



Mother Cottonmouths (*Agkistrodon piscivorus*) Alter Their Antipredator Behavior in the Presence of Neonates

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Received: February 27, 2014
Initial acceptance: April 1, 2014
Final acceptance: May 12, 2014
(S. Foster)

doi: 10.1111/eth.12265

Keywords: maternal attendance, offspring defense, parental care, pitviper, reptile

Abstract

Neonate-directed care is rare in non-avian reptiles, but female pitvipers attend their young for a period of time after birth. One of the primary functions of parental care is the protection of offspring from predators, and parents of diverse taxa are able to modulate their antipredator behavior in the presence of offspring. To test the hypothesis that the antipredator behavior of post-parturient pitvipers is altered during neonate attendance, we conducted behavioral trials on female cottonmouths (*Agkistrodon piscivorus*) in which we measured female response to a simulated predator encounter. Cottonmouths were divided into three treatment groups: (1) post-parturient, attending neonates; (2) post-parturient, not attending neonates; and (3) non-reproductive. All females were subjected to a second trial approximately 3 wk later, when females in Group 1 were no longer attending neonates. When mothers were attending offspring, they were more hesitant to engage the predator and exhibited more warning than aggressive behaviors once they did, relative to non-attending and non-reproductive females. When these same mothers were no longer attending offspring, they significantly increased their antipredator behavior by engaging the predator quickly and displaying more aggressive than warning behaviors. This change in behavior was not observed in post-parturient females who did not have neonates present during either trial, nor was it observed in non-reproductive females, indicating that the presence of neonates directly affected the antipredator behavior of attending females. We discuss hypotheses concerning the possible adaptive value of reduced antipredator behavior in female pitvipers attending neonates.

Introduction

Predation-induced mortality during early developmental stages is considered to be a major driving force in the evolution of parental care (Clutton-Brock 1991; Martin & Briskie 2009; Klug & Bonsall 2010), defined in the narrow sense as 'parental behavior that occurs post-fertilization, is directed at offspring, and appears likely to increase offspring lifetime reproductive success' (Klug et al. 2012; p. 21). In order to maximize inclusive fitness (Hamilton 1964), parents must weigh the direct costs of engaging a predator while defending offspring against the indirect benefits of offspring survival (Williams 1966; Trivers 1972; Stearns

1992), making the optimal type and intensity of offspring defense highly context dependent. For example, Campobello and Sealy (2010) found that parental reed warblers (*Acrocephalus scirpaceus*) displayed aggressive, conspicuous antipredator behaviors toward an egg-only predator, but more cautious, inconspicuous behaviors toward a hatchling/adult predator. Furthermore, the intensity of these predator-specific behaviors was greatest during the stage at which offspring were most vulnerable to each predator, which presumably minimized the costs to parents while maximizing the benefits to offspring. The ability to modulate offspring defense based on a variety of factors has been documented most widely in avian

species (Montgomery & Weatherhead 1988; Martin & Briskie 2009), but similar examples have also been found in taxonomic groups that exhibit less complex forms of parental care (e.g., long-tailed skink, Huang & Huang 2009; convict cichlid, Lavery 1995; red swamp crayfish, Figler et al. 1997).

The majority of squamate reptiles (i.e., lizards and snakes) do not display parental care, but in oviparous species, maternal egg attendance is one form of care that is widespread among certain groups (Shine 1988; Somma 2003). This behavior functions to reduce egg mortality caused by predators or conspecifics (e.g., Huang 2006) and fluctuations in environmental conditions (Somma & Fawcett 1989; Aubret et al. 2005), but typically, attendance does not continue after hatching. In contrast, viviparous species gain the protective and physiological benefits of egg attendance by retaining embryos in the uterus until fully developed (Blackburn & Stewart 2011), and consequently, parental behavior in viviparous reptiles is exceedingly rare. For example, although ~20% of all snake species are viviparous (Blackburn 1985), maternal attendance (MA) of neonates has only been documented in temperate pitvipers (Greene et al. 2002). This begs the question of why such a rare form of reptilian parental behavior would evolve in pitvipers; one possibility is that the unique defensive abilities of this group (i.e., they are venomous) make them particularly effective at protecting offspring.

In general, female pitvipers (e.g., rattlesnakes and moccasins) remain with their young at the birth site until neonates complete their first shed cycle ('postnatal ecdysis'), at which point the mother and neonates disperse (reviewed in Greene et al. 2002). Although newborn pitvipers possess fully functional fangs and venom, they are subject to a broader array of predators than adults (Ford 2002; Ernst & Ernst 2003) and might be particularly vulnerable during the attendance period, due to the general lethargy and vision impairment associated with ecdysis (King & Turmo 1997). Although direct and/or indirect predator deterrence is an intuitively likely function of MA of neonates in this group, only two empirical studies have tested this hypothesis. Graves (1989) and Greene et al. (2002) demonstrated that female rattlesnakes (*Crotalus viridis* and *Sistrurus miliarius*) relied more on crypsis and escape when pregnant, but increased defensive behavior after parturition, during the period that neonates are typically present. While these observed behavioral changes are interesting, species that do not attend offspring have also been shown to increase their defensive behavior after parturition (e.g., garter snakes, Brodie 1989) even

though neonates are not present. No empirical studies to date have examined the antipredator behavior of females attending offspring while attempting to control for the effect of reproductive state (i.e., post-parturient vs. non-reproductive); thus, whether pitviper mothers alter their behavior based on the presence of offspring, in addition to reproductive state, remains to be determined.

The goal of this study was to decouple the effect of offspring presence from the effect of reproductive state on maternal antipredator behavior in the cottonmouth (*Agkistrodon piscivorus*), a semi-aquatic pitviper native to the southeastern United States (Gloyd & Conant 1990). We conducted captive behavioral trials in which we used a live kingsnake (*Lampropeltis getula*) to simulate a predator encounter and measured the behavioral response of females that were (1) post-parturient and attending neonates; (2) post-parturient, but not attending neonates; and (3) non-reproductive. We then removed neonates from females in the first group and conducted a second set of trials on all females, which allowed us to examine the effect of neonate presence or absence on antipredator behavior in the same females, while controlling for the potential effects of reproductive state and habituation.

Methods

Pregnant ($n = 20$) and non-reproductive ($n = 10$) adult female cottonmouths (distinguished by the presence or lack of a greatly distended posterior abdomen, respectively) were collected from Clayton and Fayette counties, Georgia, during July and August 2011, and transported to the San Diego State University Sky Oaks Field Station in Warner Springs, California. All snakes were measured (snout-vent length; SVL), weighed, and housed individually in large indoor enclosures ($1.8 \times 1.8 \times 0.9$ m) containing a hide box ($0.6 \times 0.6 \times 0.6$ m). These enclosures were inside an insulated warehouse with a concrete floor and skylights, which provided a natural light/dark cycle. To eliminate the potential effects of recent feeding on antipredator behavior (Herzog & Bailey 1987), snakes were not fed during this study, but water was provided *ad libitum*.

Each pregnant female was randomly assigned to one of two treatment groups: Maternal attendance (MA; $n = 10$) or Separated (SE; $n = 10$). Upon parturition, MA females and their neonates were allowed an attendance period, whereby the litter was left in the enclosure with the mother until all neonates in that litter completed ecdysis (14–18 d post-parturition; mean \pm standard deviation: 16.29 ± 1.60). Once all

neonates of a given litter completed ecdysis, they were removed from the mother's enclosure. Females in the SE group were not allowed an attendance period, but instead were removed from their litter within 24 h of parturition and moved to a new enclosure. We moved the mother to a new enclosure, as opposed to removing the litter, to ensure the absence of any chemical cues associated with neonates that might alter the mother's behavior. Although we checked for parturition at 24-h intervals, cottonmouths typically gave birth in the early morning hours; as such, separations likely occurred within 8 h of birth. Parturition dates for pregnant females ranged from 27 August to 17 September 2011 and litters contained 2–8 neonates (mean \pm SD: 4.75 ± 1.29).

Each female was subjected to two predator trials (Trial 1 and Trial 2). For MA females, Trial 1 occurred in the presence of their neonates (6 d post-parturition) and Trial 2 occurred in the absence of neonates (1 wk after neonates had been removed; \sim 17 d after Trial 1). Females in the SE group did not have neonates present during either trial (i.e., neonates were removed shortly after birth), but the timing of Trial 1 and Trial 2 was similar to that of MA females (i.e., Trial 1 occurred 6 d post-parturition and Trial 2 occurred \sim 17 d after Trial 1). Females in the NR group ($n = 10$) were not pregnant and, thus, did not have a parturition date by which to schedule Trial 1. Instead, Trial 1 was randomly scheduled, but occurred during the weeks that pregnant females were giving birth, and Trial 2 occurred \sim 17 d after Trial 1. This two-trial design allowed us to isolate the effect of neonate presence on maternal antipredator behavior, by taking into account the potentially confounding effect of habituation (Glaudas et al. 2006). Further, the inclusion of non-reproductive females allowed us to consider the effect of reproductive state (i.e., post-parturient vs. non-reproductive), in general, on antipredator behavior.

We used a live kingsnake (*Lampropeltis getula*; a known cottonmouth predator, Gloyd & Conant 1990), to elicit antipredator behavior in female cottonmouths. Kingsnakes are active foragers, which actively hunt their prey (as opposed to ambush foragers, like cottonmouths). There is little known about kingsnake–cottonmouth interactions in the wild, but presumably encounters happen both terrestrially and aquatically. The ranges of cottonmouths and kingsnakes overlap considerably, and the cottonmouths that were used in this study were collected from sites that were within the range of kingsnakes. One of two adult male kingsnakes (SVL = \sim 950 mm; collected in Alabama and South

Carolina) was randomly assigned to each trial; kingsnakes were large enough to present a threat to both neonates and adults and were not fed during the experimental period. Before trials, the kingsnake was restrained by placing it headfirst into a plastic snake tube until its snout was at the tube opening; the body was secured to the tube end opposite the head, using electrical tape. In addition, we rubbed the kingsnake's body over the exterior of the snake tube, in an effort to enhance the transmission of volatile chemical cues used by pitvipers to recognize kingsnakes (Bogert 1941; Carpenter & Gillingham 1975; Gutzke et al. 1993). The snake tube was cleaned with a bleach solution and re-scented between trials. During trials, the restrained kingsnake was moved using snake tongs, so that the handler could remain at a distance and on the outside of the enclosure. Predator trials were only conducted when the focal female was in her hide box; in trials where neonates were present (i.e., MA Trial 1), all neonates were also in the hide box, with the exception of a single trial, during which one neonate was behind the hide box, out of view of the mother. To allow observation, the front panel of the hide box was removed and the female was left undisturbed for 10 min before the trial began. At the start of the trial, the restrained kingsnake was moved to within \sim 0.5 m from the focal female's head and slowly waved in a side-to-side motion for approximately 4 s. This allowed the focal female to orient toward the kingsnake and begin tongue-flicking. The handler then simulated nine predator 'advance-retreat' cycles, in which the kingsnake was moved toward the female until the headend of the tube made contact with the front-facing flank of the female ('advance') and then back away from the female ('retreat'). This advance-retreat cycle was repeated nine times in a slow rhythmic manner (i.e., each cycle lasted 4–5 s), after which the kingsnake was removed and the trial ended. All trials were conducted by the same handler to ensure among-trial consistency in the timing and general characteristics of predator movement. Trials lasted approximately 1 min and were recorded with a tripod-mounted video camera. All trials took place between 1 September and 13 October 2011 and during 11:30–16:30 h. Because we were unable to control temperature precisely, we noted the substrate temperature before each trial using an infrared temperature gun (Pro Exotics model PE-2). Adult cottonmouths were released at their capture site and neonates were released at their mother's capture site in the spring following this study.

Statistical Analyses

A single observer who was blind to treatment group and trial number (with exception of MA Trial 1, due to the obvious presence of neonates) analyzed video recordings of the trials with respect to the following antipredator behaviors: number of strikes and tail vibrations; latency to strike; presence of body-bridging or thrashing. Snakes engaged in bouts of tail vibrating that varied in length, but the occasional lack of visibility prevented us from accurately calculating the total time a snake spent vibrating its tail. Instead, we recorded whether or not a snake vibrated its tail at any point during the 4 s predator introduction and each predator advance-retreat cycle, to obtain a value for 'number of tail vibrations' that ranged from 0 (i.e., female did not vibrate its tail at any point) to 10 (i.e., female vibrated its tail during the predator introduction and each of the nine predator advance-retreat cycles). To compare the relative aggressiveness of females during trials, we created a composite behavioral score ('aggressiveness score') by dividing the total number of strikes (aggressive behavior) by the total number of tail vibrations (warning behavior), such that a high score indicated a more aggressive response than a low score. Because some females did not strike, and calculating a score with a numerator of 0 would effectively ignore variation in tail vibrations, we added 1 to both the strike and tail vibration values of all snakes before calculating the aggressiveness score. Latency to strike was divided into three categories: short latency (first strike occurred during first half of trial), long latency (first strike occurred during second half of trial), and no strike (snake never struck). We chose to collapse strike latency into these categories because treating it as a continuous variable would not allow us to include females that never struck in the analysis and we considered those observations to be important. Body-bridging was defined as the snake assuming an elongate posture and elevating sections of the trunk off the ground in an undulating manner (Carpenter & Gillingham 1975). This behavior is a response that is specific to threat of predation from an ophiophagous snake (Bogert 1941) and might function to direct the predator attack away from the head. Thrashing was defined as the snake assuming an elongate and raised body position, but then rapidly whipping the raised part of the trunk toward the predator. Due to the postural similarities between body-bridging and thrashing, and the fact that thrashing typically occurred during body-bridging, we did not treat the two behaviors separately in analyses, but rather scored each female as to whether they dis-

played either of the behaviors at any point during a trial.

To ensure that behavioral differences among treatment groups were not due to a significant difference in body size (e.g., Roth & Johnson 2004) or number of days between Trial 1 and Trial 2, we conducted an analysis of variance (ANOVA) on SVL and number of days between trials with treatment group as a factor. Adult female SVL ranged from 570 to 731 mm (mean \pm SD: 640.83 ± 47.08) and did not significantly differ among treatment groups ($F_{2,27} = 0.774$, $p = 0.471$), nor did the number of days between trials ($F_{2,27} = 1.69$, $p = 0.200$). To determine whether pre-trial temperature differed significantly among treatment groups, we conducted an ANOVA on substrate temperature with treatment group as a factor. There was no significant difference in substrate temperature among treatment groups for Trial 1 ($F_{2,27} = 0.46$, $p = 0.638$) or Trial 2 ($F_{2,27} = 0.32$, $p = 0.726$). Mean \pm standard error substrate temperatures were $24.35^\circ\text{C} \pm 0.23$ (Trial 1) and $23.51^\circ\text{C} \pm 0.29$ (Trial 2).

To examine differences in relative aggressiveness, we conducted a repeated-measures analysis of variance (RMANOVA), with treatment group as a factor and aggressiveness score (square-root-transformed) for Trial 1 and Trial 2 as the repeated measure. A significant interaction between treatment group and trial was further analyzed with ANOVAs (Fisher's LSD *post hoc* tests) and paired *t*-tests. *G*-tests were used to determine whether there was an association between treatment group (MA, SE, or NR) and latency to strike (short, long, no strike) or presence of body-bridging or thrashing (yes or no). The assumptions of all parametric statistics were met, and significance was assessed at $\alpha \leq 0.05$. All analyses were conducted in SYSTAT 12 (Systat Corp., Chicago, IL, USA).

Results

The RMANOVA on aggressiveness score revealed a significant between-group effect of treatment ($F_{2,27} = 3.657$, $p = 0.039$), a nonsignificant within-group effect of trial ($F_{2,27} = 2.092$, $p = 0.160$), and a significant trial \times treatment interaction ($F_{2,27} = 3.891$, $p = 0.033$). The *post hoc* ANOVA for Trial 1 indicated a significant treatment effect ($F_{2,27} = 5.236$, $p = 0.012$; Fig. 1a), in which MA females responded less aggressively (i.e., significantly lower aggressiveness score) than SE ($p = 0.049$) and NR females ($p = 0.004$), but SE and NR females did not significantly differ ($p = 0.271$). However, when MA females no longer

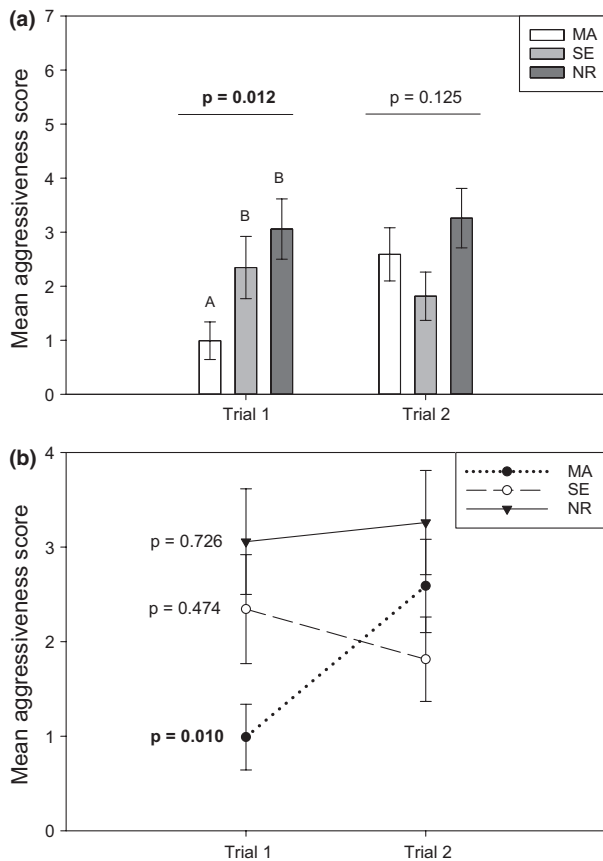


Fig. 1: Mean \pm standard error of aggressiveness scores (strike/tail vibration ratio) of adult female cottonmouths for two predator trials. Maternal attendance (MA) females were post-parturient and attending neonates in Trial 1, but not Trial 2; SE females were post-parturient, but not attending neonates in either trial; NR females were not reproductive and, thus, were not attending neonates in either trial. In graph a, above-bar p-Values were generated from within-trial ANOVAs (significant p-Values are in bold) and above-bar letters were generated from *post hoc* tests (bars that do not share a letter were significantly different). In graph b, p-Values were generated from within-treatment-group paired *t*-tests (significant p-Values are in bold).

had their neonates present (Trial 2), their aggressiveness score no longer differed from that of SE and NR females ($F_{2,27} = 2.250$, $p = 0.125$). When comparing the change in aggressiveness between trials, we found that MA females showed a significant change in aggressiveness score ($t_9 = -3.273$, $p = 0.010$; Fig. 1b), but SE and NR females did not (SE: $t_9 = 0.748$, $p = 0.474$; NR: $t_9 = -0.362$, $p = 0.726$).

There was a significant association between treatment group and latency to strike for Trial 1 ($G = 11.247$, $df = 4$, $p = 0.024$; Fig. 2), where only 50% of MA females initiated striking behavior during the first half of the trial, compared with 90% of SE females and 100% of NR females. However, in the

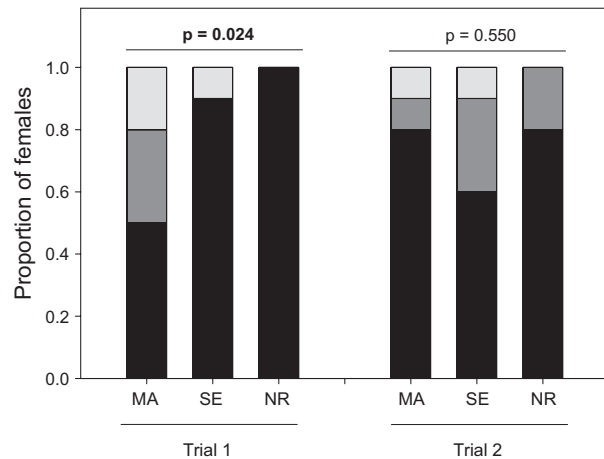


Fig. 2: Proportion of adult female cottonmouths that exhibited different strike latencies during two predator trials. Bar shades distinguish females that displayed a short latency (black), long latency (dark gray), or did not strike, at all (light gray). Maternal attendance (MA) females were post-parturient and attending neonates in Trial 1, but not Trial 2; SE females were post-parturient, but not attending neonates in either trial; NR females were not reproductive and, thus, were not attending neonates in either trial. Above-bar p-Values correspond to within-trial G-tests; significant p-Values are in bold.

absence of neonates (Trial 2), 80% of MA females struck during the first half of the trial, and there was no longer an association between treatment group and latency to strike ($G = 3.045$, $df = 4$, $p = 0.550$). Similarly, there was a significant association between treatment group and the presence of body-bridging or thrashing in Trial 1 ($G = 6.007$, $df = 2$, $p = 0.050$; Fig. 3), but not Trial 2 ($G = 0.966$, $df = 2$, $p = 0.617$). Specifically, when neonates were present (Trial 1), fewer MA females exhibited these behaviors, relative to SE or NR females (MA: 10%; SE: 60%; NR: 40%), but when neonates were absent (Trial 2), there was no longer a difference among treatment groups (MA: 40%; SE: 30%; NR: 20%).

Discussion

Mother cottonmouths exhibited more warning than overt and conspicuous aggressive behaviors toward an introduced predator when they were attending offspring. Specifically, they vibrated their tails more, struck less, and only one individual engaged in body-bridging. They were also hesitant to initiate active defense, as evidenced by 50% of attending females remaining quiescent for the first half of the simulated predator intrusion. In contrast, post-parturient females who were not attending offspring (neonates had been removed at birth) initiated defense sooner and engaged in more aggressive and conspicuous

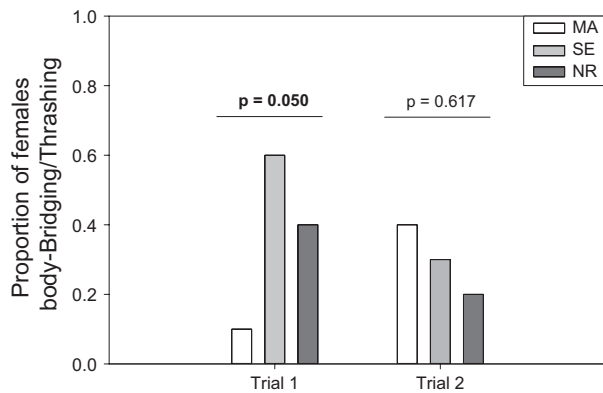


Fig. 3: Proportion of adult female cottonmouths that exhibited body-bridging and/or thrashing during two predator trials. Maternal attendance (MA) females were post-parturient and attending neonates in Trial 1, but not Trial 2; SE females were post-parturient, but not attending neonates in either trial; NR females were not reproductive and, thus, were not attending neonates in either trial. Above-bar p-Values correspond to within-trial G-tests; significant p-Values are in bold.

behaviors (e.g., all but one female thrashed or body-bridged during Trial 1), similar to non-reproductive females. After attending females were no longer in the presence of neonates, they appeared to escalate predator defense, whereby they engaged the predator quickly and displayed more aggressive than warning behaviors. This change in behavior was not observed in post-parturient females who did not have neonates present during either trial, nor was it observed in non-reproductive females.

Two previous studies quantified antipredator behavior in attending pitvipers, but differences in experimental design and methodology make direct comparisons difficult. A study on pygmy rattlesnakes (*Sistrurus miliarius*) found that a significantly larger proportion of attending females exhibited a response to an ophiophagous snake (black racer, *Coluber constrictor*), compared with non-reproductive females (Greene et al. 2002); however, behavior categories were general (i.e., 'response' was defined as movement other than tongue-flicking, including 'any head movement'), and their design did not address the potentially confounding effect of reproductive state. Similarly, a study on prairie rattlesnakes (*Crotalus viridis*) found that when approached by a surrogate predator (a human), attending females abandoned crypsis more quickly (i.e., began rattling and moving) and were less likely to escape to a refuge compared with their pre-parturient behavior (Graves 1989), but again, the direct effect of neonate presence could not be separated from the effect of reproductive state (i.e., pre- vs. post-parturient).

Controlling for the effect of reproductive state is a critical step in addressing the antipredator behavior of attending females. Due to the physiological costs associated with viviparity, post-parturient females might be either less capable of engaging in or less willing to engage in energetically expensive antipredator behavior. Specifically, a large reduction in mobility and appetite suppression during pregnancy can result in anorexia during the reproductive cycle (Macartney & Gregory 1988; Bonnet et al. 1998; Lourdaï et al. 2002; Crane & Greene 2008; Webber et al. 2012; but see Schuett et al. 2013); in conjunction with the metabolic demands of gestation, anorexia can lead to post-parturient emaciation (e.g., Madsen & Shine 1993). The intense protein catabolism and musculature loss that occurs during pregnancy has been shown to significantly reduce the physical performance of post-parturient snakes (Lourdaï et al. 2004), a consequence of which might be a reduction in antipredator behavior until a positive energy state is achieved. In our study, both groups of post-parturient females had a lower mean aggressiveness score than non-reproductive females during both trials (although not all mean differences were statistically significant), suggesting that reproductive state may influence antipredator behavior to some extent. However, during Trial 1, all MA and SE females were 6-d post-parturient, but differed in the presence or absence of neonates, respectively, so the significantly lower aggressiveness score in MA relative to SE females can be attributed to the effect of neonate presence, rather than reproductive state.

The reduced antipredator behavior of MA females attending neonates broadly corresponds to what has been observed in other pitvipers. Although both Greene et al. (2002) and Graves (1989) found that general antipredator behavior increased after parturition, the behavior of attending females was not particularly aggressive, as only 3 of 12 *S. miliarius* struck at the predator and all five *C. viridis* simply rattled. Furthermore, most anecdotal observations of MA of neonates in various pitviper species noted that the mother either remained still or was not exceptionally aggressive when approached by a human observer (reviewed in Greene et al. 2002). If attendance of neonates in pitvipers is adaptive in terms of offspring defense, reduced maternal antipredator behavior in the presence of neonates seems counterintuitive; however, the optimal level of defense during a particular encounter undoubtedly is influenced by multiple factors. For example, it is possible that attending mothers engage in less conspicuous antipredator behavior in an effort to maintain crypsis. Not only do

pitvipers rely heavily on crypsis for both ambush foraging and predator avoidance, but also pregnant pitvipers choose birth sites that are not easily detected (e.g., subterranean burrows, rock crevices; reviewed in Greene et al. 2002). As such, attending mothers might be hesitant to mount an active defense and opt for less conspicuous warning behaviors once they do to delay the predator from locating the birth site and allow exposed neonates the opportunity to take refuge. In support of this hypothesis, Graves (1989) noted that neonates quickly retreated into a refuge while their mother was rattling at the human 'predator', and numerous similar observations have been made in other pitviper species (reviewed in Greene et al. 2002). Also, the pattern of decreased or delayed parental antipredator behavior with increasing nest crypsis has been well-documented in avian species (e.g., Ricklefs 1977; Montgomery & Weatherhead 1988; Albrecht & Klavaňa 2004).

Alternatively, reduced antipredator behavior in attending females might not function to increase offspring survival, and if not, selective factors other than predator deterrence might better explain the ubiquity of parental behavior in temperate pitvipers. For example, during the attendance period, neonates undergo their first shed cycle, which requires substantial thermoregulation and hydoregulation. The postnatal shed functions to replace the highly permeable embryonic skin with thicker skin (Tu et al. 2002), so neonates must successfully complete this process to prevent desiccation. One way in which snakes can achieve the physiological requirements of shedding is by aggregating with conspecifics, which effectively reduces their surface area-to-volume ratio and thus stabilizes body temperature, while retarding cutaneous water loss (Graves & Duvall 1987; Lillywhite 1987; Reiserer et al. 2008). As aggregation size increases, via more or larger individuals, so does the thermal inertia of that aggregation (Aubret & Shine 2009). In our study, the average post-parturient mass of mother cottonmouths (mean \pm SD: 287 \pm 44.9 g) was almost twenty times that of neonates (mean \pm SD: 14.9 \pm 2.7 g), and given the small litter sizes (2–8 neonates), there was potential for neonates to realize considerable physiological benefits by aggregating with their large-bodied mother until ecdysis was completed. Further, aggregating with related individuals post-birth appears to be an important component in the development of adaptive sex-specific and kin-directed affiliative behavior in cottonmouths (S. K. Hoss, D. H. Deutschman, W. Booth & R. W. Clark, under review). In the current study, observed mothers and neonates aggregating throughout the

attendance period and this behavior appears to be the predominant characteristic of MA of neonates in other pitvipers (Greene et al. 2002).

It is possible that, had we presented cottonmouths with a more substantial threat than a restrained king snake, attending females would have exhibited elevated antipredator behavior. Even though our predator presentation was enough to elicit the full suite of antipredator behaviors observed in cottonmouths (Gibbons & Dorcas 2002; Roth & Johnson 2004; Glaudas et al. 2006; Glaudas & Winne 2007), a freely moving predator might have represented a more realistic or immediate threat; however, the trade-off would have been the inability to standardize the predator's behavior. Also, the controlled laboratory setting of our study limits our ability to speculate on how effective the different levels of antipredator behavior we observed might be at protecting offspring. For example, because trials were conducted only when neonates were in a refuge (hide box) and, typically, in an aggregation behind the mother, there was no place for neonates to further retreat. As such, even though the results of our controlled experiment demonstrate that offspring presence had a direct significant effect on maternal antipredator behavior, we cannot conclude that increased offspring survival via maternal defense was a probable selective factor leading to the evolution of MA in pitvipers. For this reason, we feel that the results of our study deserve further empirical investigation across a wider range of species and in more natural settings, where the efficacy of different antipredator strategies can be assessed. Because New World pitvipers exhibit facultative MA of young, which has been subsequently lost in tropical species, they are an ideal group for continued investigations regarding the evolution and adaptive value of MA.

Acknowledgements

All work was approved by the Institutional Animal Care and Use Committee of San Diego State University (Protocol # APF09-03-010C and 09-08-029C). Funding was provided by the San Diego State University Research Foundation. We thank P. Bryant and B. Bonano for their generous help with snake arena construction; S. Graham for assistance in the field; M. Dailey and J. Knowles for access to field sites; C. Romagosa, M. Williams, J. Graham, and S. Graham for housing during field work; and C. Guyer and M. Wines for access to the animal holding facility at Auburn University.

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