PATTERNS OF DEFENSIVE BEHAVIOR IN PITVIPERS OF THE SOUTHEASTERN UNITED STATES

by

XAVIER GLAUDAS

(Under the Direction of Whitfield Gibbons)

ABSTRACT

We investigated the importance of various factors on the defensive behavior of two pitviper species, the cottonmouth and the pigmy rattlesnake: We used cottonmouths in a controlled environment to explore the importance of thermal cues on defensive strike, and the effect of experience with a potential predator on the defensive behavior of adults and neonates. Thermal information was not of importance in eliciting a defensive strike. Adults and neonates differed in their tendencies to habituate to a confrontational but non-harmful stimulus: adults decreased defensiveness over days while neonates did not. The cost of habituation may simply be too high for neonates given their vulnerability. We also analyzed data collected on a population of free-ranging pigmy rattlesnakes: pigmy rattlesnakes were not aggressive as previously concluded. Snakes that were moving struck more frequently than coiled snakes. Fleeing behavior was affected by initial posture of the snake, the age class, and recent feeding.

INDEX WORDS: *Agkistrodon piscivorus, Sistrurus miliarius*, Cottonmouth, Pigmy Rattlesnake, Defensive Behavior, Thermal Cues, Ontogeny, Habituation, Striking, Fleeing, Displays.

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CHAPTER 1

INTRODUCTION

Animal signals and communication

Communication in the animal kingdom has been extensively studied in the field of animal behavior, and has been defined as "an action on the part of one organism (or cell) that alters the probability pattern of behavior in another organism (or cell) in a fashion adaptive to either one or both of the participants" (Wilson, 1975). Furthermore, the sender must aim to modify the receiver's behavior (Marler, 1967). Communication serves many functions. For example, it may be used to coordinate actions, to identify an individual, and to court a mate (Wilson, 1975). Another function of communication is to display an agonistic disposition, and threat displays are signals that indicate a proclivity to fight (Halliday, 1983).

Over time, a signal may become ritualized if several requirements are met: the signal must be redundant, conspicuous, and stereotyped (Wiley, 1983). Ritualization reduces ambiguity between the signaler and the receiver (Cullen, 1966), and in the case of threat displays can prevent detrimental interactions for both opponents. For instance, rattlesnakes (*Crotalus* spp.and *Sistrurus* spp.) have evolved a specialized tail structure for noise-production. The aposematic function of the rattle (Greene, 1992) allows the snake to advertise its presence and dangerousness to an approaching animal, thus reducing the necessity of physical combat.

Anti-predator behavior of snakes

Predation pressure shapes many aspects of the behavior and ecology of animals (Tuttle and Ryan, 2002). Consequently, studies of predator-prey interactions and relationships are of high significance to the scientific community if one's goal is to understand the function and evolution of behavioral traits. Snakes have been favorite subjects for studies on antipredator behavior. At least three reasons account for this trend. First, snakes cause many human deaths across the world - 30,000 to 40,000 per year (Russell, 1983) and therefore understanding their behavior may be viewed as vital. Second, they may be more easily testable compared to other animals. Third, snakes have evolved a wide variety of antipredator mechanisms (Greene, 1988). For instance, pitvipers use auditory (i.e., tail vibration, rattling), olfactory (e.g., musk), as well as visual signals (e.g., mouth gaping) to ward off potential predators. These signals may reduce the necessity of a potentially costly bite (Hayes et al., 2002) and accordingly, previous research has revealed that Viperid snakes were reluctant to strike (Prior and Weatherhead, 1994; Gibbons and Dorcas, 2002; Shine et al., 2002). These findings indirectly suggest that venom is primarily an offensive weapon used to subdue and predigest prey (Pough, 1979), and that the costs of defensive striking outweigh the benefits for venomous snakes (depending on the threat severity).

Factors that affect the defensive response of reptiles can be partitioned into intrinsic and extrinsic factors. Intrinsic factors, such as body temperature (Keogh and DeSerto, 1984), size (Hailey and Davies, 1986), sex (Scudder and Burghardt, 1983), recent feeding (Herzog and Bailey, 1987) and experience (Glaudas, in press) have been found to affect snake defensive behavior. Extrinsic factors have received less attention. Gibbons and Dorcas (2002) revealed that threat severity was the major element in releasing a striking response in cottonmouths. Other

extrinsic factors include location of the snake (Shine et al., 2002), and time of day (anecdotal reports on the elapid species *Bungarus*).

The present study

We combined both laboratory and field studies to provide insight on some of the factors that affect the defensive response of two pitviper species of the southeastern USA. In the first chapter, we will examine whether thermal cues influence the striking response of cottonmouths (*Agkistrodon piscivorus*). In a second chapter, we will ask two questions. First, is the defensive behavior of a snake affected by prior experience? In other words, can a snake habituate to a non-harmful confrontational stimulus? Second, is there an ontogenetic shift in habituation rate and/or ability? Finally, in a third chapter we will explore the defensive behavior of free-ranging pigmy rattlesnake (*Sistrurus miliarius*), the smallest North American rattlesnake, a species that has been described as fiery by Allen and Neill (1950).

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CHAPTER 2

DO THERMAL CUES INFLUENCE

THE DEFENSIVE STRIKE OF COTTONMOUTHS (Agkistrodon piscivorus)?¹

¹ Glaudas, X. and J. W. Gibbons. To be submitted to Amphibia-Reptilia

Introduction

Crotaline snakes (family Viperidae, subfamily Crotalinae) are unique among snakes in the possession of facial pits situated on each side of the head between the nostril and the eye (Klauber, 1972). As far back as the beginning of the 19th century, Desmoulins (1824) suspected the sensory function of these pits. For many years much speculation arose about their role, and Bullock and Diecke (1956) finally established the heat-sensing function of this organ: pitvipers can detect temperatures through the radiant heat energy emitted by objects and/or organisms relative to the background temperature. This thermoreceptive sense is not specific to crotalines: pythons also have the capacity to detect temperature variation but the thermal receptors are situated on the labial scales, and hence are called labial pits. Facial pits of pitvipers are depressions with highly innervated membranes at their bases (Barrett, 1970). Information is transmitted through the trigeminal nerve to the optic tectum, part of the brain that also receives visual data (Hartline et al., 1978). Previous research suggests the infrared receptor may be more widespread than first thought, and even species lacking obvious anatomical specializations (e.g., loreal or labial pits) like true vipers or anacondas react to thermal cues (Breidenbach, 1990; Smith and Kardong, 1999).

The significance of this thermoreceptive organ on the predatory strike of boid and viperid snakes has been established (De Cock Buning, 1983; Kardong and Mackessy, 1991; Shine and Sun, 2003). However, no evidence has been provided on alternative functional roles played by thermal pits (Greene, 1992), specifically on defense. It has been suggested that thermal pits might help snakes in detecting predators (De Cock Buning, 1983), in finding optimal basking sites for thermoregulation (Goris and Nomoto, 1967; Herbert and Hayes, 1992), and in locating winter dens (Sexton et al., 1992).

Because we lack empirical studies on the role of these pits in a non-predatory context, this study provides further understanding on the function of thermal pits. We conducted a simple experiment to test the influence of warm thermal cues on the defensive strike of a pitviper species, the cottonmouth (*Agkistrodon piscivorus*). Visual cues are of paramount importance in releasing a defensive strike (Scudder and Chiszar, 1977). In addition, cottonmouths are preyed upon by predators with different thermal profiles (e.g., ectotherms, endotherms). Thus, we predicted that there would be no difference in the striking response of cottonmouths tested with a warmed versus a non-warmed artificial arm.

Materials and Methods

Subjects

We collected 21 cottonmouths, 14 females and 7 males ($\bar{x} \pm SE$; SVL = 74.25 ± 3.33 cm) on the Savannah River Site, South Carolina, during spring 2003. After capture, we fasted each snake for 7 days to eliminate the effect of recent feeding on defensive behavior (Herzog and Bailey, 1987). The snakes were individually housed in identical polyethylene containers (RubbermaidTM [58 x 42 x 14-cm high]) within an environmental chamber (12L: 12D, 26°C) with water dish (provided ad-libitum) and bark mulch as a substrate. We did not disturb snakes prior to the experiment. Snout-vent length of the specimens was within a range of 60 to 100 cm SVL, and all were mature individuals (Blem, 1997).

Testing procedures

We performed all trials in the individual housing container that we previously placed in a larger plastic-walled arena (82 x 52 x 34-cm). All trials were performed between 1200 and 1600

hrs. The experimental stimulus consisted in tapping on the snake mid-body three successive times at 1-sec intervals with a pair of snake tongs (Midwest ProductionsTM) customized to look like a human arm (Gibbons and Dorcas, 2002). We wrapped a piece of heating tape (FlexwattTM) around the arm. To insure the diffusion of heat, we wrapped aluminum foil around the length of the arm. We then covered the artificial arm wrapped in aluminum foil with a shirtsleeve. We randomly assigned two treatments to each snake. We used a repeated measure design because of the high individual variation in defensive behavior (Brodie III, 1993). In the control treatment, the temperature of the arm was within a range of 22-26° C ($\overline{x} \pm SE$; T° C = 24.87 ± 0.22° C). In the experimental treatment, we heated the arm to a temperature of $32-36^{\circ}$ C (T^o C = $33.75 \pm 0.25^{\circ}$ C). We monitored the temperature of the arm with an infrared thermometer (Raytek MiniTempTM). Cottonmouths would have been unable to react to infrared radiation emanating from the tester's body because thermal receptivity is limited to 35 to 70 cm (Noble and Schmidt, 1937; Greene, 1997), and the artificial arm was approximately 1-m long. We covered the anterior part of the arm with polyethylene and replaced it between each trial so that chemical cues left on the arm did not influence the response of the subsequent snake. Because snakes could habituate to handling when tested over consecutive days (Glaudas, in press), we performed the second trial four days after the first test. The tester wore the same shade of clothing throughout the experiment so that his appearance did not influence the snake's behavior (Whitaker and Shine, 1999). We recorded the following variables: whether the snake struck, the number of strikes, and latency to strike. We recorded latency to strike with a stopwatch using the first tap as the point of reference (time 0).

Statistical analysis

We analyzed data on the occurrence of striking using Z sign-test. To investigate whether strike numbers differed among treatments, we used a non-parametric repeated measure analysis, the Friedman's test. We used Wilcoxon matched pairs test to test for differences in latency to strike between treatments.

Results

We found no difference in the occurrence of striking (Sign Test, Z = .000, P = 1.0). Fourteen of 21 control snakes (62%) struck whereas 13 (58%) of 21 struck in the experimental treatment. Only five individual snakes exhibited a change in striking behavior: three of them struck in the control experiment only and the other two did so in the experimental treatment only. We found no difference in strike numbers directed at the stimulus (Friedman's test, $\chi_r^2 = .20$, df = 1, P < 0.65): 12 of them struck once, and two struck twice in the control treatment. In the experimental treatment, 10 snakes struck once and three snakes had two strikes.

We recorded time to strike for only 8 of them (out of 11) since the videotapes did not allow us to accurately record latency to strike. Time to strike did not differ among treatments (Wilcoxon matched pairs test, Z = 0.50, P < 0.62).

Discussion

As we predicted, thermal cues did not influence the propensity of a snake to strike or not strike, the number of strikes, or latency to strike between the control and the experimental treatments. We do not find it surprising that the thermal detection capabilities of the facial pits would not be used in predator evaluation even in close terrestrial encounters. Predators of cottonmouths range from reptiles (e.g., conspecifics) whose temperatures could vary widely, to a variety of mammals and birds that have consistently higher body temperatures. For a generalized prey species, relying on the body temperature of a threat to determine its defensive response would be ill adapted.

However, because we only had 8 snakes that struck in both treatments and for which latency to strike was recordable from the tapes, we make no conclusion on the issue of latency to strike. Possibly, an increase in sample size would reveal an effect on this response variable.

Visual cues are of primary importance in releasing a defensive strike (Scudder and Chiszar, 1977). However, in situations of low visibility an alternative sensory organ may be advantageous (De Cock Buning, 1983). For example, our experiment was conducted in a well-lit environment where snakes could rely solely on visual information. It is possible that in a nocturnal setting, thermal cues may be a useful substitute to visual cues, specifically for a species like the cottonmouth, which develops nocturnal habits during late spring and summer (Ernst, 1992). Also, thermal cues may be useful during ecdysis, a period at which snakes are vulnerable partially because of a loss of visual acuity (King and Turmo, 1997). These two hypotheses could easily be tested in a controlled environment.

Previous studies have shown that thermal cues affect predatory strikes. For example, red diamondback rattlesnakes (*Crotalus ruber*) with closed nostrils and amputated tongue tips effectively struck at live mice but did not strike when thermal pits were covered (Dullemeijer, 1961). A congenitally blind northern Pacific rattlesnake (*Crotalus oreganus*) was as effective at striking live mice, as were control subjects with full sensory capacities. However, the same blind rattlesnake lost its efficiency when thermal pits were covered (Kardong and Mackessy, 1991).

To our knowledge, prey detection is the only demonstrated role of thermal pits even though other functional significance has been proposed. For example, Sexton et al. (1992) suggested that thermal pits might help rattlesnakes in detecting winter dens at varying distances because of the intense infrared radiation of rock outcrops. This is unlikely since the thermal organ is only useful at very close range.

Greene (1992) suggested that facial pits originated as defense mechanisms, and Barrett (1970) stated that thermal pits serve the same function in all crotalines. Yet crotaline species may differ in their reactivity to thermal cues because different behavioral traits could potentially lead to different micro-evolutionary pathways (e.g., sensory hierarchy). For example, many *Crotalus* species feed exclusively on endothermic prey (Rubio, 1998), and one may expect strong selection to operate on this thermoreceptive organ. In contrast, our study organism, the cottonmouth is an opportunistic feeder and eats, ectothermic and endothermic prey, as well as carrion (Ernst et al., 2003). Therefore, selection for thermal sensitivity may be decreased in the cottonmouth. Future studies are needed to investigate potential interspecific differences to infrared receptivity in a defensive context in crotalines.

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CHAPTER 3

ONTOGENY OF ANTI-PREDATOR BEHAVIORAL

HABITUATION IN COTTONMOUTHS

(Agkistrodon piscivorus)¹

¹ Glaudas, X, Fedewa, L. A. and C. T. Winne. Submitted to Animal Behaviour, 12/02/03.

Introduction

Predation can be an important factor in shaping the behavior and ecology of species (Tuttle and Ryan, 1981). For example, predation pressure has been shown to influence breeding and foraging patterns (Gotmark, 2002; Mohr et al., 2003), parental care (Fisher, 2002), and gregariousness (Gursky, 2002), among others. Over evolutionary time, behavior has been shaped to maximize net benefit as predicted by optimality models (Maynard Smith, 1978). Individuals have to make decisions based on costs and benefits when confronted by a predator (Endler, 1986). Defensive responses and their costs may affect fitness (Raberg and Stjernman, 2003) through reduction of feeding time and growth rate (Ueta, 1999; Van Buskirk, 2000), and lower reproductive success (Ueta, 1999). Alternatively, individuals can decrease these costs by not responding to the attack (e.g., if risk of injury or death is low) or by becoming gregarious (Arroyo at al., 2001).

Costs and benefits are context-dependent and factors such as the risks represented by the potential predator, as well as prior experience of the individual with the predator could influence an individual's final decision. If prior experience with a particular predator suggests that the risk of being preyed upon is low or non-existent, foregoing active defensive behavior may be an adaptive response through the minimization of defensive costs.

The dear enemy hypothesis suggests that the ability to discriminate among territorial neighbors and transient rivals allows defenders to minimize defensive costs by reducing unnecessary conflicts (Wilson, 1975). In a similar manner, we might expect individuals with the ability to adjust their defensive behavior as a result of discrimination between high and low risk threats, to be at a selective advantage. Habituation is a mechanism by which organisms modify their behavior (Bee and Schachtman, 2000) and is defined as "a stimulus-specific response

decrement resulting from repeated or constant exposure to the response-eliciting stimulus" (Wyers et al., 1973, pp. 11-12). Long-term habituation occurs when information is retained over days or weeks, demonstrating capacity for memory in experimental subjects. Previous behavioral studies have shown that rates of habituation differ among individuals, sexes, litters, and species (Desforges and Wood-Gush, 1975; Herzog et al., 1989). However, the effect of ontogeny on this behavioral mechanism has not been reported.

In most species, juveniles and neonates are at higher risk from predation and experience lower survivorship than adults, even in altricial species where parents protect their offspring (Sibly et al., 1997). Precocial species with no parental care are faced with an even greater challenge because they are independent from birth (Burghardt, 1984). Snakes are considered highly precocial species and engage in defensive displays as soon as they are born (Burghardt, 1978; Greene, 1988). Even though viperid snakes are afforded some protection by being venomous (Ford, 2002), juveniles suffer higher predator-induced mortality than adults (Li, 1995).

Our objective was to examine whether an ontogenetic shift in habituation of anti-predator behavior occurred in a venomous snake species, the cottonmouth (*Agkistrodon piscivorus*). Cottonmouths are large, heavy-bodied, semi-aquatic venomous snakes (Family Viperidae, Subfamily Crotalinae). Cottonmouths are opportunistic feeders that will eat any animal they can subdue (Ernst et al., 2003). Mature cottonmouths have few predators (Mitchell, 1994) whereas neonates are faced with a large diversity of predators including ophiophagous snakes, alligators, predatory mammals, wading birds, and birds of prey (Ernst et al., 2003). We hypothesized that unlike adults newborn snakes will not show habituation of defensive behavior; the hypothesis predicts that the overall mean scores, the number of snakes that struck, and mean strike number

decrease over time in adults whereas there would not in neonates. Venomous snakes are ideal species for investigating both anti-predator behavior and habituation because of the potentially high costs associated with striking (Hayes et al., 2002), they have evolved a large array of warning displays (Greene, 1988), and because body size varies tremendously ontogenetically although changes in morphological shape are limited (Herzog et al., 1989). Cottonmouths, in particular, exhibit a series of defensive behaviors including a stereotypical gaping behavior in which they expose the white lining of the inside of the mouth (henceforth their name) as a warning to potential predators.

Materials and Methods

Subjects

We collected 36 adult cottonmouths (17 females and 19 males, $[\bar{x} \pm SE]$; SVL = 64.69 ± 1.58 cm; mass = 357.1 ± 36.08 g) from Aiken and Barnwell counties, South Carolina on the Department of Energy's Savannah River Site from July to September 2003. For the purpose of this study, we considered snakes greater than 50 cm SVL as adults, which approximately reflects maturation size (Burkett, 1966).

After testing, we kept all gravid females in the laboratory until parturition. We obtained 33 neonates (14 females and 19 males, $[\bar{x} \pm SE]$; SVL = 23.97 ± 0.22 cm; mass = 16.8 ± 0.31 g) from 6 litters (3, 4, 5, 6, 6, 9). Neonates were removed from mothers four days after parturition. Each snake was individually housed in a polyethylene container (RubbermaidTM; adults: 58 x 42 x 14-cm; neonates: 34 x 25 x 14-cm), with water provided *ad-libitum* and bark mulch as substrate, within a walk-in environmental chamber (14 L:10 D, 26°C). We did not disturb or feed wild-caught snakes for 48 to 72 h after transfer to individual containers, to allow them to

acclimate and to eliminate the effect of recent feeding on defensive behavior (Herzog and Bailey, 1987). To minimize the presence of human scent we used latex gloves when setting-up the containers. We offered a frog to neonates the day after the last habituation trial (Day 6); however, adults were not fed for the length of the study (a total of 17-18 days). Lack of feeding probably did not affect our results because all snakes were in good condition and pitvipers typically rely on infrequent meals (Shine, 1993; Greene, 1997). We held adults for 17-18 days and neonates for 20-21 days. Subsequently we released adults at their original point of capture; we released neonates where their mothers were caught.

Testing procedures

We performed all tests in the individual containers to eliminate pre-test and post-test handling thus avoiding displacement effects (e.g., handling of snakes prior to testing). Since container heights were small (14 cm), prior to the test we placed each container in a larger plastic-wall arena to prevent escape (45 cm height). We placed the container in the arena so that the snake could see the tester (always along the same axis). All snakes either tongue-flicked or pointed their head toward the approaching stimulus, indicating that snakes were aware of the tester presence. After placement in the arena, we left the snake undisturbed in its container for 1 minute with lid on before each trial. We performed all tests in the environmental chamber between 1100 and 1400 hours. We had two testers and each tester was allocated approximately half of the total sample size. Testers performed the trials each day on the same snakes. The testers gently tapped on the snake's mid-body three successive times at 1-sec intervals with an artificial arm daily over a period of 5 days. We held the arm and approached the snake at an angle of approximately 45° from the horizontal plane. An approaching human is likely to be

perceived as a threat and trigger high defensive responses in snakes (Goode and Duvall, 1989); consequently, humans have been widely used to elicit defensive behaviours in snakes (Herzog et al., 1989). The artificial arm consisted of a pair of 1-m snake tongs (Whitney tongs, Midwest Products[™], Greenwood, MO, USA) covered with a shirtsleeve customized to look like a human arm (Gibbons and Dorcas, 2002). We covered the anterior part of the arm with polyethylene and replaced it between each trial so that chemical cues would not be left on the arm and possibly influence the response of the subsequent snake. We hand-held the polyethylene-wrapped tip for 1 min prior to each trial to insure presence of human olfactory cues. The testers wore the same shade of clothing throughout the experiment so that appearance did not influence snake's behaviour (Whitaker and Shine, 1999). However, we had no control over olfactory information. We could not record the pressure exerted on the snake; however, because both testers were blind to the experimental test day and individual snakes (e.g., snakes were caught on successive days), any inconsistency of pressure was likely randomly distributed across days and individual snakes. We gave an additional test to each snake 10 days after the last habituation trial (Day 15) to test for a recovery response. We videotaped each trial using a video camera (Canon[™] Digital Video Camcorder DM-GL1A). The observer (CTW) recorded behavioural responses during review of the tapes and was never aware of the snake identification and experimental test day it was (except for presence or absence of musk which he recorded during trials), thus removing potential bias.

Preliminary analysis indicated an effect of tester on adults (Kruskal-Wallis: H=3.73, df = 1, P = 0.05; Fig. 2.1). Snakes did not habituate to tester 1, the principal investigator (Friedman test: $\chi_r^2 = 2.89$, df = 4, P = 0.57), whereas they did with tester 2 (Friedman test: $\chi_r^2 = 12.8$, df = 4, P = 0.01). We had two testers to remove the potential bias of the PI and thus we do not view this

effect as too problematic. First, both testers standardized tapping methodology prior to tests. Second, due to logistical constraints adults were not randomly assigned to testers, but rather haphazardly assigned; testing span over 6 weeks for adults and it would have required both testers to be present everyday for that period which we unfortunately could not do. As a result, factors such as locality (e.g., snakes from different habitat types were differently defensive on Day 1), sex, and reproductive condition may have become confounded with tester. Lastly, investigating tester effect on neonates, which were randomly assigned in contrast to adults, revealed no difference (Kruskal-Wallis: H= 0.35, df = 1, P = 0.54). Thus, we dropped tester effect from further analysis.

Behavioral scores

We scored each snake following revised protocols of Arnold and Bennett (1984), Herzog and Burghardt (1986), Schieffelin and de Queiroz (1991), and Keogh and DeSerto (1994), which we adapted to fit the species and methodology in this experiment. For each cottonmouth, we observed the behavioral state of the snake's head (5 categories), tail (2 categories) and whether the snake released musk (2 categories [Table 2.1]) during the approach and the trial. We did not treat the responses to the moving stimulus (the approaching hand) separately from the responses to the tactile stimulus (tapping the snake) because tapping is dependent on approaching and, both cues may elicit a defensive response.

We obtained an overall score for each cottonmouth by adding the scores for each component with a possible range from 0 to 6. Additionally, we added +1 for each extra strike because strike number is likely to depict snake defensiveness. We recorded the most active defensive behavior exhibited (defined as the behavior with the highest score [Keogh and DeSerto, 1994]) if a snake

used several head displays during a trial. For example, if an individual snake tongue-flicked, gaped and struck during a trial, we only considered striking in the overall score since we were interested in the most defensive display. We recorded gaping behavior when the snake exposed the white lining of its mouth. We identified a feint strike as a head movement toward the stimulus without full neck extension; we recorded a strike whenever the fangs contacted the arm, as well as any motion forward by the snake with neck fully extended. We also monitored the number of strikes directed at the stimulus per snake. No distinction was made between envenomation and a dry bite (e.g., no venom injected) since no reliable methods was available to assess whether venom was injected. We monitored whether the snake twitched its tail against the substrate generating a weak auditory signal (tail vibrating), as well as whether the snake released an odoriferous substance from its cloaca producing an unpleasant odor (musking).

Statistical analysis

We used Friedman test, a repeated measure analysis for ordinal data, to test the null hypothesis (no habituation) for the first five experimental days. We used a Cochran *Q*-test to test for a significant change within individuals (e.g., repeated measures) in the presence or absence of specific attributes (striking, tail vibrating, gaping, tongue-flicking, musking) among the five experimental days. To test for differences between adults and neonates in the occurrence of behavioral displays, we used cross-tabulation tables, as well as the Kruskal-Wallis rank test. We performed non-parametric post-hoc analyses using equations from Zar (1984) to investigate when habituation began; we compared results on Day 1 to each of the other days (Day 2, 3, 4 and 5). We set the Alpha level at 0.05 and we used the statistical software *Statistica*' (StatSoft Inc. 98 Edition) for all statistical tests.

Results

Adults

Adults exhibited habituation of defensive behavior in that overall mean scores decreased significantly during the first five experimental days (Friedman test, $\chi_r^2 = 14.78$, df = 4, P = 0.001; Fig. 3.2). Post-hoc analyses revealed that Day 3 and Day 5 were different from Day 1 (P = 0.05 and P = 0.01, respectively) but not Day 4. The non-significant difference between Day 1 and Day 4 was likely due to the conservative nature of the post-hoc analysis because a pairwise comparison between these two days using Friedman test showed a significant difference (Friedman test, $\chi_r^2 = 5.76$, df = 1, P < 0.016). Strike numbers also decreased throughout the experiment (Friedman test, $\chi_r^2 = 18.75$, df = 4, P < 0.001; Fig. 3.3). Post-hoc analysis revealed a difference between Day 1 and Day 5 only (P < 0.05). Variances in scores and strike numbers on Day 1 were both significantly higher than on Day 5 (F-test (35.35) = 2.15, P = 0.01).

The number of snakes that struck decreased over the five-day trials (Cochran *Q*-test, *Q* = 17.18, df =4, *P* < 0.002; Fig. 3.4). We observed a response substitution in that the proportion of snakes that struck decreased while the proportion of snakes that tongue flicked (as the head display with the highest score) increased over the habituation test (Cochran *Q*-test, *Q* = 10.85, df =4, *P* < 0.028). In contrast, there was no change in the occurrence of tail vibrating (Cochran *Q*-test, *Q* = 4.19, df =4, *P* = 0.38; Fig. 3.5), gaping (Cochran *Q*-test, *Q* = 3.19, df =4, *P* = 0.41), or musking (Cochran *Q*-test, *Q* = 4.25, df =4, *P* = 0.37). Mean overall scores on Day 15 were significantly lower than on Day 1 (Friedman test, $\chi_r^2 = 9.14$, df = 1, *P* = 0.0025), but not different than on Day 5 (Friedman test, $\chi_r^2 = .42$, df = 1, *P* = 0.51).

Neonates

We found no habituation of defensive behavior in neonates (Friedman test, $\chi_r^2 = 6.17$, df = 4, P = 0.18; Fig. 3.2). Because some neonates were from the same mother, data points were not statistically independent (e.g., genetic effect). Therefore, we tested for the effect of litter on habituation rates but found no difference (Kruskal-Wallis, H = 6.08, df = 5, P = 0.29).

Overall, variances decreased slightly throughout the experiment but were not different between Day 1 and Day 5 (F-test $_{(32,32)} = 1.27$, P = 0.25). We found no difference in strike numbers (Friedman test, $\chi_r^2 = 3.64$, df = 4, P = 0.45; Fig. 3.3), or in the occurrence of striking among days (Cochran *Q*-test, Q = 5.27, df = 4, P = 0.26; Fig. 3.4). We observed a nonsignificant decreasing trend in the occurrence of tail vibration over the habituation test (Cochran *Q*-test, Q = 8.86, df = 4, P = 0.06; Fig. 3.5). The occurrence of gaping (Cochran *Q*-test, Q = 5.92, df = 4, P = 0.2) did not differ throughout the experiment. Among all neonates, we had only two instances of musking. We found no difference in mean overall scores between Day 5 and Day 15 (Friedman test, $\chi_r^2 = 2.58$, df = 1, P = 0.1), or between Day 1 and Day 15 (Friedman test, $\chi_r^2 =$.03, df = 1, P = 0.84). However, mean strike numbers were significantly higher on Day 15 when compared to Day 5 (Friedman test, $\chi_r^2 = 5.4$, df = 1, P = 0.02).

Adults vs. neonates

We observed no differences on Day 1 in overall scores (Kruskal-Wallis, H = 0.11, df = 1, P = 0.74) or strike numbers (Kruskal-Wallis, H = 0.37, df = 1, P = 0.54) between adults and neonates. The occurrence of strikes on Day 1 did not differ between neonates and adults (cross-tabulation tables, $\chi^2 = 1.30$, df = 1, P = 0.25; Fig. 3.4). However, on Day 1 neonates tail vibrated (cross-tabulation tables, $\chi^2 = 3.76$, df = 1, P = 0.05; Fig. 3.4) and gaped (cross-tabulation tables,

 $\chi^2 = 4.08$, df = 1, P = 0.04; Fig. 3.6) more often than adults. On Day 5, however, neonates exhibited higher incidence of striking (Cross-tabulation tables, $\chi^2 = 5.54$, df = 1, P < 0.02; Fig. 2.4), and gaping (Cross-tabulation tables, $\chi^2 = 4.29$, df = 1, P = 0.03; Fig. 3.6) than adults but there was no difference in the occurrence of tail vibration (Cross-tabulation tables, $\chi^2 = 0.61$, df = 1, P = 0.43).

Discussion

As predicted, adults habituated to a non-harmful predatory stimulus whereas neonates showed no such clear habituation. Adults showed a response decrement over the first five days of the experiment: they shifted from an active defensive response (e.g., strike) to an investigatory response (e.g., tongue flicking) suggesting that they recognized the stimulus as being nonharmful and had the cognitive ability to remember for at least a 24-hour period as previously concluded by Glaudas (in press). Habituation in adults was solely due to a decrease in striking components (e.g., number of snakes that struck, mean strike numbers per day) since other behaviors did not decrease. We also observed a reduction in individual variation over time: variances in scores and strike numbers were significantly higher on Day 1 than on Day 5, which may have been partially due to some snakes using only passive defense throughout the experiment. For venomous snakes, costs associated with striking include risk of breaking fangs, and venom expenditure (which could reduce chances of later catching prey [Hayes et al., 2002]). Lowering the cost of defense may prove a useful method to reduce risk of injury and conserve energy, making it available for other components of fitness such as reproduction and growth (Endler, 1991). The lack of recovery response between Day 5 and Day 15 indicated that 10 days

post-test was not sufficient for a recovery to occur, and snakes may have greater memory capabilities than previously reported (Chiszar et al., 1976)

In contrast to adults, neonates did not exhibit a significant decrease in overall scores or mean strike numbers throughout the habituation test. We propose two explanations for the lack of habituation in neonates. First, the costs of habituation in neonates may outweigh any potential benefit. Unlike adults, neonate cottonmouths are faced with a large diversity of predators. Neonate venomous snakes are not as dangerous as adults because they have limited striking range and their absolute striking speed is lower (Rowe and Owings, 1990). Field studies have shown that annual survival rate of neonates is approximately 30% compared to 75-100% for adults (Ford, 2002). Consequently, neonates may view all large moving objects as predators (e.g., over-general predator image [Deecke et al., 2002]). Over-general and over-specific predator images differ in their potential costs (energy or time expenditure vs. death), and predator misidentification may result in a high fitness cost (Deecke et al., 2002); natural selection might have favored a consistent use of high defensive reactions in naïve neonates since the lack of lifelong experience produces only a limited "predator-image data bank." Second, lack of habituation in neonates may be due to insufficient memory capacity to retain the information or, alternatively, neonates may not have the ability to learn as quickly as adults (i.e., neonates may have habituated if given more time). These explanations are not mutually exclusive, and failure of neonates to habituate probably involves multiple factors.

As expected, neonates gaped significantly more than adults on Day 1 and Day 5. Being more vulnerable to predation, neonates may obtain greater benefits from the use of aposematic signals than adults. We observed a marginally non-significant decrease in tail vibrating behavior in neonates. However, mouth gaping was not affected by experience in neonates, which suggests

differences of habituation among warning displays. Because of their small size, the auditory signal generated by neonate tail vibration is weak, and gaping may provide a more effective and stable warning display, thus favoring consistency in the use of this behavior. The efficiency of the auditory signal generated by a vibrating tail may also be more dependent on external factors (e.g., type of substrate) than gaping behavior, assuming that a visually concealed snake would first rely on crypsis.

Differences in habituation rates among defensive displays have been previously reported. For example, neonate hognose snakes (*Heterodon platyrhinos*) exhibit multiple warning displays that include hissing, neck flattening, striking, and death-feigning (Greene, 1988), and Burghardt (1977) reported that death-feigning did not habituate at the same rate as the other displays. Neonate cottonmouths did not habituate; therefore we could not observe a recovery response. However, there was an increase in mean strike numbers between Day 5 and Day 15 for reasons that are unclear.

In conclusion, adult cottonmouths showed behavioral plasticity in defensive behavior while neonates showed consistency. We determined that ontogeny could affect habituation ability in this precocial species. This study showed that behavioral traits are developmentally modified may be as a result of different selective forces. Differences in habituation ability when confronted with a large moving object may reflect ontogenetic changes in predation pressure, and we argue that the cost of over-specific predator images (e.g., risk of death) being much higher at early life stages, the cost of habituation may simply be too high for neonates but not for adults.

In the current study, we were not able to reliably monitor whether the snake injected venom. Therefore, we did not record the amount of venom injected. Because venomous snakes can

control the quantity of venom injected in a bite (Hayes et al., 1992, 2002; but see Young et al., 2002), examining whether venom expenditure decreased or ceased (e.g., dry bite) over days could reveal whether cottonmouths exhibit long-term habituation of venom expenditure. Additionally, to determine whether they habituated to our specific predatory stimulus or to the disturbance itself would require the introduction of a novel stimulus after habituation, which we did not do in our experiment. Introducing a new predator model following habituation would be of high interest to investigate the stimulus-specificity of their response.

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Table 3.1. Scoring index of behavioral responses in cottonmouths.

Component	Scoring Scheme	Score
Head No reaction		0
	Tongue flicking	1
	Gaping	2
	Feint strike	3
	Missed/successful strike	4
	Extra strike	+1
Tail No display		0
	Tail vibration	1
Musk No release		0
	Release	1

Figure 3.1. Tester effect on mean score of snakes. Non-significant difference for tester 1 (Friedman test, $\chi_r^2 = 2.89$, df = 4, P = 0.57). Significant difference for tester 2 (Friedman test, $\chi_r^2 = 12.8$, df = 4, P = 0.01).

Figure 3.2. Mean scores of adult and neonate cottonmouths per day. Overall significant difference for the first 5 days for adults only (Friedman test, $\chi_r^2 = 14.78$, df = 4, *P* = 0.001). * denotes a significant difference from Day 1.

Figure 3.3. Mean strike numbers of adult and neonate cottonmouths per day. Overall significant difference for the first 5 days for adults only (Friedman test, $\chi_r^2 = 18.75$, df = 4, P < 0.001). * denotes a significant difference from Day 1. ** denotes a significant difference from Day 5.

Figure 3.4. The occurrence of striking in adult and neonate cottonmouths per day. Significant decrease over the first 5 days for adults only (Cochran Q-test, Q = 17.18, df = 4, P < 0.002).

Figure 3.5. The occurrence of tail vibration in adult and neonate cottonmouths per day. Nonsignificant decreasing trend for the first 5 days in neonates (Cochran Q-test, Q = 8.86, df = 4, P = 0.06).

Figure 3.6. Comparisons of the occurrence of mouth gaping behavior in adults and neonates on Day 1 and Day 5. Significant difference on Day 1 (Cross-tabulation tables, $\chi^2 = 4.08$, df = 1, *P* = 0.04) and Day 5 (Cross-tabulation tables, $\chi^2 = 4.29$, df = 1, *P* = 0.03).



Fig 3.1. Tester effect on mean score of adult cottonmouths



Fig 3.2. Mean scores of adult and neonate cottonmouths per day



Figure 3.3. Mean strike numbers of adult and neonate cottonmouths per day



Figure 3.4. Occurrence of striking in adult and neonate cottonmouths per day

Figure 3.5. Occurrence of tail vibration in adult and neonate cottonmouths per day

Figure 3.6. Occurrence of gaping in adult and neonate cottonmouths on Day1 and 5

CHAPTER 4

THE DEFENSIVE BEHAVIOR OF FREE-RANGING

PIGMY RATTLESNAKES (Sistrurus miliarius)¹

¹ Glaudas, X., Farrell, T. M. and P. G. May. Submitted to Copeia, 10/27/03.

Introduction

Snakes have evolved some of the most elaborate and diverse antipredator mechanisms among reptiles, and these mechanisms can be classified into several functional categories: crypsis, mimicry, locomotory escape, bluffs and threats, diversion of attack, removal of cues (e.g., head-hiding), and active defense (Greene, 1988). The diversity of antipredator behaviors among snakes, combined with the fact that venomous snakes are responsible for approximately 30,000 to 40,000 human deaths worldwide per year (Russell, 1983) has resulted in increased attention to defensive behaviors of snakes.

Increasing human pressures on the environment, through habitat destruction and fragmentation, and development of outdoor recreation have increased interactions between humans and certain species of animals (Aune, 1991; Lammers et al., 2000). Even though most animals do not present a direct threat to humans, venomous snakes are an exception. As a result, rattlesnakes have suffered from a reputation of being aggressive and vicious creatures (Klauber, 1972).

Factors that affect the defensive response of reptiles can be partitioned into intrinsic and extrinsic factors. Intrinsic factors, such as body temperature (Keogh and DeSerto, 1984; Layne and Ford, 1984; Goode and Duvall, 1989), size (Hailey and Davies, 1986; Whitaker and Shine, 1999), sex (Scudder and Burghardt, 1983), recent feeding (Herzog and Bailey, 1987) and experience (Glaudas, unpubl.) have been found to affect snake defensive behavior. Extrinsic factors have received less attention. Gibbons and Dorcas (2002) revealed that threat severity was the major element in releasing a striking response in cottonmouths. Other extrinsic factors include location of the snake (Shine et al., 2002), and time of day (anecdotal reports on the elapid species *Bungarus*).

Most studies show that venomous snakes are reluctant to bite (Prior and Weatherhead, 1994; Gibbons and Dorcas, 2002; Shine et al., 2002). Pitvipers, in particular, are known to exhibit a large repertoire of defensive displays that may be used to reduce the necessity of biting, including body flattening, release of musk, tail vibration or rattling, and mouth gaping. Although several studies have quantified the defensive behaviors of snakes, more studies are needed to draw general conclusions about the antipredator behavior in the Viperidae.

Humans have been widely used as a stimulus to elicit defensive responses in snakes; a human hand elicits high levels of antipredator behavior (Herzog et al., 1989). Size, as well as movement, is of primary importance in releasing a predatory or a defensive response (Scudder and Chiszar, 1977). Therefore, an approaching human is likely to be perceived as a threat and elicit defensive behaviors in wild snakes, as would any large, moving animal (Goode and Duvall, 1989).

Our study organism, *Sistrurus miliarius barbouri*, has been described as fiery and irritable (Allen and Neill, 1950), and is responsible for many snakebites in Florida (Minton, 1987). The purpose of this study was to investigate whether this snake deserves its aggressive reputation, and to determine the factors that influence the likelihood of a snake being defensive with the goal of elucidating the adaptive significance of defensive behavior patterns in a small species of rattlesnake. We took advantage of a large population of dusky pigmy rattlesnakes (*S. miliarius barbouri*) in central Florida to obtain adequate sample sizes. We hypothesized that 1) the location of the snake would have an effect on defensive response in that snakes located in vegetation would react more defensively (e.g., strike) than snakes on the ground; 2) moving snakes would flee and strike more often than stationary snakes; 3) snakes that had recently fed on large prey items would not escape as often; 4) there would be an ontogenetic shift in defensive behavior in that smaller/younger snakes would strike more often than larger/older snakes; 5)

there would be no differences between sexes; and finally, that 6) gravid females would strike more often and flee less often than non-gravid females.

Materials and Methods

Study organism

Sistrurus miliarius is North America's smallest rattlesnake (typically 40-55 cm total body length) and inhabits a variety of habitats (Ernst et al., 2003) throughout the southeastern USA (from North Carolina to the Florida Keys), and as far west as central Texas and Oklahoma (Palmer, 1978). In the study population, snakes were active year-round with peaks of activity in March-April and September-November. Snakes in the study population feed mostly on lizards (*Anolis carolinensis, Eumeces inexpectatus, Scincella lateralis*) and frogs (*Hyla* spp., *Rana utricularia*), and in turn are probably consumed by a variety of snakes (e.g., *Coluber constrictor*), carnivorous mammals (e.g., *Lynx rufus, Procyon lotor*), and birds of prey (e.g., *Buteo lineatus, Strix varia* [Farrell et al., 1995; May et al. 1996]).

Study site

The study site was an 8-ha semi-deciduous woodland located in Volusia County, Florida. We performed the behavioral experiments in wet hammock and pine flatwoods habitats. The site was surrounded by a freshwater marsh adjacent to the Saint John's River. Sabal palm (*Sabal palmetto*), live oak (*Quercus virginianus*), and red bay (*Persea borbonia*) were the dominant vegetation (May et al., 1996).

Testing procedures

We collected data two or three times a week from January 1993 to October 1995. Two to 10 persons (usually 3-6) conducted censuses on foot. Upon detection of the rattlesnake, one of us approached the rattlesnake, gently tapped it on the snout with a gloved hand and recorded if the snake fled, rattled, or struck. We recorded data on each of the following intrinsic variables: location of the snake (on the ground vs. off the ground), age class, sex, reproductive condition of mature females, initial posture (moving or coiled), and whether the snake had an obvious prey item in its gut. We later removed the rattling response variable from the analysis since very few snakes rattled (Rowe at al., 2002). Beginning in 1991, rattlesnakes were PIT-tagged once they reached a year of age, allowing a determination of the age of most of the snakes born in or after 1991. Because neonate snakes were too small to be PIT-tagged, we randomly selected 40 neonates from available data so as to have representation of all size-classes. Therefore, a single individual may have been included twice in the analysis even though this was not likely to happen.

After behavioral testing as above, we recorded body temperature (using a thermocouple wire thermometer HH508 Digital thermometer Type K, OmegaTM), sex (using a cloacal probe), size (snout-vent length using a squeeze box), mass (using a spring scale), reproductive condition (by palpating the ventral region of females), time of day, and PIT tag number. Additionally, we established presence of food by ventral palpation. Even though small prey items may have been overlooked, we wanted to detect large prey items that were more likely to have an impact on locomotory abilities. We recorded air temperature one meter above the ground in the shade using a thermocouple wire thermometer. We were able to insure independence of observations by

including individual snakes only once in the analyses. Snakes were processed immediately after the trial and released where they were found.

Statistical analysis

Data were analyzed using Chi-square Contingency tables. Each independent variable was analyzed separately in a 2 X 2 table. We tested for differences in body temperature using a oneway ANOVA. If data did not meet the assumption of the homogeneity of variance, we used a non-parametric Kruskal-Wallis by rank test. The experiment-wise alpha level was set at 0.05. *P*values were adjusted accordingly using the sequential Bonferroni technique (Rice, 1989) since we performed multiple tests on the same data set. All tests were performed using the statistical software *Statistica*' (StatSoft Inc. 98 Edition). Values given are means \pm SE.

Results

Most of the *S. miliarius* did not react to our approach and test. Of the 616 different snakes tested over the course of this study, 447 (72.6 %) did not strike or flee. They usually remained in the position where found and did not respond to stimuli. Of the 616, 119 (19.4 %) fled but did not strike, 25 (4%) struck and fled, and another 25 (4%) struck but did not flee. Disregarding the fleeing variable, only 50 rattlesnakes (8.1%) struck. There was no overall effect of body temperature on the two response variables, fleeing (One-way ANOVA test, F = 1.89, df = 1, P = .16, n = 340) and striking (Kruskal-Wallis test, H = .44, df = 1, P = .50, n = 340), so body temperature was not included in further analysis.

Movement

Moving snakes were more likely to flee than snakes that were coiled upon initial encounter $(\chi^2 = 21.74, df = 1, P < .0001, n = 392; Fig. 4.1)$. Movement also influenced the frequency with which snakes struck, and was the only variable that was significantly associated with a snake's striking response ($\chi^2 = 16.83, df = 1, P < .0001, n = 392;$ Fig. 4.1). Since this variable was so important in the way a snake reacted, we tested for differences in the proportion of snakes that were initially moving for each independent variable.

Age class

Age class had an effect on the likelihood that a snake would flee ($\chi^2 = 10.78$, df = 2, P = .0045, n = 428) but not on the striking response ($\chi^2 = 1.41$, df = 2, P = .49, n = 428; Fig. 4.2). Age class significantly influenced snout-vent length (Kruskal-Wallis test, H = 238.47, df = 2, P < .001). Mean snout-vent lengths for snakes in age classes 1, 2, and 3 were 25 cm ± 0.29, 32.2 cm ± 0.29, and 38.2 cm ± 0.65, respectively. Snakes of age class 1, however, were less likely to be moving than the two other classes ($\chi^2 = 18.79$, df = 2, P < 0.0001, n = 299), and this might have contributed to this significant difference in defensive reactions.

Prey

Snakes containing large prey fled less often than snakes without large prey ($\chi^2 = 6.55$, df = 1, P = .01, n = 383) but did not strike more often ($\chi^2 = .00075$, df = 1, P = .97, n = 383; Fig. 4.3). As expected, snakes with large prey items weighed significantly more than snakes that did not have a large prey item (ANCOVA test, F = 52.65, df = 1, P < .0001, n = 309). The proportion of

snakes that were moving ($\chi^2 = .0042$, df = 1, P = .95, n = 349) did not differ between these two categories.

Location

The location of the snake, either on the ground or above ground did not affect propensity to flee ($\chi^2 = 6.55$, df = 1, P = .017, n = 601) or strike ($\chi^2 = .0026$, df = 1, P = .95, n = 601), even though there was a trend in which "terrestrial" snakes fled more often than "arboreal" snakes (Fig. 4.4).

Sex

Females fled more often than males but the difference was not significant ($\chi^2 = 4.86$, df = 1, P = .027, n = 613; Fig. 4.5). We observed no effect of sex on striking response ($\chi^2 = 1.19$, df = 1, P = .27, n = 613). Interestingly, males were significantly more likely to be moving than females ($\chi^2 = 6.5$, df = 1, P = .01, n = 389) but still, females fled more often.

Pregnancy

The reproductive condition of mature females (gravid vs. non-gravid) did not affect either the fleeing ($\chi^2 = .044$, df = 1, *P* = .83, *n* = 123) or striking responses ($\chi^2 = .08$, df = 1, *P* = .76, *n* = 123).

Discussion

Because of the large sample size used in these observations convincing conclusions can be reached regarding the defensive behavior of *S. miliarius*. Our results indicate that the species is

not aggressive in nature, as previously concluded for other species of venomous snakes (Whitaker and Shine, 1999; Gibbons and Dorcas, 2002).

In this study only 8.1% of the 616 snakes struck. Massasaugas (*Sistrurus catenatus*) never struck when the researchers either passed by or stepped on the snake (Prior and Weatherhead, 1994). The authors pointed out that massasaugas would bite if grasped, but unfortunately no quantitative data were provided. An experimental study by Gibbons and Dorcas (2002) revealed that cottonmouths usually (*Agkistrodon piscivorus*) did not bite unless they were physically picked up. Shedao pitvipers (*Gloydius shedaoensis*) were also relatively docile to human disturbances, with less than 20% of the snakes striking at a stick (Shine et al., 2002). These findings suggest that venomous snakes bite as a last resort in human encounters, and indirectly suggest that venom is primarily an offensive weapon used to subdue and predigest prey (Pough, 1979). Presumably, the costs of defensive striking generally outweigh the benefits for venomous snakes (Hayes et al., 2002).

The influence of body temperature on the defensive behavior of snakes has been widely discussed, and results are contradictory (Arnold and Bennett, 1984; Schieffelin and de Queiroz, 1991; Passek and Gillingham, 1997). In our study body temperature had no influence on the striking and fleeing responses, and other studies failed to detect an effect of body temperature on defensive responses of snakes (Goode and Duvall, 1989; Gibbons and Dorcas, 2002).

The initial posture of the snake (coiled vs. uncoiled) was the only variable that significantly affected the striking response of *S. miliarius*. Increased likelihood of detection and vulnerability to attack may be reasonable explanations since natural selection would favor more active defensive responses with increased threat of predation. To humans, crypsis in *S. miliarius* is less effective when a snake is stretched out and moving. The reputation for aggressive defense

responses by *S. miliarius* may result from causal observers whom are more likely to find individuals of this small cryptic species when they are moving. *S. miliarius* is much more likely to respond defensively when it is encountered out-stretched on open trail or road than when coiled in its typical hunting posture.

Previous studies have shown that other factors influenced the striking response of snakes. Shine et al. (2002) found that juvenile Shedao pitvipers struck and displayed more often than adults. We expected small snakes to react more defensively because predation pressures on small snakes are expected to be higher, and natural selection should favor more active defensive displays. However, we observed no difference in striking response between smaller and larger snakes. Perhaps, adult *S. miliarius* are so small that the size factor is not as important as it could be in species of rattlesnakes with a larger range of body size. Estimating predator-induced mortality by age classes in this population would be useful and may help explain this phenomenon. We also expected "arboreal" snakes to strike more often than terrestrial snakes because the former fled less often than the latter, and striking could potentially be an adaptive alternative to fleeing. In our study, this was not the case. Shine et al. (2002) revealed that "arboreal" Shedao pitvipers were actually less likely to strike than snakes on the ground.

Three of the six independent variables we tested – initial posture, age class, and presence or absence of large prey - had an influence on the snakes' fleeing response. Whether the snake was moving when first discovered was of paramount importance. It has been suggested that moving snakes would resume fleeing because crypsis would be less efficient (Arnold and Bennett, 1984). Given the stimulus we used (tapping on the snake's snout), this was unlikely to be true since both coiled and moving snakes were presumably aware that they were detected.

Younger snakes were less likely to flee than older snakes. A higher proportion of snakes in their second and third year of life was moving, and could account for the observed significant difference. However, an ontogenetic shift in fleeing behavior has been documented for the eastern brown snake (*Pseudonaja textilis* [Whitaker and Shine, 1999]), and it has been suggested that sub-adult snakes fled more often because they would not be as efficient at defending themselves as adults (Ford and Burghardt, 1993; Whitaker and Shine, 1999).

Laboratory studies have shown that locomotory abilities in snakes are reduced after the ingestion of a meal (Garland and Arnold, 1983). In our study, snakes that recently fed on a large prey item fled less often than snakes with no large food item in the gut.

S. miliarius in our population are often found in vegetation, which they use as refugia from water during late summer flooding. Snakes in vegetation did not flee as often as those on the ground, but that difference was not significant. This trend may have been due to the lower proportion of "arboreal" snakes that were moving, their poor abilities at climbing trees and thus at escaping on branches, and also because escape routes are more limited or longer, as suggested by Shine et al. (2002).

No significant difference in defensive behavior among sexes could be detected but females may be behaviorally different to some extent. Females fled more often than males even though males were more likely to be moving. Differences in defensive behavior among sexes may be expected if males and females are morphologically and/or ecologically different, and has been reported for the sexually dimorphic banded water snake (*Nerodia fasciata* [Scudder and Burghardt, 1983]). However, since male and female *S. miliarius* exhibit almost no sexual dimorphism (Bishop et al., 1996), it remains difficult to explain why females fled more often. Because pregnancy applies constraints on locomotion similar to ingesting a large food item

(Shine et al., 2002), an alternative strategy to fleeing may be adaptive for gravid females, and sex-related behavioral differences in rattling and striking responses have been reported for prairie rattlesnakes (Goode and Duvall, 1989; Kissner et al., 1997). However, we did not detect any differences between gravid and mature non-gravid females.

Interspecific differences in defensive behavior have been shown (assuming that this is not an artifact of different methodologies used), other species should be tested, especially those that are responsible for many snakebites. *S. miliarius* is responsible for many snakebites in the southern United States (Minton, 1987). Because of its small size *S. miliarius* is more likely to go undetected by humans than the larger eastern diamondback rattlesnake (*Crotalus adamanteus*) or cottonmouth (*Agkistrodon piscivorus*). Therefore, *S. miliarius*/human accidental interactions are likely to be higher than interactions with the previous two species. However, findings of this study reveal that the species is rather non-aggressive.

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Fig. 4.1. The influence of movement on snakes' defensive behavior. Significant differences for the fleeing (χ^2 analysis, P < .0001) and striking variables (χ^2 analysis, P < .0001).

Fig. 4.2. Age class and defensive behavior. Significant difference for the fleeing variable only (χ^2 analysis, *P* < .005).

Fig. 4.3. The influence of a large prey item. Significant difference for the fleeing variable only (χ^2 analysis, *P* < .01).

Fig. 4.4. The location of the snake and defensive behavior. Significant difference for the fleeing variable only (χ^2 analysis, *P* < .02).

Fig. 4.5. Sex and defensive behavior. Non-significant difference for both response variables. However, this difference is very close to significance for the fleeing response (χ^2 analysis, *P*< .028)

Figure 4.1. Influence of movement on pigmy rattlesnake defensive behavior

Figure 4.2. Influence of age class on pigmy rattlesnake defensive behavior

Figure 4.3. Influence of recent feeding on pigmy rattlesnake defensive behavior

Figure 4.4. Influence of location on pigmy rattlesnake defensive behavior

Figure 4.5. Influence of sex on pigmy rattlesnake defensive behavior

CHAPTER 5

CONCLUSION

In this study, we answered several questions regarding the defensive behavior of two species of pitvipers, the cottonmouth (*Agkistrodon piscivorus*) and the pigmy rattlesnake (*Sistrurus miliarius*). In a first part, we determined that thermal information did not influence the defensive strike of cottonmouths. Science is biased toward supportive evidence but reporting the non-importance of a factor may be viewed as important. Because few experimental studies have tested specific hypothesis on the alternative function of thermal pits, many speculations have arisen. We argue that visual as well as tactile cues are sufficient to release a defensive strike, at least in the cottonmouth.

In a second part, we answered two questions. First, adult cottonmouths have the cognitive ability to retain specific information regarding a potential predator for at least a 24 hour period. They learned that we were not a threat and adjusted their defensive responses accordingly. Second, we demonstrated that learning abilities vary ontogenetically in that neonate cottonmouths did not modify their defensive behavior over time. It is likely that this difference is due to a higher predation pressure on neonates since adults are relatively safe from predators. Habituation to a large moving object may be simply too high because predator misidentification by neonates may result in death in contrast to adults.

Lastly, we demonstrated that pigmy rattlesnakes were not aggressive as previously concluded by some herpetologists. We determined that pigmy rattlesnakes were more likely to strike when

they were moving (e.g., crossing a road). We believe that the aggressive reputation attributed to this rattlesnake species may result from causal observers whom are more likely to find individuals of this small cryptic species when they are moving. In addition, we found that the posture of the snake (stretched out vs. coiled), the presence of a prey item in the gut, as well as the age class of the snake influenced their fleeing behavior.

In conclusion, we provided answers regarding the defensive behavior of these pitviper species, and we hope that it will motivate other researchers to investigate patterns of defensive behavior in other venomous snake species. Because of high species diversity in pitvipers or more generally in Viperid snakes, we may expect interspecific differences. There remain many questions to be asked and answered, and hopefully future studies will provide further understanding of the adaptive significance of defensive behavior in Viperid snakes.