

Ontogeny of Anti-Predator Behavioral Habituation in Cottonmouths (*Agkistrodon piscivorus*)

Xavier Glaudas*†, Christopher T. Winne* & Luke A. Fedewa*‡

* University of Georgia's Savannah River Ecology Laboratory, Aiken, SC, USA

† Department of Biological Sciences, University of Nevada, Las Vegas, NV, USA

‡ Partners in Amphibian and Reptile Conservation, Phoenix, AZ, USA

Correspondence

Xavier Glaudas, Department of Biological Sciences, University of Nevada, Las Vegas, 4505 Maryland Parkway Las Vegas, NV 89154, USA. E-mail: glaudasx@unlv.nevada.edu

Received: June 29, 2005

Initial acceptance: August 24, 2005

Final acceptance: October 17, 2005

(S. K. Sakaluk)

doi: 10.1111/j.1439-0310.2005.01183.x

Abstract

Few studies of venomous snakes have addressed how anti-predator behavior may be affected by experience with a potential predator. Because defensive strikes may be costly to snakes, individuals with the ability to learn to discriminate among potentially harmful and non-harmful predatory stimuli should be favored by natural selection. In large venomous snakes, adults are capable of successfully defending themselves against most potential predators, whereas neonates suffer higher predator-induced mortality and are faced with a large diversity of predators. Consequently, we hypothesized that the relative costs of habituation to potential predatory stimuli should vary ontogenetically. This hypothesis predicts that adults should habituate rapidly to non-harmful predatory stimuli, whereas neonates should consistently employ active defensive displays (e.g. striking) because they are at higher risk. To test this prediction, we examined daily changes in the defensive behavior of adult and neonate cottonmouths (*Agkistrodon piscivorus*) towards a standardized non-harmful predatory stimulus. As predicted, adults and neonates differed in their tendencies to habituate: adults decreased defensiveness over days while neonates did not. Adults showed habituation of striking components but not of warning displays. Our results support the hypothesis that there may be ontogenetic differences in predator perception.

Introduction

Predation can be an important factor in shaping the behavior and ecology of species (Tuttle & Ryan 1981). Over evolutionary time, behavior is shaped to maximize net benefits as predicted by optimality models (Smith 1978). Because the cost of defense may affect fitness (Ueta 1999; Van Buskirk 2000; Raberg & Stjernman 2003), we might expect individuals to make behavioral decisions based on context-dependent costs and benefits when confronted by a predator (Endler 1986). Accordingly, factors such as the risks represented by a predator, as well as prior experience of the individual with the predator could influence an individual's final decision.

One way in which individuals can adjust their behavior is through habituation, a mechanism by which organisms display a stimulus-specific response decrement resulting from exposure to the response-eliciting stimulus (Wyers et al. 1973). Long-term habituation (as opposed to short-term habituation) occurs over multiple training sessions and is typically measured over days or weeks (Owen & Perrill 2000), demonstrating capacity for memory in experimental subjects. Intuitively, 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. For instance, if prior experience with a potential predator suggests that the risk of being preyed upon is

low or non-existent, the best strategy may be to simply not engage in active defense (e.g. striking) and, therefore, minimize the costs associated with it. Alternatively, if the risk of being preyed upon is very high, the cost of defense may be trivial compared to an individual's need to maximize survival. Conceptually, this scenario is analogous to the well-known dear-enemy hypothesis, which suggests that the ability to discriminate among territorial neighbors and transient rivals allows territory holders to minimize costs of defense by reducing unnecessary conflicts (Wilson 1975; Owen & Perrill 1998).

In most vertebrate species, juveniles and neonates suffer higher risk of 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 highly precocial and engage in defensive displays as soon as they are born, making them important model organisms for studies of defensive behavior and habituation (Burghardt 1978; Greene 1988). More specifically, venomous snakes are ideal for investigating both ontogenetic shifts in anti-predator behavior and habituation rates: they presumably have high costs associated with striking (e.g. venom expenditure, fang breakage, increased vulnerability to predator due to outstretched head and neck, muscle fatigue; Hayes et al. 2002), they have evolved a large array of warning displays (Greene 1988), and body size varies tremendously ontogenetically without large changes in shape (Herzog et al. 1989a). Furthermore, in some large viperid snakes, adults are capable of successfully defending themselves against most potential predators (Mitchell 1994; Ford 2002), whereas juveniles, due to their smaller body size, suffer higher predator-induced mortality (Li 1995) and are faced with a larger diversity of predators (Ernst & Ernst 2003). Consequently, we might expect these differences in predation pressures to result in ontogenetic differences in costs and benefits of habituation.

Because defensive strikes may be costly to snakes (Hayes et al. 2002), individuals with the ability to learn to discriminate among potentially harmful and non-harmful predatory stimuli may be favored by natural selection. However, any potential benefits accrued by forgoing unnecessary defensive strikes through habituation may be outweighed by the costs of misclassifying potential predators as non-harmful. Given that neonatal pitvipers suffer much higher rates of predator-induced mortality and are faced

with a larger diversity of predators than adults, there are more opportunities for neonates to wrongly display habituation during potential predation events. Consequently, we hypothesized that the relative costs of habituation to potential predatory stimuli should vary ontogenetically. We predicted that adults should habituate rapidly to non-harmful predatory stimuli whereas neonates should consistently employ active defensive displays (i.e. not habituate). Our objective was to test this prediction by examining whether an ontogenetic shift in anti-predator habituation occurs in a locally abundant viperid venomous snake, the cottonmouth (*Agkistrodon piscivorus*).

Materials & Methods

Model Species

Cottonmouths are large, heavy-bodied, semi-aquatic pitvipers (Family Viperidae, Subfamily Crotalinae). They are opportunistic feeders that eat almost any animal they can subdue (Ernst & Ernst 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 & Ernst 2003). Cottonmouths of all ages exhibit a series of defensive behaviors, including a stereotypical gaping behavior in which they expose the white lining of the inside of the mouth (hence their name) as a warning to potential predators.

Experimental Subjects

We collected 36 adult cottonmouths [17 females and 19 males, ($\bar{x} \pm \text{SE}$); snout-vent length (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 Jul. to Sep. 2003. We defined snakes >50 cm SVL as adults, which approximately reflects maturation size (Burkett 1966).

After testing, all gravid females were kept in the laboratory until parturition. We obtained 33 neonates [14 females and 19 males, ($\bar{x} \pm \text{SE}$); SVL = 23.97 ± 0.22 cm; mass = 16.8 ± 0.31 g] from 6 litters (3, 4, 5, 6, 6, 9). Four days after parturition, we removed neonates from their mother and placed them in individual containers. Prior to the trials, we housed all snakes individually in polyethylene containers (Rubbermaid™, Rubbermaid Commercial Products, Winchester, VA, USA; adults: $58 \times 42 \times$

14 cm; neonates: 34 × 25 × 14 cm), with bark mulch as substrate. We housed all containers within a walk-in environmental chamber (14L:10D, 26°C) and provided water ad libitum. To minimize the presence of human scent, we used latex gloves when setting up the containers. All snakes were allowed to acclimate to their individual containers for 48–72 h prior to testing. We did not feed the snakes during the experiment to eliminate the effect of recent feeding on defensive behavior (Herzog & Bailey 1987). Immediately following the completion of the trials, we released all snakes at their original point of capture.

The Predatory Stimulus

An approaching human is likely to be perceived as a threat and trigger strong defensive responses in snakes (Goode & Duvall 1989); consequently, humans have been widely used to elicit defensive behaviors in snakes in general (Herzog et al. 1989a,b; Glaudas et al. 2005) and cottonmouths in particular (Gibbons & Dorcas 2002; Glaudas 2004; Roth & Johnson 2004; Glaudas & Gibbons 2005). In this study, we approached each snake with an artificial human arm at an angle of approximately 45° from the horizontal plane, and then gently tapped the snake's midbody three successive times at 1-s intervals. This stimulus has the advantage of being a highly repeatable standardized test that elicits and allows cottonmouths the opportunity to deploy their full range of defensive responses.

The artificial arm consisted of a pair of 1-m snake tongs (Whitney tongs, Midwest ProductsTM, Greenwood, MO, USA) covered with a shirtsleeve customized to look like a human arm (Gibbons & Dorcas 2002). In this study, we did not warm the artificial arm because previous studies using the same stimulus have demonstrated that thermal cues do not influence the defensive strike of cottonmouths (Glaudas & Gibbons 2005). 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 ensure the presence of human olfactory cues.

We tested adults and neonates with the same stimulus because we wanted to compare habituation rates of the different age classes to an identical threat (hence, we did not adjust the size of the stimulus or the force of the taps to the body length of adults vs. neonates).

Testing Procedures

We performed all tests in the individual housing containers to eliminate pre- and post-test handling. Since container heights were short (14 cm), we placed each container within a larger plastic arena to prevent escape. We left the closed container undisturbed for 1 min prior to the test. Containers were always oriented in the arena with the snake facing the tester. After removing the container lid, the tester gently tapped the snake's midbody three successive times at 1-s intervals with the artificial arm (see above). At the start of each trial all snakes tongue-flicked and/or pointed their head toward the approaching stimulus, indicating that snakes were aware of the tester's presence. We tested each snake daily over a period of 5 d between 11:00 and 14:00 hours. All trials were videotaped using a Digital Video Camcorder (Canon, USA, Lake Success, NY, USA) and later reviewed by an independent observer.

To safeguard against potential biases and tester effects, we used two testers and an independent observer throughout the experiment. Each tester was allocated approximately half of the adults and half of the neonates. Initial trials were staggered among individuals (e.g. Day 1 snakes might be tested on the same day as Day 2, 3, 4, and/or 5 snakes) and on each test day the independent observer randomly chose the order of trials. Consequently, the testers did not know which individual was being tested, nor the day in the habituation sequence on which it was being tested. Although we could not record the pressure exerted on the snake, both testers practiced the protocol prior to the experiment so that their approach was standardized. Nonetheless, because both testers were blind to the experimental test day and the identity of the subject, any inconsistency of pressure was likely randomly distributed across days and individual snakes. Both testers wore the same shade of clothing throughout the experiment so that their appearance did not influence the snakes' responses (Whitaker & Shine 1999). After all of the trials were completed, the independent observer, blind to the identity of the subject and experimental test day, recorded behavioral responses during review of the videotapes.

Defensive Behaviors

We recorded the number of strikes, and the occurrence (presence/absence) of strikes, faint strikes, gaping, tail vibrations, and musking, daily over the 5-d testing period for each snake. We defined a strike as

any motion forward by the snake with its mouth open and neck fully extended, regardless of whether strikes successfully contacted the arm (i.e. we assumed accurate and inaccurate strikes were derived from an equal intent to strike the arm). A separate category was used for feint (i.e. 'half-hearted' or 'bluff') strikes, which consisted of head movement, with either opened or closed mouth, toward the stimulus without fully extending the neck. Because feint strikes occurred infrequently (22 out of 345 trials) and because the intent of feint strikes was not as clear as strikes, we did not include this variable in the analyses (although including feint strikes did not alter our results). We made no distinction between envenomation and a dry bite (i.e. no venom injected) because no reliable methods were available to assess whether venom was injected. We recorded gaping behavior when the snake exposed the white lining of its mouth. 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 unpleasant odoriferous substance from its cloaca (musking).

Data Analysis

We used contingency tables to test for an effect of tester on the proportions of snakes that struck, and on the number of strikes per snake for Day 1 and Day 5, separately.

We performed direct comparisons between the defensive responses of adults and neonates at the start of the trials (Day 1) and the end of the trials (Day 5) using a separate contingency table for each response variable. This set of tests examines if adults and neonates differ in initial defensive behavior on Day 1, and whether they differ in defensive behavior at the end of the habituation trials (Day 5).

Additionally, we performed a second group of tests (repeated measure analyses) to determine if habituation occurred over the course of the trials. Specifically, we used a Cochran Q-test to test for a significant change within individuals (i.e. dichotomous repeated measures) of each experimental group in the presence or absence of specific attributes (striking, tail vibrating, gaping, musking) among the five experimental days. We used Friedman's ANOVA, a repeated-measures analysis for ordinal data, to test the null hypothesis of no difference in the number of strikes among the five experimental days. Subsequently, we performed non-parametric post hoc

analyses using equations from Zar (1984) to determine the onset of habituation by comparing results on Day 1 to each of the other days (Days 2, 3, 4 and 5). To compare within group variances on Day 1 and Day 5 we used the F-ratio test.

To alleviate concerns that our neonatal data come from a limited number of litters we used a Kruskal–Wallis test to examine whether habituation rates differed among litters. Similarly, to determine whether adult body size might be related to habituation rates we also used a Kruskal–Wallis test. In both of these cases we used the numerical difference between the striking responses on Day 1 and Day 5 as the dependent variable. In the latter test, we assigned each individual to one of three size categories of SVL and used size class as the independent variable: 60–70 cm ($N = 12$), 70–80 cm ($N = 17$), and 80–90 cm ($N = 6$). Furthermore, we used a one-way ANOVA to test for litter effects on neonate size.

We used the false discovery rate method (Benjamini & Hochberg 1995) to ensure an Alpha level of 0.05 throughout each set of analyses. All statistical tests were performed using Statistica' 6.0 software (StatSoft 2000, Tulsa, OK, USA).

Results

Testers

We found no effect of tester on the occurrence of striking on Day 1 (Contingency table: adults: $\chi^2 = 1.80$, $df = 1$, $p = 0.18$; neonates: $\chi^2 = 0.004$, $df = 1$, $p = 0.95$) or Day 5 (adults: $\chi^2 = 0.360$, $df = 1$, $p = 0.55$; neonates: $\chi^2 = 1.91$, $df = 1$, $p = 0.16$). Additionally, testers did not affect the number of strikes on Day 1 (adults: $\chi^2 = 2.47$, $df = 3$, $p = 0.47$; neonates: $\chi^2 = 1.71$, $df = 3$, $p = 0.63$) or Day 5 (adults: $\chi^2 = 0.360$, $df = 1$, $p = 0.55$; neonates: $\chi^2 = 1.93$, $df = 2$, $p = 0.37$). Consequently, tester effect was not considered in other analyses.

Initial Defensive Response

The proportion of individuals that struck the artificial arm ($\chi^2 = 1.30$, $df = 1$, $p = 0.25$; Fig. 1a) and the number of times they struck ($\chi^2 = 4.96$, $df = 3$, $p = 0.17$; Fig. 1b) did not differ between adults and neonates on Day 1. In addition, musking behavior did not differ between the two groups ($\chi^2 = 1.67$, $df = 1$, $p = 0.19$). However, neonates vibrated their tails ($\chi^2 = 4.07$, $df = 1$, $p = 0.05$; Fig. 1c) and gaped ($\chi^2 = 4.08$, $df = 1$, $p = 0.04$; Fig. 1d) significantly more often than adults.

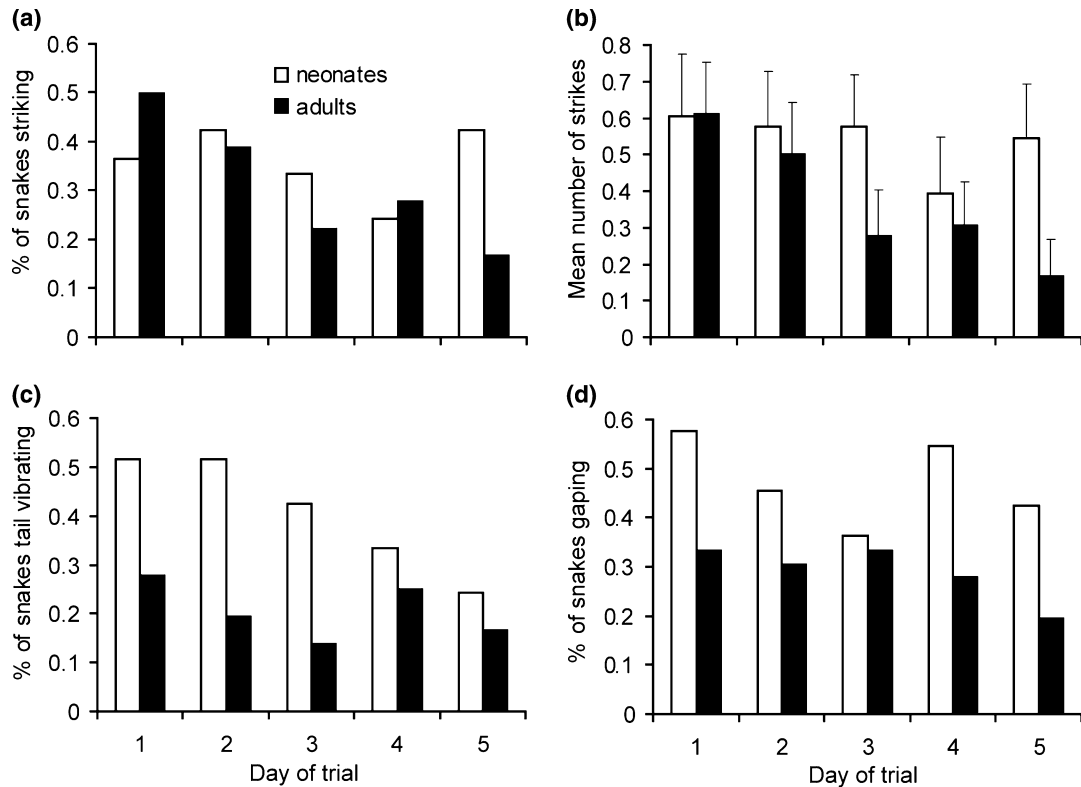


Fig. 1: (a) Percentages of adults and neonates that struck during trials. There was a significant decrease in the number of adults, but not neonates, that struck throughout the trial which resulted in significantly fewer adults than neonates striking on Day 5. (b) Mean number of strikes per snake (± 1 SE) by adults and neonates. Adults, but not neonates, showed a significant decrease in the number of strikes by Day 5 which resulted in a significant difference in number of strikes between adults and neonates on Day 5. (c) Percentages of adults and neonates that vibrated their tails. Although there was a trend for fewer neonates to exhibit tail vibrations towards the end of the trials, the results were not significant for either age class. (d) Percentages of adults and neonates that displayed mouth-gaping during trials. Adults and neonates did not exhibit any change in gaping behavior over time. However, neonates displayed significantly more than adults on Day 1 and Day 5

Adults' Habituation Test

The number of adult snakes that struck in response to the stimulus decreased over time (Cochran Q-test: $Q = 17.18$, $df = 4$, $p = 0.007$; Fig. 1a). Additionally, adults exhibited habituation of defensive behavior by decreasing the number of times they struck over the 5-d experiment (Friedman test: $\chi_r^2 = 18.75$, $df = 4$, $p = 0.007$; Fig. 1b). Post hoc analyses revealed a difference between Day 1 and Day 5 only ($p < 0.05$). We also observed a decrease in variance of striking behavior between Day 1 and Day 5 (F-ratio test: $F_{35,35} = 3.71$, $p = 0.0001$). Size class did not affect the habituation responses of adults in the occurrence of striking (Kruskal-Wallis: $H = 0.76$, $df = 2$, $p = 0.68$) or in strike number ($H = 0.648$, $df = 2$, $p = 0.72$). We found no habituation of tail vibration ($Q = 4.19$, $df = 4$, $p = 0.38$; Fig. 1c), gaping ($Q = 3.19$, $df = 4$, $p = 0.41$; Fig. 1d), or musking behavior ($Q = 4.25$, $df = 4$, $p = 0.37$).

Neonates' Habituation Test

Neonates did not exhibit habituation in any defensive behaviors over the 5 d of trials. The number of snakes that struck ($Q = 5.27$, $df = 4$, $p = 0.26$; Fig. 1a) and the number of times they struck did not differ among days ($\chi_r^2 = 3.64$, $df = 4$, $p = 0.45$; Fig. 1b). Moreover, variances in strike number did not differ between Day 1 and Day 5 ($F_{32,32} = 1.72$, $p = 0.13$). More neonatal snakes vibrated their tails on Day 1 than on Day 5, but this trend was not statistically significant ($Q = 8.86$, $df = 4$, $p = 0.06$; Fig. 1c). Neonates did not exhibit daily differences in gaping ($Q = 5.92$, $df = 4$, $p = 0.2$; Fig. 1d) or musking frequency (there were only two instances of musking throughout all of the neonate trials).

We found no litter effect in habituation of striking ($H = 5.59$, $df = 5$, $p = 0.37$) or strike number ($H = 3.77$, $df = 5$, $p = 0.58$). We detected a significant effect of litter on mean neonate size (one-way ANOVA:

$F_{5,27} = 4.68$, $p = 0.003$). Post hoc analysis revealed that one litter was significantly different from three other litters. However, removing this litter from the analysis did not alter our results in that neonates did not exhibit any changes in occurrence of striking ($Q = 3.52$, $df = 4$, $p = 0.47$) or number of strikes ($\chi^2_r = 2.09$, $df = 4$, $p = 0.71$) over time.

Final Defensive Response

The proportion of snakes that struck the artificial arm ($\chi^2 = 5.54$, $df = 1$, $p = 0.04$; Fig. 1a) and the number of times individuals struck ($\chi^2 = 7.35$, $df = 2$, $p = 0.04$; Fig. 1b) were significantly higher in neonates than in adults on Day 5. We found no difference between neonates and adults in the frequency of tail vibration ($\chi^2 = 0.61$, $df = 1$, $p = 0.43$; Fig. 1c). However, we observed gaping more frequently in neonates than in adults ($\chi^2 = 4.29$, $df = 1$, $p = 0.04$; Fig. 1d).

Discussion

General Response to Stimulus

On the whole, both adults and neonates responded defensively towards the artificial arm stimulus, with many individuals deploying their full range of anti-predator behaviors over a single trial. This suggests that (1) our artificial stimulus was perceived by cottonmouths to be a potential threat (at least on Day 1) and (2) our testing procedure provided enough time for snakes to exhibit all defensive behaviors during each trial. Furthermore, the lack of statistical difference between our two testers suggests that our protocol provided highly repeatable results. Consequently, this stimulus provides an appropriate method for testing defensive behavior in this species (Gibbons & Dorcas 2002; Glaudas 2004; Roth & Johnson 2004).

Initial Defensive Response

Adults and neonates did not differ in their striking responses on Day 1. This similarity among neonates and adults was important for the rest of this study by providing the same potential for habituation in adults and neonates. However, neonates initially used warning displays (i.e. tail vibration, gaping) significantly more often than adults. Being more vulnerable to predation, neonates may simply obtain greater benefits from the use of aposematic signals than adults. Our results are consistent with Roth &

Johnson's (2004) findings in which they reported no effect of body size on striking behavior, and observed a negative relationship between body size and the use of warning displays in cottonmouths. Similarly, Herzog et al. (1992) isolated effects of age on defensive behavior independent of habituation and reported no developmental changes in the first month of life in the striking behavior of neonates of three snake species, *Nerodia rhombifer*, *Thamnophis butleri*, and *T. radix*. However, in the same study, two other *Thamnophis* species reacted more defensively at 20 d of age than at 1 d of age, suggesting interspecific differences in ontogenetic trajectories of initial defensive behavior.

Habituation Tests

As predicted, adults habituated to a non-harmful predatory stimulus. There was a clear decrease in both the number of snakes that struck and in the number of times they struck. This suggests that adults recognized the stimulus as being non-harmful and retained this learned association for at least a 24-h period (Glaudas 2004). Reducing or eliminating the frequency of unnecessary strikes through habituation to non-harmful stimuli may be an adaptive strategy to reduce risk of injury and conserve energy, thus increasing the energy available for other components of fitness such as reproduction and growth (Endler 1991). In addition, adults showed no habituation of warning displays, which may have been partially related to the infrequent use of warning displays by adults on Day 1 or to the presumably minor costs of performing warning displays in comparison to the high costs associated with striking.

We observed high individual variation in initial defensive behavior, a pattern that is consistent with previous investigations (Arnold & Bennett 1984; Brodie 1993). During this study, variation in adult strike numbers decreased significantly over the 5-d trials. This decrease in variance is explained by some adults relying primarily on a passive defense (i.e. not striking) throughout the experiment, whereas other individuals switched from striking frequently during the beginning of the trial towards a more passive defense at the end of the trial. The net result was that individual variation decreased as a function of time and experience and resulted in behavioral convergence by Day 5.

In contrast to adults, neonates showed no habituation in striking response. We propose three possible explanations for the lack of habituation in neonate

cottonmouths, all of which may be interrelated. First, the costs of habituation in neonates may outweigh any potential benefit: in neonates, which experience significantly greater mortality and vulnerability to a higher diversity of predators due to their small body size, habituation may have little benefit because the risk of wrongly identifying a potential predator as 'non-dangerous' is presumably higher than in adults. Indeed, field studies have shown that annual survival rate of neonate cottonmouths is approximately 30% compared to 75–100% for adults (Ford 2002). As a result, unlike adults, neonates may view all large moving objects as predators resulting in a consistent use of active defense (e.g. striking). Second, the differences we observed in the habituation rates of neonates and adult cottonmouths may be due to our stimulus being relatively more intense to neonates (same stimulus to different-sized animals). However, we re-emphasize that our choice of experimental design is ecologically relevant to understanding the alternative views of adults and neonates towards a potential predator. In nature, any given predator is larger and relatively more aggressive towards a neonate, which is exactly why neonates are more susceptible to a larger diversity of predators. Consequently, even if the differences in habituation rate between adults and neonates are due to differences in realized stimuli, we would still interpret this result as evidence for different rates of habituation in nature. Nonetheless, a similar study with a size-scaled stimulus would be of great interest to decouple the effect of stimulus intensity from other predictor variables. Finally, lack of habituation in neonates may be due to an insufficient memory capacity (but see Herzog et al. 1989a), or an inability to learn as quickly as adults (i.e. neonates may have habituated if given more time). Failure to habituate to a confrontational stimulus has been previously reported for neonates of other snakes including colubrid (Herzog et al. 1989a) and elapid snake species (Bonnet et al. 2005). It should be noted that this behavioral mechanism varies interspecifically (e.g. even among congeneric species), among litters within species (Herzog et al. 1989a), and possibly at the population level (Bonnet et al. 2005).

Conclusions

Our experiment clearly demonstrates that adults and neonates differ in their ability to habituate to a similar-sized predatory stimulus, and therefore, provides a necessary first step towards understanding how

habituation varies ontogenetically within snakes. Further our study is, to the best of our knowledge, the first report of the effect of ontogeny on long-term habituation of anti-predator behavior. We encourage future studies to experimentally decouple traits that may drive this ontogenetic change in habituation rates. For example, carefully designed experiments could isolate the key predictor(s) of habituation rates from the various factors known to vary with ontogeny (age, size, memory, physiology, etc.). Ultimately, understanding which factors affect ontogenetic shifts in habituation of defensive behavior may lead to important discoveries about the evolution of anti-predator behavior in snakes.

Acknowledgements

We thank J. Whitfield Gibbons, Patricia A. Gowaty, John Pickering, and I. Lehr Brisbin for providing useful comments on the manuscript. Manuscript preparation was aided by the Environmental Remediation Sciences Division of the Office of Biological and Environmental Research, US Department of Energy through the Financial Assistant Award No. DE-FC09-96SR18546 to the University of Georgia Research Foundation. This research was completed as a part of a Master's thesis (XG) at the University of Georgia and approved by the University of Georgia IACUC under IACUC No. A2003-10144-0. Specimens were collected under scientific research permit #56-2003 provided by the South Carolina Department of Natural Resources.

Literature Cited

- Arnold, S. J. & Bennett, A. F. 1984: Behavioural variation in natural populations. III. Anti-predator displays in the garter snake *Thamnophis radix*. *Anim. Behav.* **32**, 1108–1118.
- Benjamini, Y. & Hochberg, Y. 1995: Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* **57**, 289–300.
- Bonnet, X., Aubret, F., Lourdais, O., Ladyman, M., Bradshaw, D. & Maumelat, S. 2005: Do 'quiet' places make animals placid? Island vs. mainland tiger snakes. *Ethology* **111**, 573–592.
- Brodie, E. D., III 1993: Consistency of individual differences in antipredator behavior and colour pattern in the garter snake, *Thamnophis ordinoides*. *Anim. Behav.* **45**, 851–861.
- Burghardt, G. M. 1978: Behavioural ontogeny in reptiles: where, whither and why? In: *The Development of Behaviour: Comparative and Evolutionary Aspects*

- (Burghardt, G. M. & Bekoff, M., eds). STPM Garland Press, New York, pp. 149–174.
- Burghardt, G. M. 1984: On the origin of play. In: *Play in Animals and Humans* (Smith, P. K., ed.). Blackwell, London, pp. 5–41.
- Burkett, R. D. 1966: Natural history of cottonmouth moccasin, *Agkistrodon piscivorus* (Reptilia). Univ. Kansas Publ. Mus. Nat. Hist. **17**, 435–491.
- Endler, J. A. 1986: Defense against predators. In: *Predator–Prey Relationships: Perspectives and Approaches from the Study of Lower Vertebrates* (Feder, M. E. & Lauder, G. V., eds). Univ. of Chicago Press, Chicago, pp. 109–134.
- Endler, J. A. 1991: Interactions between predators and prey. In: *Behavioural Ecology, An Evolutionary Approach* (Krebs, J. R. & Davies, N. B., eds). Blackwell Scientific Publications, Oxford, pp. 169–196.
- Ernst, C. H. & Ernst, E. M. 2003: *Snakes of the United States and Canada*. Smithsonian Institution Press, Washington D.C.
- Ford, N. B. 2002: Ecology of the western cottonmouth (*Agkistrodon piscivorus leucostoma*) in Northeastern Texas. In: *Biology of the Vipers* (Schuett, G. W., Hoggren, M., Douglas, M. E. & Greene, H. W., eds). Eagle Mountain Publishing, Eagle Mountain, UT, pp. 167–178.
- Gibbons, J. W. & Dorcas, M. E. 2002: Defensive behavior of cottonmouths (*Agkistrodon piscivorus*) toward humans. *Copeia* **2002**, 195–198.
- Glaudas, X. 2004: Do cottonmouths (*Agkistrodon piscivorus*) habituate to human confrontations? *Southeast. Nat.* **3**, 129–138.
- Glaudas, X. & Gibbons, J. W. 2005: Do thermal cues influence the defensive strike of cottonmouths (*Agkistrodon piscivorus*)? *Amphib.–Reptil.* **26**, 264–267.
- Glaudas, X., Farrell, T. M. & May, P. G. 2005: The defensive behavior of free-ranging pygmy rattlesnakes (*Sistrurus miliarius*). *Copeia* **2005**, 196–200.
- Goode, M. J. & Duvall, D. 1989: Body temperature and defensive behaviour of free-ranging prairie rattlesnakes, *Crotalus viridis viridis*. *Anim. Behav.* **38**, 360–362.
- Greene, H. W. 1988: Anti-predator mechanisms in reptiles. In: *Biology of the Reptilia*, Vol. 16 (Gans, C. & Huey, R. B., eds). Alan R. Liss, New York, pp. 1–152.
- Hayes, W. K., Herbert, S. S., Rehling, G. C. & Gennaro, J. F. 2002: Factors that influence venom expenditure in viperids and other snake species during predatory and defensive contexts. In: *Biology of the Vipers* (Schuett, G. W., Hoggren, M., Douglas, M. E. & Greene, H. W., eds). Eagle Mountain Publishing, Eagle Mountain, UT, pp. 207–233.
- Herzog H. A., Jr & Bailey, B. D. 1987: Development of anti-predator responses in snakes. II. Effects of recent feeding on defensive behaviors of juvenile garter snakes (*Thamnophis sirtalis*). *J. Comp. Psychol.* **100**, 233–242.
- Herzog H. A., Jr, Bowers, B. B. & Burghardt, G. M. 1989a: Development of antipredator responses in snakes. IV. Interspecific and intraspecific differences in habituation of defensive behavior. *Dev. Psychobiol.* **22**, 489–508.
- Herzog H. A., Jr, Bowers, B. B. & Burghardt, G. M. 1989b: Stimulus control of antipredator behavior in newborn and juvenile garter snakes (*Thamnophis*). *J. Comp. Psychol.* **103**, 233–242.
- Herzog H. A., Jr, Bowers, B. B. & Burghardt, G. M. 1992: Development of antipredator responses in snakes. V. Species differences in ontogenetic trajectories. *Dev. Psychobiol.* **25**, 199–211.
- Li, J. L. 1995: *China Snake Island*. Liaoning Science and Technology Press, Dalian, People's Republic of China.
- Mitchell, J. C. 1994: *The Reptiles of Virginia*. Smithsonian Institution Press, Washington, DC.
- Owen, P. C. & Perrill, S. A. 1998: Habituation in the green frog, *Rana clamitans*. *Behav. Ecol. Sociobiol.* **44**, 209–213.
- Owen, P. C. & Perrill, S. A. 2000: Habituation and neighbor–stranger discrimination in green frogs: a reply to Bee and Schachtman. *Behav. Ecol. Sociobiol.* **48**, 169–171.
- Raberg, L. & Stjernman, M. 2003: Natural selection on immune responsiveness in blue tits *Parus caeruleus*. *Evolution* **57**, 1670–1678.
- Roth, E. D. & Johnson, J. A. 2004: Size-based variation in anti-predator behavior within a snake (*Agkistrodon piscivorus*) population. *Behav. Ecol.* **15**, 365–370.
- Sibly, R. M., Collett, D., Promislow, D. E. L., Peacock, D. J. & Harvey, P. H. 1997: Mortality rates of mammals. *J. Zool.* **243**, 1–12.
- Smith, J. M. 1978: Optimization theory in evolution. *Annu. Rev. Ecol. Syst.* **9**, 31–56.
- Tuttle, M. D. & Ryan, M. J. 1981: Bat predation and the evolution of frog vocalizations in the neotropics. *Science* **214**, 677–678.
- Ueta, M. 1999: Cost of nest defence in azure-winged magpies. *J. Avian Biol.* **30**, 326–328.
- Van Buskirk, J. 2000: The costs of an inducible defense in anuran larvae. *Ecology* **81**, 2813–2821.
- Whitaker, P. B. & Shine, R. 1999: Responses of free-ranging brown snakes (*Pseudonaja textilis*: Elapidae) to encounters with humans. *Wildl. Res.* **26**, 689–704.
- Wilson, E. O. 1975: *Sociobiology: The New Synthesis*. Harvard Univ. Press, Cambridge, MA.
- Wyers, E. J., Peeke, H. V. S. & Herz, M. J. 1973: Behavioral habituation in vertebrates. In: *Habituation*, Vol. 1. Behavioral Studies (Peeke, H. V. S. & Herz, M. J., eds). Academic Press, New York and London, pp. 1–57.
- Zar, J. H. 1984: *Biostatistical analysis*. Prentice-Hall, Inc., Englewoods Cliffs, NJ.