonmouth (*Agkistrodon piscivorus leucostoma*) population within a steam/riparian habitat. Using radio-telemetry, I examined the spatial distribution of males, gravid females, and non-gravid females. Although 83% of all snake observations were within 10 m of the stream, population subunits exhibited different patterns of spatial use. Gravid females provided most of the distant observations, inhabiting the surrounding terrestrial habitat up to 94 m from the shoreline. Thus, disturbances to terrestrial areas surrounding the riparian habitat would likely have the greatest impact on gravid females. These results further establish a need for buffer zones around riparian ecosystems, and highlight the importance of considering spatial use differences between population subunits when outlining buffer zone applications for conservation management.

Introduction

Although many conservation efforts have focused on preservation of wetland and/or riparian habitats and their rich biodiversity (e.g., Rudolph and Dickson, 1990; Semlitsch and Bodie, 1998; Russell et al., 2002), numerous studies suggest that terrestrial areas adjacent to wetlands and/or riparian habitats may also be critical to the persistence of riparian taxa (e.g., Burke and Gibbons, 1995; Naiman et al., 1988; Semlitsch, 1998). Buffer zones, relatively undisturbed vegetated areas located between a natural resource and areas subject to human disturbance, help reduce anthropogenic impacts on wetlands, streams, and other riparian habitats (Castelle et al., 1994). Riparian taxa may directly use surrounding terrestrial areas for foraging, overwintering, or reproductive activities (e.g., Burke and Gibbons, 1995; Richter et al., 2001; Roe et al., 2003). Riparian taxa may also indirectly depend on terrestrial buffer zones for maintenance of environmental attributes, such as habitat diversity, community structure, and water quality (e.g., Saunders et al., 1991; Castelle et al., 1994; Houlahan and Findlay, 2003)

Researchers have begun to examine the potential influence of buffer zones on a variety of biotic communities (e.g., Hodges and Krementz, 1996; Semlitsch and Bodie, 2003; Wilson and Dorcas, 2003). However, data are relatively sparse and additional comparative studies are needed to enhance general understanding of terrestrial use by riparian taxa. Such studies may strengthen arguments for legislative change aimed at extending environmental protection to areas around wetlands and riparian habitats.

Cottonmouths (*Agkistrodon piscivorus)* are large bodied, relatively abundant, semi-aquatic snakes that occur in riparian ecosystems throughout the southeastern United States. Cottonmouths often inhabit shoreline, using both aquatic and terrestrial resources (Gloyd and Conant, 1990). Thus, Cottonmouths may be ideal comparative subjects for addressing questions regarding terrestrial buffer zone applications for riparian snakes. The relative importance of terrestrial buffer zones was assessed for a Cottonmouth population by examining spatial distribution patterns around a stream/riparian habitat.

Materials and Methods

Study site and experimental subjects.—I conducted this study from May 2000 to August 2001 at Sam Houston State University's Center for Biological Field Studies (CBFS). The CBFS was adjacent to the Sam Houston National Forest and consisted of a small creek system within a pine woodlands habitat, dominated by Loblolly Pine (*Pinus taeda*). The streambed was immediately surrounded by deciduous forest, primarily dominated by Box Elder (*Acer negundo*) and American Elm (*Ulmus americana*). This riparian zone generally receded into a pine woodlands habitat within approximately 20 m from the stream. See Dent and Lutterschmidt (2001) for a detailed description of the CBFS. Cottonmouths (*Agkistrodon piscivorus leucostoma*) were collected from the study site at the beginning (April-June) of each radio-tracking season. Each radio-tracked snake weighed greater than 160 g, and each 5.5 g transmitter comprised less than 5% of total body weight. From May to August 2000, I radio-tracked two gravid females, two non-gravid females, and one male. In the following year (May to August 2001), I radio-tracked three gravid females, three non-gravid females, and four males. Thus, the total sample is represented by five gravid females, five non-gravid females, and five males.

Surgical Procedures.—Holohil Systems Inc. SB-2 transmitters were coated with a 1:1 mixture of paraffin and beeswax and surgically implanted into the body cavity using methods described by Reinert and Cundall (1982). Prior to surgery, I administered isoflurane (inhalation gas) until subjects were unresponsive to touch. After surgery, I maintained snakes in the laboratory for approximately one week. Water was provided *ad libitum* until snakes were released at original capture locations.

Data Collection.—Radio-tagged snakes were usually located every other day, during the early morning and afternoon hours (0500-1500). Upon locating each snake, geographic coordinates (UTM, Datum WGS 84) were recorded with a global positioning system (GPS) receiver (Magellan GPS 320: accuracy ± 15 m). If snake locations were within 10 m (333 of 402 observations: 83%) from the stream, distances were recorded with a measuring tape. Locations approximately 10-50 m (53 observations: 13%) from the stream were estimated using calibrated paces (accuracy ± 1 m for every 10 m estimated) and then compared to distances estimated within ArcView 3.3 (Environmental Systems Research Institute, Inc., 1999) from GPS coordinates. If these two distance estimates differed by more than 10%, distances were measured again. This was only necessary for two observations. Distances from the stream in excess of approximately 50 m (16 observations: 4%) were all estimated within ArcView. Individual snakes were never relocated twice on a single day and at least 15 hours elapsed between each observation.

For each of the 15 individuals, the proportions of total observations within a 10 m, 20 m, 30 m, 40 m, 50 m, and 60 m buffer radius around the stream were calculated and arcsin transformed prior to statistical analysis (Sokal and Rohlf, 1995). A two-factor repeated measures ANOVA was applied to compare population subunits (factor 1: males, non-gravid females, and gravid females) in respect to the proportion of observations (dependent variable) recorded for each buffer radius size (factor 2).

Results

Individuals were radio-tracked for 51.10 ± 4.00 (mean ± SE) days with an average of 26.4 ± 0.97 observations per individual. Cottonmouths were observed from 0 to 94 m from the stream. Significant main effects for buffer radius size (repeated measures ANOVA: $F_{5,60} = 10.695$, $P < 0.0001$) were reflective of clumped distributions near the stream, as 82.8% of all observations were within a 10 m buffer radius (Fig. 1). Spatial patterns varied among the population subunits ($F_{2,12} = 4.101$, $P < 0.05$)), as gravid females were more often observed in terrestrial habitats further from the stream (Fig. 1). The distribution of gravid females significantly differed from non-gravid females (Fisher's

Protected Least Significant Difference: $P < 0.05$). Differences between gravid females and males were marginal ($P = 0.098$). No interaction effects were observed ($F_{10,60} = 1.189$, $P = 0.317$).

Discussion

Although 83% of all observations were within 10 m of the stream, population subunits were non-randomly distributed. Gravid females comprised most of the distant terrestrial observations up to 94 m from the stream. Thus, similar to pond salamanders (Semlitsch, 1998) and freshwater turtles (Burke and Gibbons, 1995), a critical reproductive component of this snake population inhabited adjacent terrestrial areas beyond the immediate riparian habitat. Factors driving increased terrestrial use by gravid females remain unclear. Additional studies are needed to address the influence of reproductive state on Cottonmouth spatial use.

Cottonmouths may be useful taxa for future comparative studies addressing buffer zone implications for riparian snake populations, but how do cottonmouths compare to other riparian snakes? While some spatial studies have been conducted on semiaquatic species (e.g., Fraker, 1970; Brown and Weatherhead, 1999; Roe et al., 2003), very few have focused on spatial differences within population subunits in terms of terrestrial habitat use and relative proximity to aquatic areas. Massasaugas (*Sistrurus catentatus*) are known to inhabit swamps and marshes, as well as other habitat types. Reinert and Kodrich (1982) demonstrated that Massasaugas select wet areas and vegetated zones in the spring and fall but chose dryer terrestrial upland areas during the summer. Again, this trend was especially evident in gravid females. In another study, Michot (1981) reported a winter mean distance from water of 18.7 m for Diamondback Water Snakes (*Nerodia rhombifera*), suggesting some degree of seasonal terrestrial use.

Studies of other semiaquatic snakes are not suggestive of extensive terrestrial use. Tiebout and Cary (1987) described Brown Water Snakes (*Nerodia sipedon*) as strictly edge species with no observations beyond 6 m from the shoreline. Whiting et al. (1997) compared the spatial use of males, gravid females, and non-gravid females within a Concho Water Snake (*Nerodia harteri paucimaculata*) population and reported a mean distance from water of less than 3 m for each group. Similarly, a survey study by Scott et al. (1989) on Harter's Water Snake (*Nerodia harteri*) reported all observations within 3 m of the shoreline.

Although some wetland snakes may have minimal terrestrial requirements and others may be strictly aquatic (e.g., *Acrochordus arafurae*; Shine and Lambeck, 1985), their persistence may still depend on adequate buffer zones. For many wetland snakes, a large element of the prey base includes amphibians (e.g,. Mushinsky and Hebrard, 1977; Kofron, 1978), which are often dependent on terrestrial habitats (e.g., Semlitsch, 1998; Richter et al., 2001). Furthermore, amphibians, fishes, and other elements of the prey base can be sensitive to water quality (e.g., Moring, 1982; Chessman et al., 2002; Jansen and Healey, 2003), that is also influenced by the surrounding terrestrial habitat (e.g., Gilliam, 1994; Houlahan and Findlay, 2003). Finally, riparian snakes may also rely on terrestrial sites for overwintering (e.g., Michot, 1981; Roe et al., 2003).

In conclusion, similar to studies addressing other vertebrate taxa, this case study provides arguments for the importance of terrestrial buffer zones to Cottonmouths and other riparian snakes. More importantly, this study stresses the importance of considering differences in population substructure before proposing buffer attributes and implementing conservation management plans. Additional studies are needed to enhance our understanding of buffer zone applications for preserving biodiversity and environmental integrity. Many snake species commonly inhabit riparian/terrestrial ecotones and may provide useful spatial models for comparative buffer zone studies.

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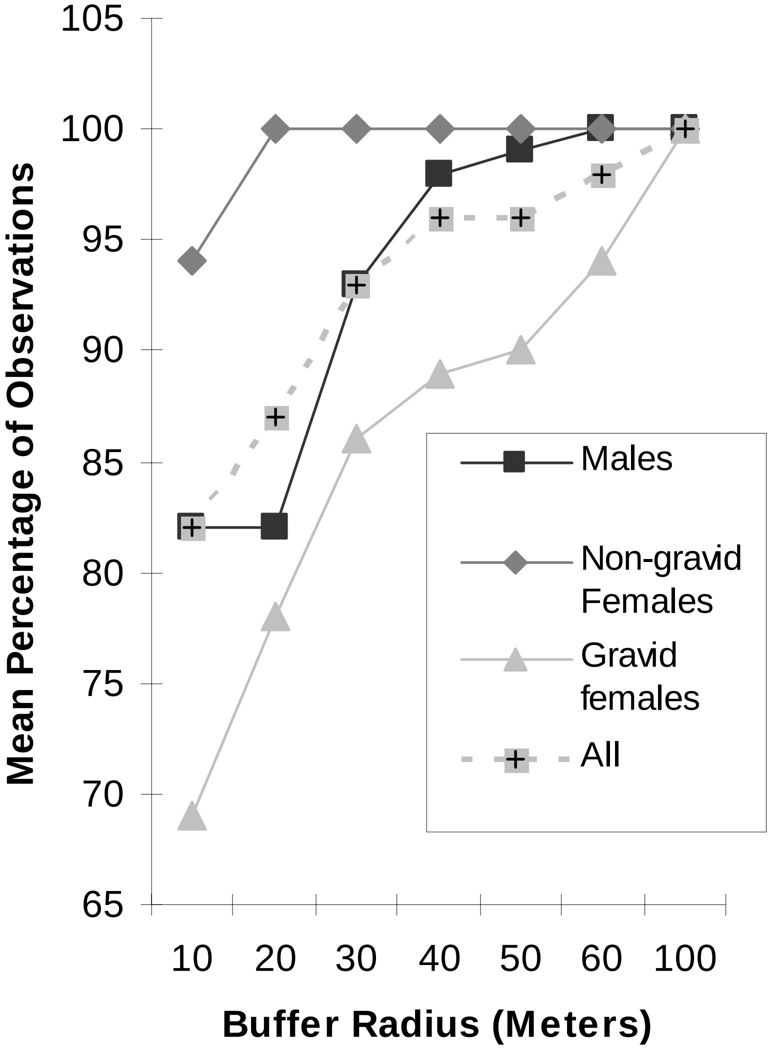
Figure Captions

Figure 1. The mean percentage of observations for each population subunit (gravid females, non-gravid females, and males) is displayed for each buffer radius size.

Figure 1



105
100
95
90
85
80
75
70
65
Mean Percentage of Observations
10
20
30
40
50
60
100
Buffer Radius (Meters)
Males
Non-gravid Females
Gravid females
All

Chapter 3

Sex Differences in Spatial Dispersion Patterns within a Cottonmouth (*Agkistrodon piscivorus*) Population in East Texas

Abstract

Social interactions often play a significant role in determining patterns of spatial use. Although snakes are generally thought of as non-social, recent spatial dispersion studies suggest that the spatial ecology of snakes may be more strongly influenced by social interactions than previously believed. I investigated spatial dispersion patterns within a cottonmouth (*Agkistrodon piscivorus*) population in east Texas. Although causal mechanisms remain unclear, results from radio-telemetry observations on free ranging *A. piscivorus* and spatial dispersion data in outdoor arenas both indicate that females are more gregarious than males. Thus, similar to other vertebrate groups, a growing body of literature suggests that social interactions in snakes should be strongly considered when interpreting spatial ecological data.

Introduction

Social interactions, including territoriality, aggressive behavior, and active avoidance or attraction to conspecifics, may influence home range size, movements, spatial distribution, and other patterns of spatial use (Brown and Orians, 1970). In squamates, social interactions commonly influence the spatial ecology of many lizard species (Stamps, 1977, 1988). In comparison, snakes are generally considered to be relatively non-social (Brattstrom, 1974), but recent field and laboratory studies (e.g., Clark, 2004a; Webb and Shine, 1997; Whitaker and Shine, 2003) suggest that social interactions may more strongly contribute to patterns of spatial use than previously believed. Further research is needed to explore the role of social interactions in the spatial ecology of snakes.

Although causal mechanisms remain unclear, intra-specific aggressive interactions or "combat bouts" have often been observed in snakes, especially between males (Gillingham, 1987). These combat bouts generally conclude with the rapid retreat of the presumably subordinate individual (Gillingham, 1980; Gillingham et al., 1983). Radio-telemetric field studies further suggest that aggressive interactions may influence patterns of spatial dispersion. Webb and Shine (1997) suggest that a lack of temporal and spatial overlap in movement patterns of *Hoplocephalus bungaroides* may indicate that these snakes actively avoid conspecifics. Similarly, Whitaker and Shine (2003) found that adult male *Pseudonaja textiles* had smaller spatial overlap in home ranges than females, cohabited less, and upon arrival of a male to an occupied burrow, prior conspecific residents were rapidly displaced.

In addition to aggressive interactions, gregarious behavior is often observed during a variety of seasonal and daily activities (Ford and Burghardt, 1993; Gillingham, 1987; Gregory, 2004; Gregory et al., 1987). For example, aggregations of gravid females have been commonly observed prior to parturition (Graves and Duvall, 1995), and inter-sexual aggregations have been observed during periods of foraging (Arnold and Wassersug, 1978; Wharton, 1969) and ecdysis (Ashton, 1999; Gregory et al., 1987). However, it is often unclear whether these aggregations form in response to a mutual attraction for other conspecifics or a shared resource.

Many aggregations are likely driven by chemosensory perception of available resources, as snakes often use chemical cues to converge on resources such as prey (e.g., Burghardt and Goss,1988; Clark, 2004b; Kubie and Halpern, 1979; Roth et al., 1999) and potential mates (e.g., Graves et al., 1991; Heller and Halpern, 1981; Kubie, 1978). However, other studies suggest that some aggregations outside the contexts of mating and foraging are, in part, facilitated by a mutual attraction to conspecifics. In the field (Graves et al., 1986; Reinert and Zappalorti, 1988) and laboratory (e.g., Brown and Maclean, 1983; Heller and Halpern, 1981; Scudder et al.,1988) neonates will follow scent trails of conspecifics. Other laboratory studies have demonstrated that some snakes prefer areas soiled by conspecifics (e.g., Allen et al., 1984; Halpin, 1990; Scudder et al., 1980, but see Porter and Czaplicki, 1974). Additionally, a laboratory spatial dispersion study by Clark (2004a) demonstrated that female sibling *Crotalus horridus* were more gregarious than non-siblings, suggesting a mutual attraction between kin. Similarly, Yeager and Burghardt (1991) found that social recognition of prior competitors affected aggregation patterns. Together, these studies suggest that social interactions may play a significant role in the spatial ecology of snakes.

Previous spatial studies have demonstrated sex differences in home range size (Roth 2005b, In press: Chapter 1) and spatial distribution (Roth 2005a: In press: Chapter 2) within a cottonmouth (*Agkistrodon piscivorus*) population in east Texas. In the present study, I examined patterns of spatial dispersion within this population. Spatial overlap patterns among males and females are reported for free ranging individuals and snakes confined to outdoor arenas under semi-natural conditions.

Materials and Methods

Experiment 1: Free ranging spatial distribution

From May 2000 to January 2002, I conducted a radio-telemetry study on a Cottonmouth (*A. piscivorus)* population at Sam Houston State University's Center for Biological Field Studies (CBFS). Detailed descriptions of radio-telemetric methods, data acquisition, and resulting home range estimations for 15 (5 males, 5 gravid females, 5 non-gravid females) radio-tagged *A. piscivorus* have previously been reported (See Chapter 1: Roth 2005b In press). To address sex differences in spatial dispersion, the present study builds upon these previous results and incorporates data from all chance encounters with other (non-radio-tagged) *A. piscivorus* at the CBFS during that radio-telemetry study. Upon capturing each non-radio-tagged individual, I recorded their geographic location, injected a passive integrated transponder tag for future identification, and released each snake at the capture location. Geographic coordinates of non-radio-tagged *A. piscivorus* captured during this study period were imported into ArcView 3.3 (Environmental Systems Research Institute, Inc., 1999) and overlaid onto home ranges (95% minimum convex polygons) reported for the radio-tagged *A. piscivorus* in Roth (2005b In Press) (Figure 1). For each of the 15 radio-tagged *A. piscivorus*, I recorded the number of other *A. piscivorus* that were of the same sex and captured within the subject's plotted home range area during that same time period. This produced a quantitative representation of clustering tendencies or spatial overlap in free ranging snakes. A Mann-Whitney U test was used to compare clustering tendencies of males and females.

Experiment 2: Spatial distribution in confined field arenas

Experimental subjects and laboratory conditions:

Approximately one year prior to experimental testing, I collected 20 adult *A. piscivorus*: 10 males, 10 non-gravid females) from the CBFS and maintained them in the laboratory in individual 51x38x18 cm plastic cages on a diet of approximately one mouse per week. The light dark cycle varied naturally with the time of year and temperatures in the laboratory ranged from 21-32°C. Seven days prior to experimental testing, I fed each snake one final mouse.

Testing arenas:

At the CBFS within habitat commonly occupied by *A. piscivorus*, I constructed two outdoor testing arenas approximately 61 m apart. Each arena (Figure 2) consisted of a circular area (6 m: diameter) enclosed with a hardware cloth fence (0.9 m: height). The arena floor primarily consisted of a sandy substrate intermixed with leaf litter. Around the internal perimeter of the arena, I placed four plastic water bowls (33X21X10 cm) demarcating the outermost North, South, East, and West locations. Finally, I placed rows and columns of string across the arena at 0.5 m intervals to create a visual reference grid approximately 0.1 m above the arena floor.

Experimental trials:

Over the duration of the study (July-August 2003), I performed five experimental trials. Each trial consisted of location observations on a pair of males in one arena and a pair of females in another arena. At the onset of each trial, I filled all water bowls with 1.5 L of distilled water and released two adult male cottonmouths of approximately the same size (differences in snout-vent length ≤ 6%) at opposite ends (North and South) of one arena. This process was then repeated with females in the other arena. After release, snakes were given approximately 36 hours for habituation prior to data collection. For each observation, I recorded the location of the two individuals within each arena. I collected observations three times a day (morning, afternoon, and evening) for approximately 4.5 days (14 observations per arena). At least 5 hours elapsed between each observation. After each trial was completed, I collected all subjects from the arenas and manually overturned and intermixed arena substrates to randomly redistribute and homogenize chemical cues and other substrate contents. Each new trial began with the introduction of new naive snakes to the arenas. Methods were identical for all trials except arena sex assignments were counterbalanced (i.e., if an arena contained males in Trial 1, it was assigned females for Trial 2).

Statistical analysis

For each observation, Image J software was used to digitize snake locations within each arena and measure the distance between subjects (DBS) for males and DBS for females. For each trial, I averaged DBS observations for each arena to generate an average DBS value for each sex. I used the Mann-Whitney U test to compare DBS values between sexes for the five trials.

Results

Experiment 1: Spatial dispersion among free ranging snakes

No differences (Mann-Whitney U: $p = 0.831$) in clustering tendencies were observed between gravid and non-gravid females. Thus all females were grouped together and compared to males. Sex comparisons (Figure 3) revealed greater spatial overlap among females (Mann-Whitney U: $p < 0.05$). Furthermore, at a smaller spatial scale I had many anecdotal observations of females coiled directly adjacent to another female. Conversely, males were never observed within sight of another male at a given time. Experiment 2 was designed to explore these anecdotal observations and further examine sex differences in spatial dispersion at a smaller spatial scale.

Experiment 2: Spatial dispersion within confined arenas

Female spatial distributions within the arena were more clumped as DBS values were significantly smaller (Mann-Whitney U: $p < 0.05$) than males (Figure 4). Furthermore, similar to field observations on free ranging snakes, females had strong tendencies to select retreat sites directly adjacent to or even on top of each other. To address this prevailing pattern, I conducted a post hoc analysis examining sex differences in the number of observations per trial that resulted in direct physical contact between subjects. For each trial, of the 14 total observations females averaged 4.4 ± 1.36 SE observations in direct physical contact, whereas males averaged only 0.6 ± 0.4 SE observations in direct physical contact (Figure 5: Mann-Whitney U: $p = 0.052$).

Discussion

In free ranging snakes spatial overlap among females was greater than among males. Thus, similar to field radio-telemetric studies on *P. textilis* (Whitaker and Shine 2003) and *H. bungaroides* (Webb and Shine, 1997), patterns of spatial dispersion may suggest a significant role of social interactions in snake spatial ecology. However, in the present field study many potential confounding variables are uncontrolled. For example, resources are patchily distributed throughout the study site. Thus, females may be simply clustering around patchy resources generating clumped distributions through non-social mechanisms. These results may also reflect sampling biases. It is unlikely that all resident snakes were captured and chance encounters may be biased by behavioral sex differences.

Under more controlled conditions in the outdoor arena study, patterns of spatial dispersion among males and females were replicated at a smaller spatial scale. Similar to free ranging snakes, spatial overlap among females was greater than among males. Females maintained smaller inter-individual distances (i.e., DBS values) and were more likely to be in physical contact with each other. These findings under confined semi-natural conditions are similar to laboratory observations on *C. horridus* within much smaller arenas (Clark, 2004a).

One explanation for these results is that males actively avoid each other and/or larger inter-individual distances are maintained through aggressive interactions. However, aggressive interactions were never observed between males and movements around the arenas lacked discernable patterns and appeared to be irrespective of the other male. Another explanation for the observed spatial dispersion patterns is that females cluster around a shared resource generating clumped distributions. However, discernable resources within the arena, such as water, sunlight, and substrate type, were rather homogeneously distributed. It is plausible that female movements may be in response to detectable patchy resource distributions outside the arena, but again, female affinities for a particular region within the arena were not evident.

Finally, mutual attraction of females to each other may provide another explanation for spatial dispersion patterns. In both the present study and Clark (2004a), this explanation seems likely given the strong tendencies for females to be in direct physical contact with each other.

From small laboratory arenas (*C. horridus*: Clark, 2004a) to larger outdoor semi-natural arenas (*A. piscivorus*: present study) to free ranging snakes (*A. piscivorus*: present study; *H. bungaroides*: Webb and Shine, 1997; *P. textilis*: Whitaker and Shine, 2003), similar patterns of spatial dispersion are beginning to emerge at different levels of spatial scale. Although causal mechanisms are yet unclear, results suggest that social interactions in snakes may play a significant role in snake spatial ecology and warrant further research.

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Figure Legends

Figure 1. Example analysis of spatial overlap among same sex individuals. The minimum convex polygon home range is plotted for a female monitored by radio-telemetry. Other females captured during this time period are overlaid onto the home range map. The number of females captured within the home range were recorded.

Figure 2. (2A) Side view depicting general shape and size of outdoor arenas. (2B) Top view depicting arena attributes and layout of water bowls (grey rectangles). For each observation, the distance between subjects (DBS) was recorded.

Figure 3. Mean number (± standard error) of same sex individuals captured within the home range of males and females monitored by radio-telemetry. Example analysis depicted in Figure 1. Spatial overlap among females was significantly greater than among males.

Figure 4. Mean distance between subjects per trial for males and females in outdoor arenas. Distances between females were significantly less than males.

Figure 5. Mean number of observations of physical contact between subjects per trial for males and females. Observations of females in physical contact significantly exceeded such observations in males.

Figure 1

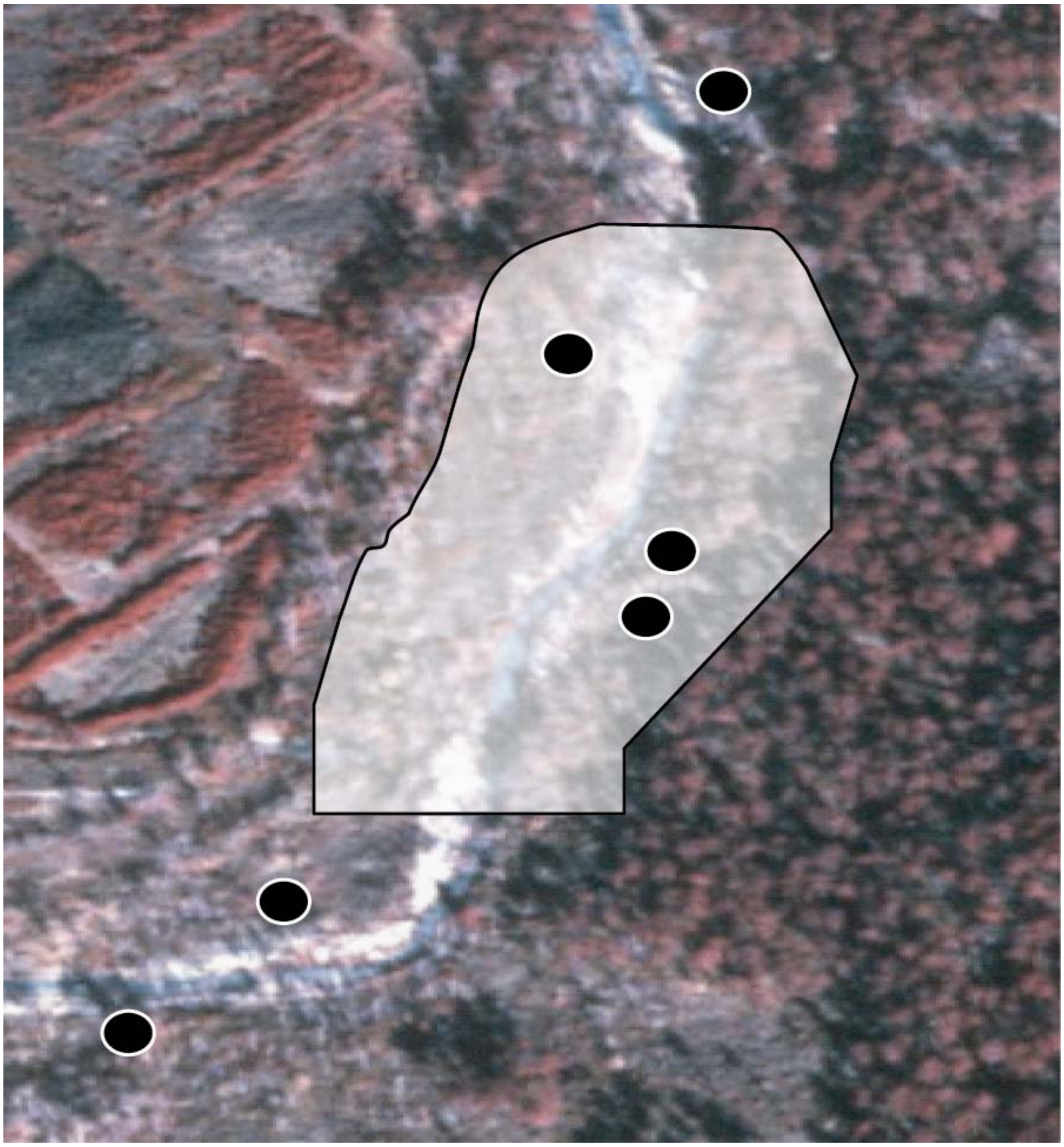


Figure 2

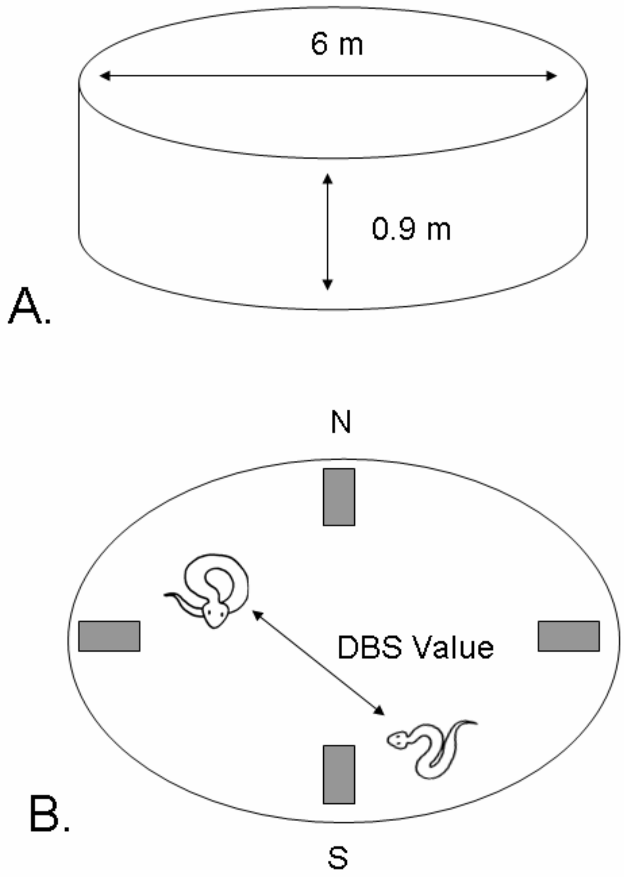


Figure 3


Free Ranging Snakes
Mean Number of Same Sex Individuals
2.0
1.5
1.0
0.5
0.0
Female
Male

Figure 4

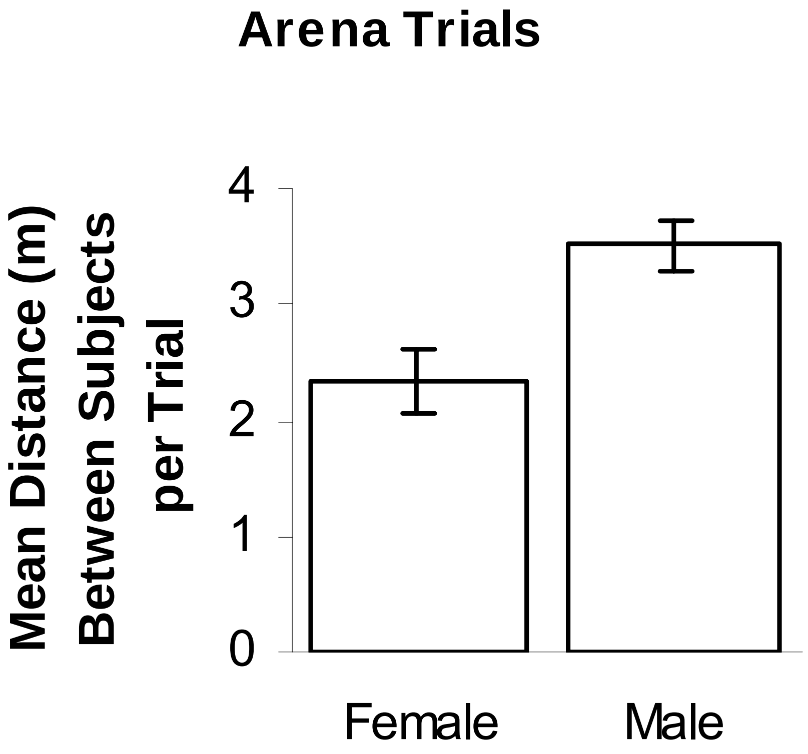
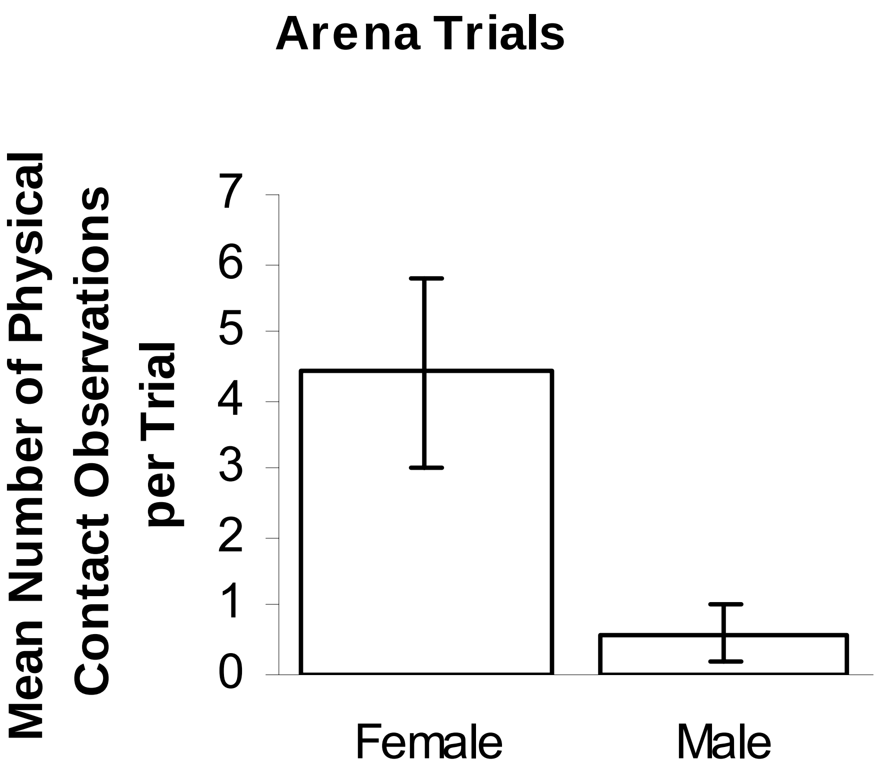

Arena Trials
Mean Distance (m) Between Subjects per Trial
4
3
2
1
0
Female
Male

Figure 5


Arena Trials
Mean Number of Physical Contact Observations per Trial
0
1
2
3
4
5
6
7
Female
Male

Chapter 4

Size-based Variation in Antipredator Behavior within a Snake (*Agkistrodon piscivorus*) Population

Abstract

Variation in an animal's response to a predator likely reflects the complex interaction of factors that influence predation risk. Due to their high degree of behavioral variation and simplified *bauplan*, snakes offer a unique model for investigating the influence of sex and body size on antipredator behavior. We examined variation in antipredator behavior within a cottonmouth (*Agkistrodon piscivorus leucostoma*) population. Behavioral responses to human induced predation risk were compared across a continuous scale of body size. Defensive responses significantly declined with increasing body size. After controlling for body size, no differences between the sexes were detected. While our study suggests that variation in antipredator behavior is, in part, related to body size, some studies on snakes have not found this relationship. Likewise, some studies have demonstrated differences between sexes. Such disparate patterns of variation indicate a need for future comparative studies examining the complex interaction of factors that may influence predator-prey relationships. *Key words: Agkistrodon piscivorus leucostoma*, body size, cottonmouth, intraspecific variation, sex, snakes.

Introduction

Variation in antipredator behavior has been demonstrated across a diversity of taxa, both between and within species or populations (reviewed by Edmunds, 1974; see also Arnold and Bennett, 1984; Ducey and Brodie, 1991; Labra and Leonard, 1999; Randall et al., 1995). Examining patterns of variation may enhance our understanding of predator-prey interactions and provide insight into factors influencing antipredator behavior. Optimality theory suggests that the behavioral response of an individual to a predator is influenced by the risk of predation (Cooper and Vitt, 2002; Lima and Dill, 1990; Ydenberg and Dill, 1986). Furthermore, differences in individual physiology, morphology, and ecology may affect an individual's response to predation risk. For example, studies have demonstrated that antipredator behaviors can vary relative to sex (Clutton-brock, 1991; Magurran and Nowak, 1991; Shine et al., 2000) and body size (Gomes et al., 2002; Krause et al., 1998; Puttlitz et al., 1999). However, these factors operate within an ecological context and are subject to complex interactions with other intrinsic (e.g., age, reproductive condition, and prior experience or learning) and extrinsic factors (e.g., predator type and density, habitat characteristics, temperature, and social context) (reviewed by Endler, 1986). Variation in antipredator behavior likely reflects these complex interactions and their influence on predation risk (reviewed by Lima and Dill, 1990; Lima, 1998). The complexity of predator-prey relationships dictates a need for comparative studies from which further generalizations of antipredator behavior may be established.

Snakes exhibit a diverse suite of antipredator behaviors (reviewed by Greene, 1988; see also Weldon et al., 1992), that can vary with body size (Carpenter and Gillingham, 1975; Gutzke et al., 1993; Hailey and Davies, 1986; Shine et al., 2002; Sweet, 1985; Whitaker and Shine, 1999; Whitaker et al., 2000), sex (Herzog and Burghardt, 1986; King, 2002; Scudder and Burghardt, 1983; Shine et al., 2000; Webb et al., 2001), reproductive state (Goode and Duvall, 1989; Graves, 1989), body temperature (Brodie and Russell, 1999; Goode and Duvall, 1989; Layne and Ford, 1984; Passek and Gillingham, 1997; Shine et al., 2000, 2002; Webb et al., 2001; Whitaker and Shine, 1999), body condition (Andrén, 1982), and a variety of extrinsic factors (Duvall et al., 1985; Shine et al., 2000, 2002; Whitaker and Shine, 1999). Furthermore, relationships among these variables are often inconsistent between species and populations. Given the numerous factors that may influence antipredator behavior, interpreting variation among and within taxa is problematic. However, it is likely that some factors are more influential than others, and may be more useful when constructing general models of antipredator behavior.

In reptiles, antipredator behavior has been shown to vary through ontogeny (reviewed by Greene, 1988; see also Vitt, 2000), specifically with body size (Cooper and Vitt, 1985; Fox, 1978; Huey and Pianka, 1977). Differential survivorship of alternate strategies can lead to divergent behavioral phenotypes, especially in prereproductive stages (Fox, 1978). Since predation is most likely the greatest threat to juvenile survivorship, behavioral phenotypes that reduce the effects of predation are no doubt subject to natural selection. Given that body size may strongly influence predation risk (e.g., Blomberg and Shine, 2000; Janzen et al., 2000; Shine et al., 2001; Tucker et al., 1999; Vitt, 2000), variation in antipredator behavior likely reflects different selection pressures on different body sizes. Snakes are ideal models to investigate the effects of body size on behavioral variation because, unlike birds and mammals, morphology remains relatively constant with sex and age, but body size varies enormously.

Investigations into ontogenetic variation of snake antipredator behavior have typically focused on differences between small and large or juvenile and adult snakes (e.g., Shine et al., 2002; Sweet, 1985; Whitaker and Shine, 1999; Whitaker et al., 2000). While such studies are effective in demonstrating variation, dichotomous comparisons are limited in their ability to adequately address variation in antipredator behavior across the entire continuum of body size or age. For example, variation within groups may be further explained by body size, but these patterns may remain obscured by simple between group comparisons. Furthermore, comparative studies including neonates must be interpreted with caution. Neonates may have difficulty in perceiving and responding to predation risk because of inexperience with predatory encounters and underdeveloped morphological and physiological traits (reviewed by Morafka et al., 2000; see also Pough 1977, 1978). Additionally, neonates may possess a limited behavioral repertoire simply because certain behaviors have yet to develop (Greene, 1988; and references therein). Due to developmental limitations and extreme ontogenetic differences in ecology, direct comparisons in antipredator behavior between neonates and other age groups may further confound behavioral interpretations. Thus, studies that explore variation within the non-neonate component of a population may be of great comparative value.

Herein we examined the influence of body size and sex on antipredator behaviors of a western cottonmouth (*Agkistrodon piscivorus leucostoma*) population. We explored variation in antipredator behavior across a continuous scale of body size and attempted to minimize the influence of developmental limitations by examining only individuals greater than one year in age.

Methods

We collected 46 western cottonmouths from Huntsville, Texas, at the Center for Biological Field Studies, Sam Houston State University. We maintained the snakes in the laboratory for 3-16 months prior to experimental testing in individual 51x38x18 cm plastic cages. We fed cottonmouths one mouse per week except for the two weeks prior to testing, as recent ingestion may alter antipredator behavior (Herzog and Bailey, 1987; Shine et al., 2002). Water was provided *ad libitum*. The light dark cycle varied naturally with the time of year and temperatures in the laboratory ranged from 21-32°C. Upon completion of the study, animals were sexed with a cloacal probe and snout-vent length (SVL) was recorded using the squeeze box technique (Quinn and Jones, 1974). Subjects were maintained in the laboratory for future observations.

Experimental Design

Forty-six snakes, ranging from 30.5-82.5 cm in snout-vent length (SVL), were tested in a randomized order on 16 November 2002, between 1300-2000 hrs in their individual cages. Two hours before testing, all subjects were habituated to room temperature (27-28°C). At this temperature, we subjected individual cottonmouths (23 males, 23 non-gravid females) to a 60 s experimental trial executed in three consecutive 20 s stages separated by one minute intervals. The three stages of the experiment were designed to mimic a predation event with an escalating level of predation risk over time. Similar to other antipredator snake studies (e.g., Burghardt and Greene, 1988; Herzog et al., 1989; Gibbons and Dorcas, 2002; Scudder and Burghardt, 1983; Shine et al., 2000; 2002), humans were used as the threat stimulus to generate antipredator responses. During the first stage, we removed the top of the cage exposing the snake to the two observers. During the second stage, we approached and harassed the snake with gentle nudges to the snout using a welding glove placed on the end of a pair of 1 m snake tongs as the threat stimulus (similar to methods of Gibbons and Dorcas, 2002). For the third stage, we picked up the snake at mid-body with the tongs for approximately five seconds, released the snake, and then repeated this process.

Seven common behaviors (i.e., escape behavior, defensive posturing, tail vibrations, musk release, mouth gapes, strikes, and bites) often considered antipredator responses (e.g., Gibbons and Dorcas, 2002; Greene, 1988, 1997; Klauber, 1997) were quantified with each behavioral category receiving a score of 0-2 based on predefined scoring criteria. Categorical scores were awarded by a consensus of the two observers at the end of each stage. For each category, individuals failing to exhibit a response were awarded zero points. Otherwise, we scored the categories as follows. Escape behavior was defined as any attempt to move away from the threat stimulus. Slow but directed movements away from the threat stimulus were awarded 1 point, whereas quick movements in the same manner were awarded 2 points. Defensive posturing was defined as the classical striking coil (Klauber 1997) or S-curve in the neck as the snake appears ready to strike. Brief or intermittent defensive posturing < 10 s was scored as 1 point., whereas, defensive posturing ≥ 10 s was scored as 2 points. Vibrating the tail generally in short bursts of 1-2 s was defined as a tail vibration. We awarded 1 point for 1-2 tail vibrations and 2 points for > 2 tail vibrations. Because musking behavior was difficult to quantify, we scored any musk release as 2 points. Cottonmouths will briefly mouth gape to display the white inside lining of the mouth. We scored 1 mouth gape as 1 point and > 1 mouth gape as 2 points. A strike was defined as a quick forward thrust towards the threat stimulus. This action was occasionally followed with biting behavior. Bites and strikes were scored in the same manner as mouth gapes.

Total points were recorded for each 20 s stage with a maximum of 2 points for each behavioral category (i.e., maximum of 14 points per 20 s stage). Scores from the three stages were then totaled for the 60 s trial to create a total behavioral index score for each cottonmouth. This score represents a measure of cumulative response to escalating levels of predation risk.

Data Analysis

While index scores for each behavior were based on an ordinal scale, the numerous combinations of ordinal values allowed for by our experimental protocol generated a large spread of possible values in the dataset simulating a pseudo-interval value (total behavioral index score). Total behavioral index scores and SVL measurements were then log base 10 transformed to further conform to assumptions of normality (Sokal and Rohlf, 1995). Although parametric analysis on pseudo-interval data is problematical, the F-test is fairly robust to violations of normality and related assumptions (discussed in Underwood 1997). Thus, we report parametric statistics when analyzing total behavioral index scores.

We used a simple regression to test for a relationship between body size (log SVL: independent variable) and antipredator behavior (log total behavioral index score: dependent variable). We performed an ANCOVA to test for behavioral differences (log total behavioral index score: dependent variable) between the sexes (independent variable) with body size (log SVL) as the covariate. For each individual we calculated the proportion of points each behavioral category contributed to the total behavioral index score. All proportions were arcsine transformed prior to statistical analysis (Sokal and Rohlf, 1995). We performed a repeated-measures ANOVA to test for proportional differences between behavioral categories. If behaviors are considered separately, index scores for each behavior more closely resemble ordinal data and do not meet the assumptions of normality. Thus, to further examine each behavior separately, Spearman rank analyses (corrected for ties) were applied to test for correlations between non-transformed index scores and SVL.

Results

Time in captivity was randomly distributed across all body sizes and was not correlated with total behavioral index scores (Spearman rank: $r_s = 0.063$, $p = 0.6713$). Thus any effects due to differences in time in captivity were negligible and were not considered in further analyses. A negative relationship (Fig. 1) exists between body size and antipredator behavior (simple regression: $r^2 = 0.23$, $F_{1,44} = 12.78$, $p < 0.001$). No interaction between body size and sex was evident (ANCOVA: $F_{1,42} = 0.52$, $p = 0.47$). Thus the interaction term was removed from the model and main effects were compared. Effects of body size (SVL : covariate) were significant (ANCOVA: $F_{1,43} = 12.28$, $p < 0.005$). However, no significant differences in relative antipredator behavior were detected between sexes (ANCOVA: $F_{1,43} = 0.01$, $p = 0.96$). The repeated-measures ANOVA revealed differences in the relative contribution of each behavioral category ($F_{6,270} = 23.59$, $p < 0.0001$; Fig. 2) to the total behavioral index score. The most common behavior was escape, which significantly differed from all other behavioral categories (Fisher's Protected Least Significant Difference: $p < 0.0001$). Graphical relationships between body size (SVL) and each behavior are depicted in Fig. 3. Spearman rank correlations revealed significant negative relationships with body size for tail vibrations ($r_s = -0.308$, $p < 0.05$) and musk (r_s

= -0.513, $p < 0.001$). The negative correlations with body size for posture ($r_s = -0.265$, $p = 0.0758$) and mouth gapes ($r_s = -0.272$, $p = 0.068$) were marginally significant. No correlation was found with body size for escape ($r_s = 0.117$, $p = 0.4308$), strikes ($r_s = -0.121$, $p = 0.4174$), or bites ($r_s = -0.09$, $p = 0.5457$).

Discussion

As body size increased, total behavioral index scores decreased. To interpret this result, it is important to consider how behavioral index scores were generated. Index scores for each individual were based upon the number of antipredator behaviors recorded and the degree to which each behavior was exhibited throughout the trial. Essentially, this index score represents behavioral diversity with varying levels of intensity. However, the sequence of behavioral categories is not random, but occurs in a generalized progression. For example, an individual usually responds to an initial threat stimulus with an escape behavior or another passive response. An aggressive defense, such as a strike or bite, rarely occurs without an escape attempt or some initial progression of passive behaviors, which are presumably intended to warn, threaten, or distract the predator (Greene, 1988). This hierarchical system of antipredator behavior is common among snakes (for discussion see Duvall et al., 1985). An increase in diversity and intensity of behavior is indicative of a hierarchical progression of antipredator tactics. Our behavioral index scores provided a quantitative measure of defensive response, recorded across a sequence of events, which were designed to mimic an escalating predation threat.

Our results demonstrated that antipredator behavior varied with body size and increases in body size were associated with a decrease in the level of defensive response. Ontogenetic variation in antipredator behavior is common among reptiles (reviewed by Greene, 1988), but reasons why defensive response declines with increasing body size are unresolved. This may result from reduced predation risk in larger individuals (e.g., Blomberg and Shine, 2000; Janzen et al., 2000; Shine et al., 2001; Tucker et al., 1999; Vitt, 2000) simply because small predators that could easily kill a small cottonmouth might not be able to subdue and consume a larger cottonmouth. Furthermore, smaller individuals may have locomotor limitations related to speed or endurance (reviewed by Carrier, 1996, see also Garland and Losos, 1994) that affect their ability to escape. Indeed, as a general rule, body size in snakes is positively related to locomotor performance (Finkler and Claussen, 1999; Hailey and Davies, 1986; Jayne and Bennett, 1990; Kelley et al., 1997; Pough, 1977, 1978; Scribner and Weatherhead, 1995), and inversely related to predation risk (Mushinsky and Miller, 1993; Shine et al., 2001). Similar to our results, many snake studies indicate that defensive responses decline with increasing body size (Bogert, 1941; Carpenter and Gillingham, 1975; Gutzke et al., 1993; Hailey and Davies, 1986; Shine et al., 2002; Sweet, 1985; but see Layne and Ford, 1984). Shine et al. (2000) found no direct effects of body size on defensive response, but suggested that body size indirectly influenced antipredator behavior through temperature, because larger snakes were typically warmer.

This explanation does not account for our size-based behavioral variation because all snakes were habituated to the same temperature prior to testing.

Behavioral variation was exhibited across a continuous scale of body size (SVL) within the non-neonate component of a cottonmouth population. Although a highly significant relationship with antipredator behavior was demonstrated, body size only accounted for a small portion of the variance ($r^2 = 0.225$). Some residual variation is likely attributable to relatively crude behavioral quantification and possibly could be minimized with refined scoring techniques and detailed video analysis. Nonetheless, given the complexity of interactions (physiological, morphological, and ecological) that can influence antipredator behavior, it is not surprising that body size alone accounted for some of the variation.

With the effect of body size removed, we found no differences in behavioral index scores between sexes. Numerous other snake studies also failed to find sex-based differences in antipredator behavior (Hailey and Davies, 1986; Layne and Ford, 1984; Whitaker and Shine, 1999). However, our study is limited in scope as it does not include gravid females and only compares the sexes within a laboratory setting at a snapshot in time during the fall season when reproductive activity is presumably low. How reproductive state and experimental conditions interact to influence motivational state and physiology of our study subjects is unknown. Gravid snakes may exhibit reduced locomotor performance (Seigel et al., 1987, but see Brown and Weatherhead, 1997) and differences in antipredator behavior (Goode and Duvall, 1989). Additionally, other studies have suggested sex differences in snake antipredator behavior

(Herzog and Burghardt, 1986; King, 2002; Scudder and Burghardt, 1983; Shine et al., 2000, Webb et al., 2001) and warrant future research of sex-based influences.

Similar to the results of a field study on cottonmouth defensive behavior (Gibbons and Dorcas, 2002), proportional comparisons of different antipredator behaviors reveal a general pattern of hierarchical decision making: 1) if detected by predator, then retreat (escape behavior), 2) if threatening stimulus persists, employ passive deterrents (tail vibration, defensive posturing, mouth gape, musk release), 3) if the threat further escalates, then engage in aggressive defense (strikes, bites). Our data support this interpretation as behavioral proportions generally declined across this hierarchical gradient (Fig. 2). Within this gradient, mouth gapes represented the lowest mean proportion of behavioral index scores. The mouth gape is a common cottonmouth antipredator behavior (Gibbons and Dorcas, 2002) and is frequently exhibited by our experimental subjects in response to threat stimuli. The low mean proportion of mouth gapes in this study was likely an artifact of our testing methods. Threat stimuli, such as gentle nudges to the snout, were applied in close proximity to the subject and may have inhibited this type of warning behavior.

Examination of each antipredator behavior independently may provide further insight into why total behavioral index scores based on the additive quantification of seven antipredator behaviors generally declined with increasing body size. As body size increased, snakes were equally likely to exhibit escape behavior. This may be expected since escape behavior is an early response in the hierarchical progression. All snakes, despite differences in body sizes, were tested in the same size arena or cage. This method controlled for any confounding effects of absolute cage size but may be prone to influences of relative cage size (i.e., larger snakes were tested in arenas that were smaller relative to their SVL). Escape behavior results demonstrate that all snakes are equally likely to flee despite differences in body size and relative cage size. However, further experiments examining the influence of absolute and relative testing arena size would be of interest.

The next sequence of hierarchical responses, which include passive deterrents or warning behaviors, generally declined with increasing body size. Although a gradual decline is graphically exhibited, aggressive defense behaviors (strikes and bites) did not significantly vary with body size. Thus most of the variation in total behavioral index scores may be explained by the significant negative correlation between passive deterrents or warning behaviors and body size. Reasons for the lack of correlation between aggressive defense behaviors and body size are unclear. Natural selection pressures due to predation risk may still vary across body sizes but may be mediated by different mechanisms producing convergent behaviors. For example, larger snakes may be reluctant to strike simply because perceived predation risk is much lower. Smaller snakes may have a higher perceived predation risk, but a strike effectively decreases the distance between a snake and the predator and increases the vulnerability of the head and neck region to predatory attack. In both cases low index scores for strikes and bites may be predicted.

While behavioral index scores generally declined with increasing body size, we can not rule out that the relationship between body size and antipredator behaviors was indirect, as body size is related to numerous other confounding variables (Peters, 1983; Werner and Gilliam, 1984). For example, an alternate explanation for our results is that the significant negative relationship between behavioral index scores and body size is reflective of experience, thus differences in age. Younger snakes may exhibit an elevated response to all potential predators, whereas older snakes are better able to evaluate predation risk from prior experience and respond accordingly. However, this explanation assumes that the smaller snakes from our study were younger and less experienced. All snakes from our study were greater than one year in age (subadult to adult). Body size is a relatively poor predictor of age, especially in adult snakes. In reptiles, body size and growth rates are asymptotic (Andrews, 1982; Shine and Charnov, 1992) and may be influenced by rates of resource acquisition, daily temperature patterns, and other intrinsic and extrinsic variables (reviewed by Andrews, 1982; see also Madsen and Shine, 2000). Additionally, age is not always a good predictor of experience with predators. Experience may also be dependent upon relative predator densities and activity patterns within an individual’s home range.

Another alternative explanation is that lower behavioral index scores exhibited by larger snakes are the result of differential survivorship and simply represent a subset of behaviors exhibited by smaller snakes. However, given this explanation, we would expect a greater variance in behavioral scores at smaller sizes with a decreasing variance in behavioral scores for the larger sizes. Our results do not support this interpretation as the variance around the best-fit line (Fig. 1) is relatively uniform through all sizes. Furthermore, this explanation again assumes that larger snakes are older, which as we have previously stated, may be an improper assumption.

Although handling and maintaining animals in captivity may affect antipredator behavior (Greene, 1988), our laboratory setup allowed us to reduce potential influences of extrinsic factors. Under these controlled conditions, we were able to demonstrate a size-based relationship with antipredator behavior. While such size-based relationships are common among snakes (Bogert, 1941; Carpenter and Gillingham, 1975; Gutzke et al., 1993; Hailey and Davies, 1986; Shine et al., 2002; Sweet, 1985), some field studies on cottonmouths (Gibbons and Dorcas, 2002) and other snakes (Layne and Ford, 1984; Shine et al., 2000) revealed no size-based differences. It is plausible that complex ecological interactions in a natural setting may negate the influence of body size. However, failure to detect body size influences on antipredator behavior in free ranging snakes may instead result from logistical difficulties in controlling for confounding variables (e.g., differences in microhabitat type, body temperature, recent prior experience), and obtaining adequate sample sizes. Complementary comparative field and lab studies are needed to explain variation in behavioral responses and address patterns of antipredator behavior within a fluctuating environment.

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Figure Legends

Figure 1. Relationship between body size (SVL) and antipredator behavior (behavioral index score) ($r^2 = 0.23$; $p < 0.001$; $n = 46$).

Figure 2. Representation of the mean contribution of a behavior to the overall behavioral index score.

Figure 3. Mean behavioral index scores for each of seven antipredator behaviors across 4 categories of body size (SVL). Significant spearman rank correlations are indicated for each behavior: * = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.001$.

Figure 1

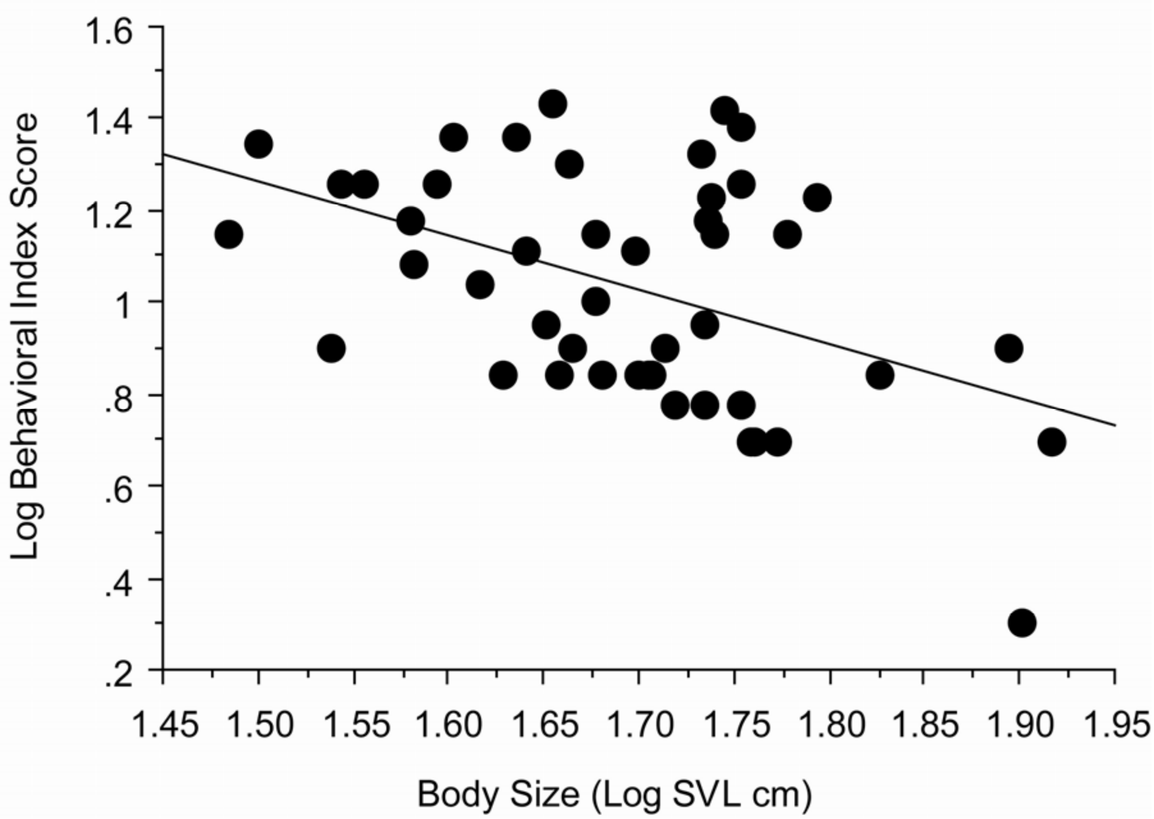

1.6
1.4
1.2
1
.8
.6
.4
.2
Log Behavioral Index Score
1.45
1.50
1.55
1.60
1.65
1.70
1.75
1.80
1.85
1.90
1.95
Body Size (Log SVL cm)

Figure 2

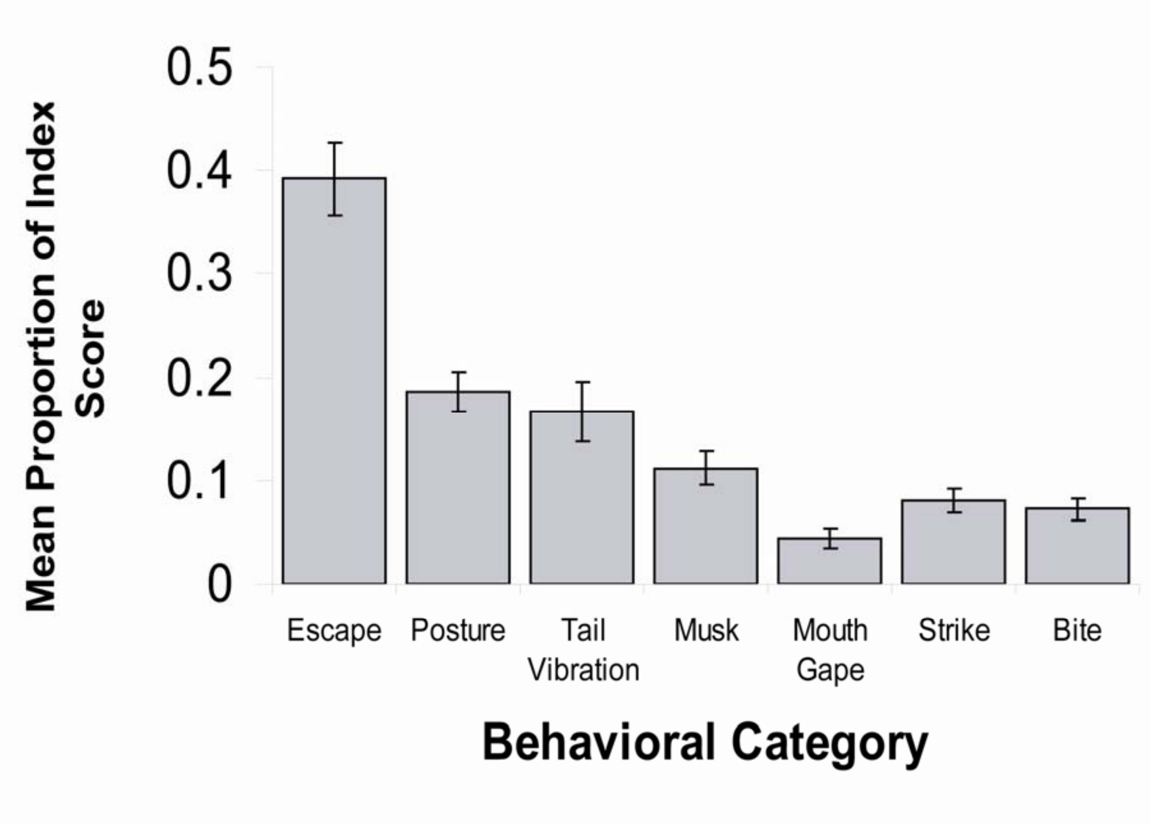
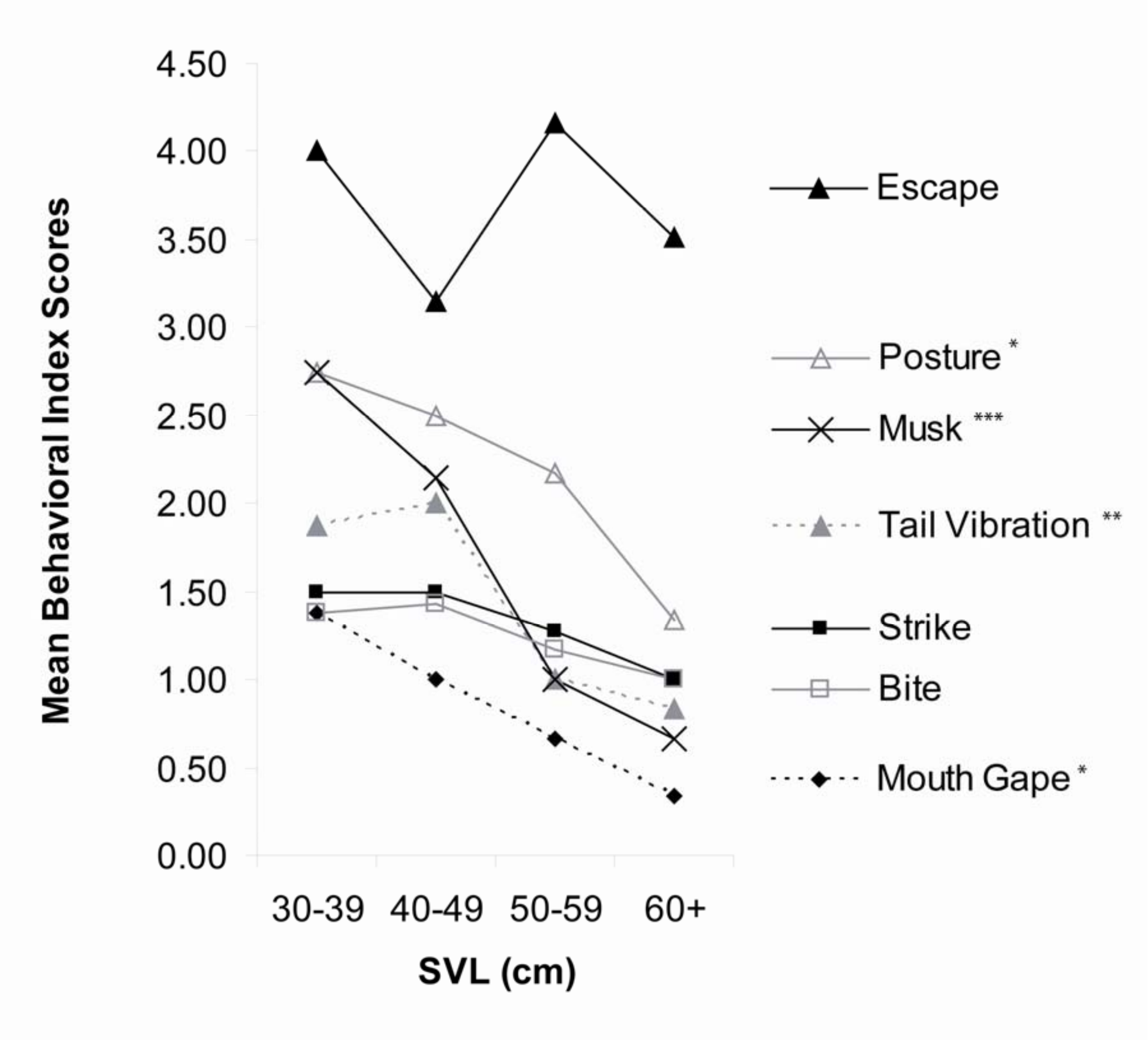

0.5
0.4
0.3
0.2
0.1
0
Mean Proportion of Index Score
Escape
Posture
Tail Vibration
Musk
Mouth Gape
Strike
Bite
Behavioral Category

Figure 3



4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Mean Behavioral Index Scores
30-39
40-49
50-59
60+
SVL (cm)
Escape
Posture *
Musk ***
Tail Vibration **
Strike
Bite
Mouth Gape *

Chapter 5

Relative Medial and Dorsal Cortex Volume in Relation to Sex Differences in Spatial Ecology of a Snake Population

Abstract

In non-avian reptiles the medial and dorsal cortices are putative homologues of the hippocampal formation in mammals and birds. Studies on mammals and birds commonly report neuro-ecological correlations between hippocampal volume and aspects of spatial ecology. We examined the relationship between putative homologous cortical volumes and spatial use in a population of the squamate reptile, *Agkistrodon piscivorus*, which exhibits sex differences in spatial use. Do male *A. piscivorus* that inhabit larger home ranges than females, also have larger putative hippocampal volumes? Male and female brains were sectioned and digitized to quantify regional cortical volumes. Although sex differences in dorsal cortex volume were not observed, males had a significantly larger medial cortex relative to telencephalon volume. Similar to studies on mammals and birds, relative hippocampal or medial cortex volume was positively correlated with patterns of spatial use. We demonstrate volumetric sex differences within a reptilian putative hippocampal homologue. Although definitive causal mechanisms are yet unclear, results from our study parallel results of studies on neural structure and function in other vertebrates.

Introduction

The hippocampal formation (hereafter hippocampus) in mammals and birds plays an important role in spatial learning and memory [O'Keefe and Nadel, 1978; Morris, 1982; Sutherland et al., 1983; Sherry and Duff, 1996; Burgess et al., 1999; Hollup et al., 2001]. Enhanced performance on spatial tasks is often correlated with hippocampal dimensions [Crusio and Schwegler, 1987; Crusio et al., 1987; Biegler et al., 2001]. Studies have begun to establish neuro-ecological links between hippocampal volume and aspects of spatial ecology.

Polygynous voles, which occupy larger home ranges than monogamous voles, also have enhanced spatial abilities [Gaulin and Fitzgerald, 1986] and a larger hippocampus [Jacobs et al., 1990]. Food-storing birds, which are dependent on spatial learning and memory associated with locating storage sites, often exhibit a larger hippocampus than non-food-storing species [Krebs et al., 1989; Clayton, 1995; but see Volman et al., 1997; Brodin and Lundborg, 2003]. Within food storing birds, species that store more food often have a larger relative hippocampus than species that store less food [Healy and Krebs, 1992; Hampton et al., 1995; Basil et al., 1996; Healy and Krebs, 1996]. This enlarged hippocampus may reflect induced neurogenesis from spatial learning [Patel et al., 1997] and may be associated with enhanced memory persistence [Biegler et al., 2001]. Parasitic birds, which presumably require enhanced spatial abilities to locate and return to host nests, have a larger relative hippocampus than nonparasitic birds [Sherry et al., 1993; Reboreda et al., 1996; Clayton et al., 1997]. In humans, taxi cab drivers must perform extensive spatial tasks associated with learning, memory, and navigation. London taxi cab drivers exhibit a larger relative posterior hippocampus than control subjects and volume of the posterior hippocampus positively correlates with amount of time spent as a taxi cab driver [Maguire et al., 2000].

In non-avian reptiles, the medial cortex (MC) and dorsal cortex (DC) are believed to be hippocampal homologues [Butler and Hodos, 1996], but relatively few studies have examined their structure and function. Lesions to the MC [Rodriguez et al., 2002a; 2002b; Lopez et al., 2003a; 2003b] and DC [Blau and Powers, 1989; Avigan and Powers, 1995] of turtles and MC of lizards [Lopez-Garcia et al., 1992] can disrupt cognitive spatial tasks [but see Grisham and Powers, 1989; 1990; Day et al., 2001], However, to our knowledge only one study has examined ecological correlations with cortical volume in squamate reptiles. Day et al. [1999a] report larger relative MC and DC volumes in an actively foraging lizard than volumes observed in a congeneric, relatively sedentary, sit and wait predator, but suggest that in light of a previous behavioral study [Day et al., 1999b], this neuro-ecological correlation may simply reflect nonspatial cognitive demands. Contradictory lesion and behavioral results and a lack of neuro-ecological studies inhibit meaningful comparisons with mammals and birds. Additional comparative studies are needed to enhance understanding of cortical structure and function among divergent taxa.

Cottonmouths (*Agkistrodon piscivorus*) are semi-aquatic snakes that occur throughout the southeastern United States [Gloyd and Conant, 1990]. In the present study population, male *A. piscivorus* occupied larger home ranges than females (Roth 2005, In Press: Chapter 1), a trend common among snakes [Macartney et al., 1988; Reinert and Zappalorti, 1988; Fitzgerald et al., 2002; Whitaker and Shine, 2003]. Given sex differences in spatial use within this cottonmouth population, are there sex differences in relative MC and DC volume?

Materials and Methods

The methods described below were approved by the University of Oklahoma Animal Care and Use Committee (permit number A3240-01).

From 6/21/03 to 7/16/03, 21 adult Cottonmouths (*Agkistrodon piscivorus*: 11 males, 10 females) were collected from the Center for Biological Field Studies (CBFS), Sam Houston State University, Huntsville, Texas. Within 48 h of capture, each snake was deeply anesthetized with isoflurane (inhalation gas) and perfused transcardially with 0.9% NaCl followed by 10% formalin. Brains were removed from the skull, postfixed in 30% sucrose 10% formalin for at least 48 h, and stored at 4°C until embedded and cryoprotected in 10% gelatin 30% sucrose. Brains embedded in gelatin were then postfixed in 30% sucrose 10% formalin for at least 24 h prior to sectioning. Frozen brains were sectioned in a coronal plane at 50 μm on a sliding microtome. Sections were mounted and stained with cresyl violet.

Brain sections were digitized (Fig. 1). Boundaries of brain regions, including the medial cortex (MC), dorsal cortex (DC), and remainder of the telencephalon, were defined as described by Halpern [1980]. Cortical subdivisions such as large cell (dorsomedial) and small cell (medial) portions of the MC were not considered. Regional brain areas were quantified in approximately every other section (100 µm) using NIH Image software. Ventricular area was measured and subtracted from telencephalic area prior to volume calculations. The formula for a truncated cone was used to calculate regional brain volumes. Volumes of both hemispheres were added to generate total volumes.

Statistical Analysis

An ANCOVA was used to test for sex differences in relative total volume of the medial and dorsal cortices. To control for variation in brain size among individuals, telencephalon volume (TV) was used as a covariate in all tests. Within the MC, TV was quantified by subtracting MC volume from the total telencephalon volume on the corresponding brain slices. This provided a measure of telencephalon size independent of the MC. This process was then repeated for the DC to create a unique set of covariates for each brain region investigated.

Results

To test the assumption of homogeneity of regression slopes, ANCOVA tests originally included interaction terms in statistical models. All interaction terms were nonsignificant ($p > 0.2$) and removed from the models prior to reporting traditional ANCOVA results [Sokal and Rohlf, 1995]. In all tests, the main effect for the covariate (TV), was highly significant (ANCOVA: $F_{(1,18)} > 67.0$, $p < 0.0001$). Sex differences (fig. 2) in DC volumes were not observed (ANCOVA: $F_{(1,18)} = 0.309$, $p = 0.585$). However, males had a larger relative MC (fig. 3) than females (ANCOVA: $F_{(1,18)} = 9.645$, $p = 0.006$). Given previous results demonstrating regional structural and functional variation within the mammalian hippocampus [Moser and Moser, 1998; Holscher, 2003; Bannerman et al., 2004], we conducted a post hoc regional examination within the MC. The elongated snake MC was further subdivided into rostral and caudal subregions and statistically reanalyzed. The termination of the connection between the telencephalon and thalamus was arbitrarily chosen as a standardized division point between rostral and caudal regional classifications (fig. 1). Within the rostral MC, sex differences (fig. 4) were not observed (ANCOVA: $F_{(1,18)} = 0.162$, $p = 0.692$). Thus, most of the variation between the sexes in MC volume occurred in the caudal MC (ANCOVA: $F_{(1,18)} = 10.085$, $p = 0.0052$; fig. 5). All statistically significant results remain below Bonferroni-corrected significance levels ($p < 0.0125$).

Discussion

For the first time, sex differences in MC volume were demonstrated within a squamate reptile. Similar to mammals and birds, sex differences in spatial ecological demands correlate with hippocampal volume. Although it is plausible that sex differences in MC volume are already present from early stages in development, many proximate mechanisms associated with learning and memory may also explain correlations between functional demands and neural volume. Functional and morphological plasticity within the brain are often correlated with learning or experience [e.g., Bennett et al., 1964; Polley et al., 2004]. Within the hippocampus, learning may induce neurogenesis [Gould et al., 1999a; Shors et al., 2001], enhance neuron survival [Leuner et al., 2004], and increase dendritic spine density [Moser et al., 1994; O'Malley et al., 2000, Leuner et al., 2003]. Thus, if the MC plays a role in spatial learning and memory, MC volume likely reflects the animal's reliance on spatial information [Gaulin, 1992; Sherry et al., 1992]. Male cottonmouths, which navigate and reside within areas more than twice as large as females (Roth 2005, In Press: Chapter 1), must presumably process more spatial information. As predicted by spatial ecological demands, male cottonmouths in our study exhibited a larger relative MC.

Although our results reveal a correlation between MC volume and spatial ecological demands, the hippocampus also participates in non-spatial functions [Day, 2003; Holscher, 2003]. Furthermore, larger MC volume in males may be related to hormonal influences [Foy et al., 1984; Gould et al., 1990; Roof and Havens, 1992; Woolley and McEwen, 1992; Galea et al., 1999] or other sex related differences. Many studies have also demonstrated sex differences in hippocampal volume among mammals and birds [Healy and Krebs, 1992; Sherry et al., 1996; Clayton et al., 1997; Lavenex et al., 2000], but definitive causal mechanisms remain unclear. A few comprehensive studies [Jacobs et al., 1990; Sherry et al., 1992; Sherry et al., 1993; Jacobs and Spencer, 1994; Reboreda et al., 1996] examining multiple species with contrasting or reversed patterns of spatial use offer more compelling arguments that sex differences in hippocampal volume are related to differences in spatial use or spatial ability. Nonetheless, additional comparative studies are needed to enhance our understanding of mechanisms driving these neuro-ecological relationships.

After subdividing the MC into rostral and caudal subregions, it was evident that sex differences may be primarily attributed to the caudal MC. This result parallels mammalian studies that report functional and structural regional specializations within the hippocampus [Moser and Moser, 1998; Holscher, 2003; Bannerman et al., 2004]. Place cells are concentrated in the dorsal hippocampus [Jung et al 1994], and lesion studies suggest the dorsal, but not ventral, hippocampus is critical for spatial memory [e.g., Moser et al., 1995; Hock and Bunsey, 1998; Bannerman et al., 2002; Pothuizen et al., 2004]. Similar to the mammalian hippocampus, the MC is a complex organization of multiple cell types and layers that perform specialized functions [Ulinski, 1974; Halpern, 1980; Butler and Hodos, 1996]. Thus, potential increases in MC volume in response to increased experience or higher demands for spatial learning and memory are likely localized within select MC subregions. Indeed, our results confirm sex differences in regional cortical organization within the MC, but additional research is required to definitively link these structural differences with spatial cognition.

Contrary to our results within the MC, we did not detect sex differences in relative DC volume. The DC is considered to be homologous to the mammalian entorhinal cortex, which primarily serves as a gateway for interactions between the hippocampus and other brain regions [Butler and Hodos, 1996]. In contrast, the MC is more similar to the mammalian dentate gyrus and Ammon's horn, which are common sites of enhanced structural plasticity associated with putative mechanisms for learning and memory, including neurogenesis, synaptogenesis, and enhanced neuronal survival [Kaplan and Hinds, 1977; Moser et al., 1994, Gould et al., 1999b; Cameron and McKay, 2001, Shors et al., 2001; Leuner et al., 2003; 2004; Shors, 2004]. The DC receives many peripheral inputs and then interacts with other brain centers including the MC [Ulinsky, 1976; Halpern, 1980; Hoogland and Vermeulen-Van der Zee, 1988], where high levels of neurogenesis and structural plasticity have been reported [Lopez-Garcia et al., 1988; Font et al., 1991; Lopez-Garcia et al., 1992; Penafiel et al., 2001; Lopez-Garcia et al., 2002]. Thus, similar to the entorhinal cortex in mammals, lesions to the DC may impair spatial cognition [Blau and Powers, 1989; Avigan and Powers, 1995; but see Day et al., 2001] by limiting projections to the MC. However, in comparison to the MC, increases in DC volume associated with neurogenesis, synaptogenesis, or enhanced neuron survival in response to spatial learning and memory may be relatively limited. This may explain our lack of sex differences in DC volume, despite differences in spatial ecological demands.

Similar to studies on mammals and birds, we have demonstrated correlations between spatial ecological demands and hippocampal (i.e., MC) volume in a squamate reptile. These results support putative evolutionary and functional cortical relationships with the mammalian brain, but many alternative mechanisms may explain these patterns. Nonetheless, neuro-ecological correlations from the present study combined with previous behavioral and neuro-anatomical studies present interesting parallels to similar spatial learning and memory studies in other vertebrates. Such studies highlight the potential importance of future neuro-ecological research. Establishing links between neuro-anatomy and ecological interactions provides a powerful integrative approach for addressing proximate and ultimate mechanisms influencing animal behavior and evolutionary biology.

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Figure Legends

Fig. 1. Examples of digitized brain sections including boundaries of the medial cortex (MC) and dorsal cortex (DC) are illustrated sequentially (A-D) from rostral to caudal telencephalon. Section C depicts the start of the caudal telencephalon, as the connection between the telencephalon and thalamus (T) is terminated.

Fig. 2. Dorsal cortex volume is depicted for each individual relative to residual telencephalon volume. No significant sex differences were observed ($p = 0.585$).

Fig. 3. Medial cortex volume is depicted for each individual relative to telencephalon volume. Males exhibited a significantly larger relative medial cortex ($p < 0.01$) than females.

Fig. 4. Rostral medial cortex volume is depicted for each individual relative to telencephalon volume. No significant sex differences were observed ($p = 0.692$).

Fig. 5. Caudal medial cortex volume is depicted for each individual relative to telencephalon volume. Males exhibited a significantly larger relative caudal medial cortex ($p < 0.01$) than females.

Figure 1

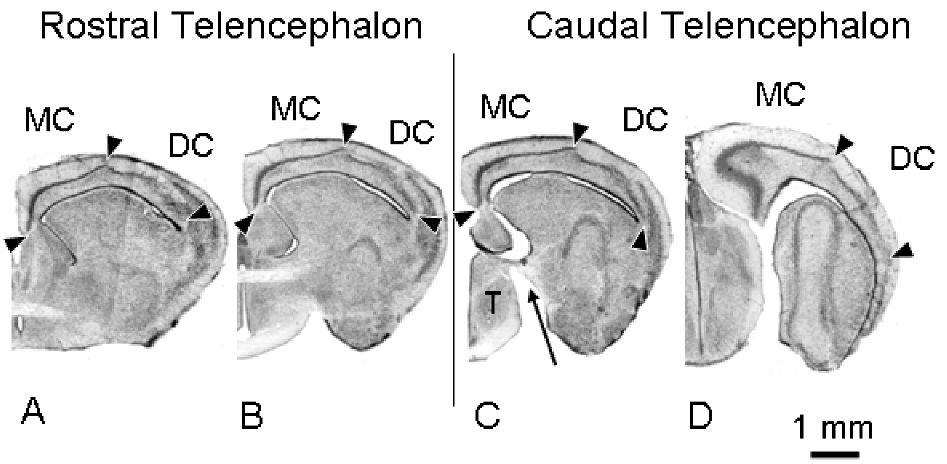
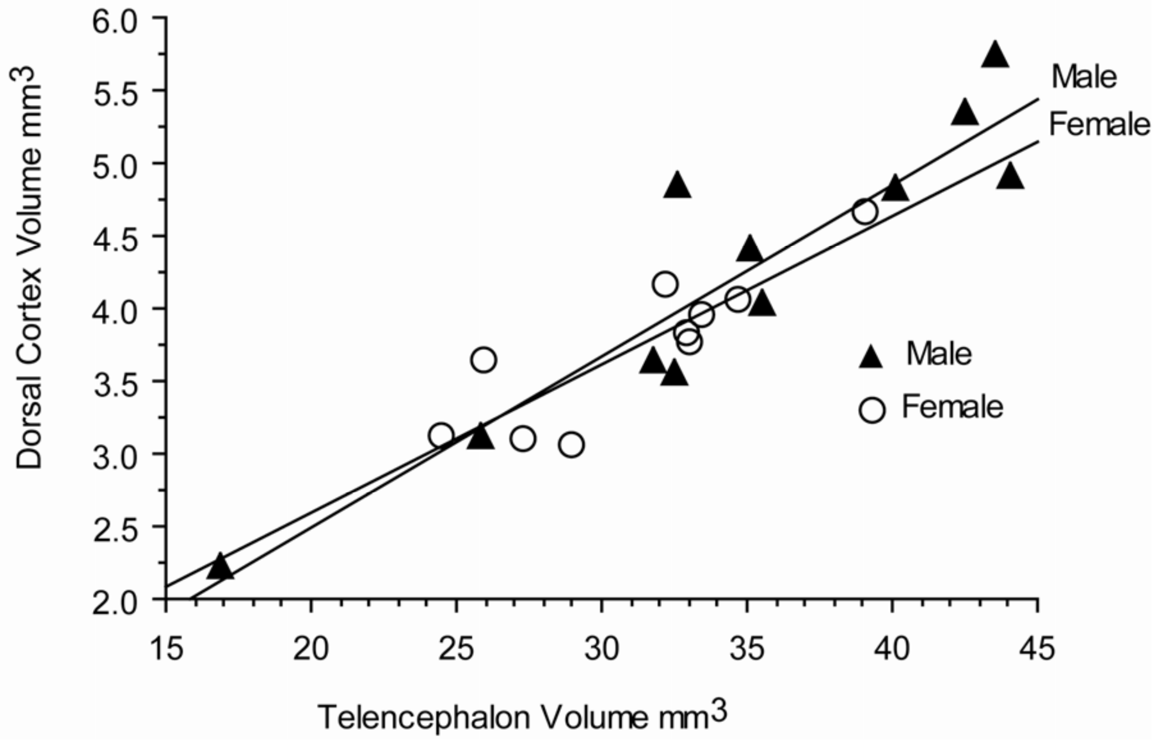

Rostral Telencephalon
Caudal Telencephalon
MC
DC
MC
DC
MC
DC
T
MC
DC
A
B
C
D
1 mm

Figure 2



6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Dorsal Cortex Volume mm3
15
20
25
30
35
40
45
Telencephalon Volume mm3
Male
Female
▲ Male
○ Female

Figure 3

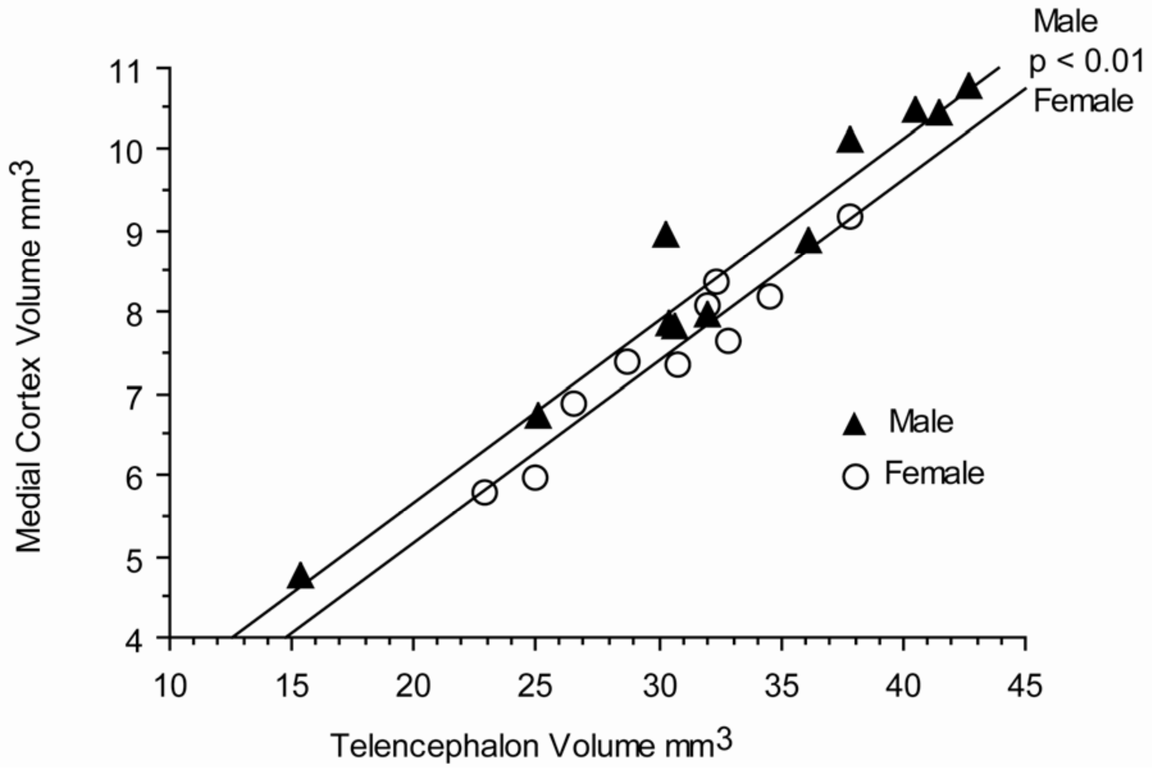

Male
p < 0.01
Female
Medial Cortex Volume mm3
11
10
9
8
7
6
5
4
10
15
20
25
30
35
40
45
Telencephalon Volume mm3
▲ Male
○ Female

Figure 4

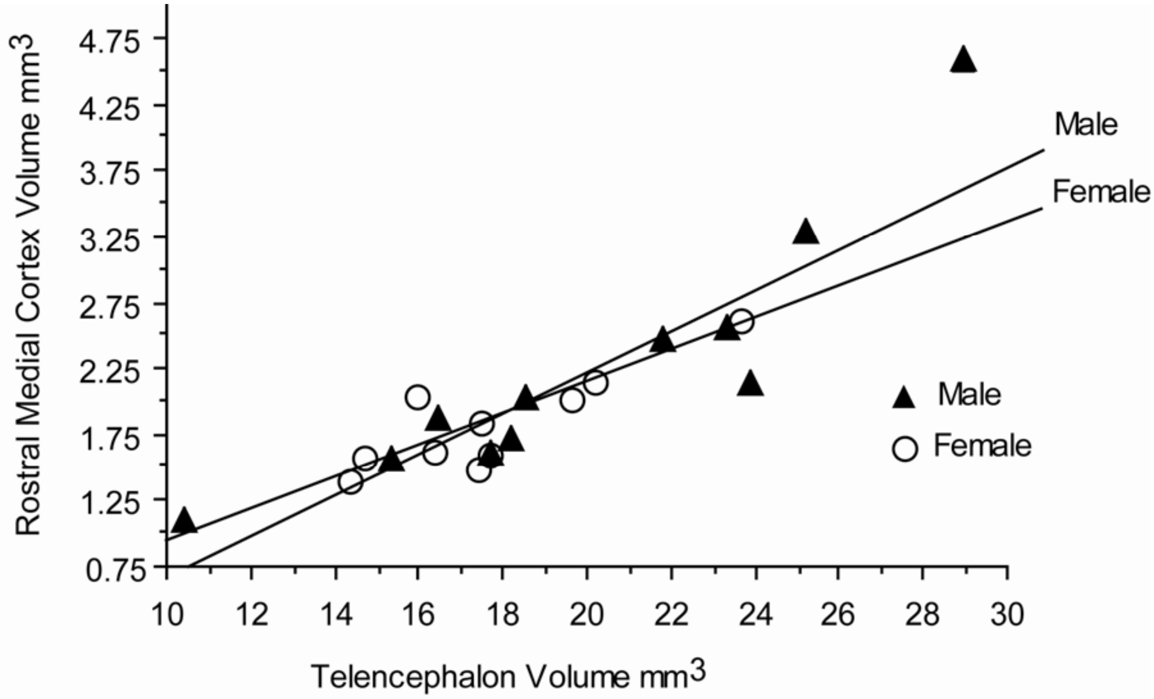
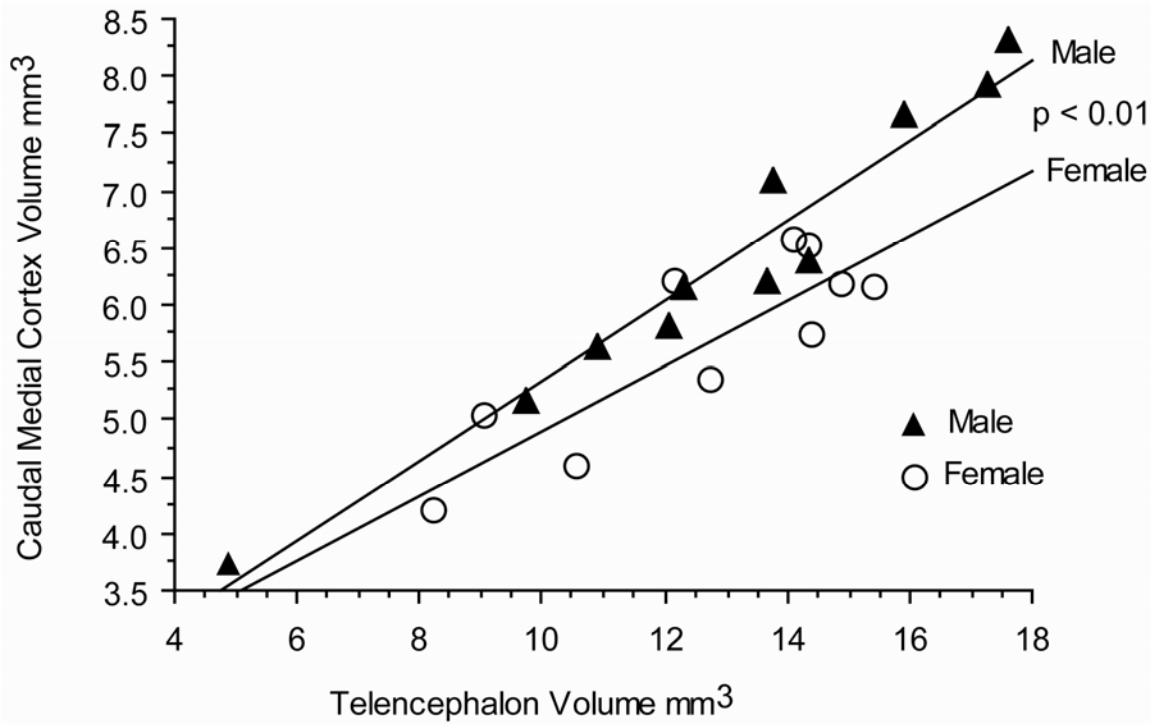

Rostral Medial Cortex Volume mm3
4.75
4.25
3.75
3.25
2.75
2.25
1.75
1.25
0.75
10
12
14
16
18
20
22
24
26
28
30
Telencephalon Volume mm3
Male
Female
▲ Male
○ Female

Figure 5


8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
Caudal Medial Cortex Volume mm3
4
6
8
10
12
14
16
18
Telencephalon Volume mm3
Male
p < 0.01
Female
▲ Male
○ Female