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# Behavioral Responses By Cottonmouths (*Agkistrodon Piscivorus*) To Chemical And Visual Predator Cues

Kristen Lee Kohlhepp

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**BEHAVIORAL RESPONSES BY COTTONMOUTHS (*AGKISTRODON*  
*PISCIVORUS*) TO CHEMICAL AND VISUAL PREDATOR CUES**

A Masters Thesis

Presented to

The Graduate College of  
Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Natural and Applied Sciences, Biology

By

Kristen Lee Kohlhepp

May 2016

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Biology

Missouri State University, May 2016

Master of Science

Kristen Lee Kohlhepp

**ABSTRACT**

Predator detection and assessment of predation risk have important survival consequences for animals. However, responses to predatory threats can vary with different stimuli. Chemical cues are important for predator recognition but visual cues may elicit defensive responses. Cottonmouths (*Agkistrodon piscivorus*) exhibit an array of antipredatory behaviors that have been thoroughly characterized in response to human aggressors, but their responses to cues from other predators are poorly known. I conducted three experiments to evaluate cottonmouth responses to visual and chemical stimuli from predators and non-predators. Snakes exposed to predator chemical cues exhibited elevated tongue-flick rates compared to controls but did not perform any antipredatory behaviors. Snakes exposed to mobile silhouettes of predators and non-predators performed significantly more defensive displays in response to red-tailed hawk models than controls. However, snakes exposed to visual models of terrestrial predators and non-predators exhibited elevated responses to taxidermed mink and muskrat compared to an inanimate object of similar size and color. My results are consistent with risk-sensitivity predictions in that cottonmouths can use chemical cues to recognize predators and use visual cues to distinguish predatory bird silhouettes from those of non-predatory birds. However, snakes generalized their antipredator responses to stationary mammals based on visual cues alone.

**KEYWORDS:** threat-sensitivity, chemical detection, visual detection, anti-predator response, behavior, snake, cottonmouth, *Agkistrodon piscivorus*

This abstract is approved as to form and content

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Brian Greene, PhD  
Chairperson, Advisory Committee  
Missouri State University

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## TABLE OF CONTENTS

Introduction.....	1
Methods.....	8
Study Animals.....	8
General Experimental Design.....	8
Experiment 1: Behavioral Response to Chemical Stimuli .....	10
Experiment 2: Behavioral Response to Visual Stimuli.....	13
Statistical Analyses .....	14
Results .....	18
Behavioral Response to Chemical Stimuli .....	18
Behavioral Response to Visual Stimuli: Aerial .....	18
Behavioral Response to Visual Stimuli: Ground.....	19
Discussion.....	36
References.....	41

## LIST OF FIGURES

Figure 1. Testing arena set-up for chemical trials.....	16
Figure 2. Testing arena set-up for visual trials .....	17
Figure 3. Percentage of individuals that fed during chemical trials .....	20
Figure 4. Mean latency to feed during chemical trials.....	21
Figure 5. Mean latency to cross midpoint during chemical trials.....	22
Figure 6. Mean handling time during chemical trials.....	23
Figure 7. Mean tongue-flick rate during chemical trials.....	24
Figure 8. Number of individuals that performed a defensive display during visual aerial trials .....	25
Figure 9. Number of individuals that performed a defensive posture during visual aerial trials .....	26
Figure 10. Number of individuals that maintained eye contact with the stimulus during visual aerial trials .....	27
Figure 11. Mean time spent in the shelter during visual aerial trials .....	28
Figure 12. Mean time spent freezing during visual aerial trials .....	29
Figure 13. Mean time spent in defensive posture during visual aerial trials .....	30
Figure 14. Number of individuals that displayed a defensive posture during visual terrestrial trials .....	31
Figure 15. Number of individuals that maintained eye contact with the stimulus during visual terrestrial trials.....	32
Figure 16. Mean time spent in the shelter during visual terrestrial trials .....	33
Figure 17. Mean time spent freezing during visual terrestrial trials .....	34
Figure 18. Mean time spent in a defensive posture during visual terrestrial trials .....	35

## INTRODUCTION

Predation risk influences the behavior and ecology of most animal species. For predation to be successful, a sequence of events must be implemented where prey is encountered, detected, identified, approached, subjugated, and consumed (Endler 1986). As a consequence of the selection pressures of predation, prey species have evolved an array of warning signals and antipredatory strategies that increase their survival chances (Lima and Dill 1990; Glaudas et al. 2005). Antipredatory adaptations interfere with one or more stages of the predation sequence and reduce the chances that a predation event will go to completion (Endler 1986; Lima and Dill 1990; Brodie et al. 1991). For prey that are noxious or possess a physical defense, warning signals may be used as a first line of defense to discourage a predator from pursuing a prey individual (Glaudas and Winne 2007). Some examples of warning signals include aposematic traits, tail vibration in rattlesnakes, and stotting in springbok (Pitcher 1979; Stuart-Fox et al. 2003). Crypsis and immobility are effective in reducing detection by predators. Once prey are detected, rapid escape, active defensive behaviors, and/or chemical deterrents are employed to repel predation attempts (Endler 1986; Greene 1988; Brodie et al. 1991; Kraemer and Adams 2013). The type of antipredator defense that an individual utilizes will depend upon the species, age, and sex of prey and often multiple defense mechanisms may be adopted (Endler 1991). However, defense mechanisms effective on one predator may not be effective against another and some defenses may be deployed at different stages of the predation sequence (Bateman et al. 2014).

Prey should benefit by recognizing predators and distinguishing them from non-predators to avoid wasting time and energy reacting to non-threatening stimuli (Endler 1986; Mathis and Vincent 2000; Glaudas et al. 2005). Prey that fail to recognize a significant predatory threat reduce their chances of survival while those that exhibit high sensitivity to minor threats may compromise fitness through lost foraging or mating opportunities. The threat-sensitivity hypothesis suggests that natural selection should promote scaling of antipredator responses to the level of threat posed by a particular organism or situation (Helfman 1989; Gonzalo et al. 2008). For instance, threespot damselfish (*Stegastes planifrons*) adjust their behavior according to the level of threat perceived so that the magnitude of avoidance responses varied with size, proximity, and posture of predatory trumpetfish (*Aulostomus maculatus*) (Helfman 1989). Threat sensitivity may not be exhibited by all types of cues as demonstrated by newt larvae that distinguished between predatory and nonpredatory chemical cues, but not predatory and nonpredatory visual cues (Mathis and Vincent 2000). Predicting how an organism will respond to a predator is not always straightforward since responses may vary with age, physiological state, environmental factors (Roth and Johnson 2004) and experience (Glaudas 2005; Crane et al. 2012).

Prey can recognize predators using a variety of cues corresponding to different sensory modalities. Chemical detection of predators is particularly important when visual cues are not available, such as when predators are nocturnal, cryptic, or encountered in turbid water (Kats and Dill 1998). Chemical assessment may lead to conserved rationales, such as avoidance of an area because of the persistence of a particular cue (Amo et al. 2006). However, lingering chemical cues may also benefit prey by providing information

about where a predator has been, which is useful for evaluating predation risk associated with habitats containing or lacking predator scents (Downes and Shine 1998a; Downes and Shine 1998b; Kats and Dill 1998; Webb et al. 2009). Visual cues, such as body size and activity, are commonly utilized by prey organisms to assess predation risk (Scudder and Chiszar 1977; Fine 1999; Chivers et al. 2001; Cooper and Sherbrooke 2010). For instance, if the potential threat is moving toward a prey organism, greater threat may be perceived than if the potential threat was stationary. Mammals and birds are relatively proficient at recognizing predators by shape, whereas reptiles often rely on size, speed, activity pattern, and the presence of eyes (Fine 1999; Chiszar et al. 2009; Cooper and Sherbrooke 2010). For instance, western fence lizards (*Sceloporus occidentalis*) responded equally to a kestrel silhouette and a large circle, but only when the objects were moving at a particular speed, suggesting that a combination of motion and size influences threat perception more than the shape of a predator (Fine 1999). Black iguanas (*Ctenosaura similis*) exhibit a shorter latency to flee from humans with larger eye masks than those with smaller eye masks, and flee sooner from individuals with eyes compared to individuals lacking eyes (Burger et al. 1991; Burger et al. 1993).

Many amphibians and reptiles use odor as a primary source of predator recognition (Bevelander et al. 2006; Cooper and Secor 2007; Chiszar et al. 2009; Crane et al. 2012). Being able to distinguish between predators and non-predators is essential to increase the probability of survival (Downes 2002). For instance, plethodontid salamanders have been known to avoid substrates where a predator odor is detected (Crane et al. 2012). Avoidance behavior has also been reported in the velvet gecko (*Oedura lesueurii*), which will select cold retreat-sites over thermally favorable retreats

containing predator odor (Downes and Shine 1998b). Snake responses to predator odors typically include increased tongue flicking, delayed movement, avoidance, and defensive postures (Kats and Dill 1998; Cooper and Secor 2007).

Many organisms use visual cues to detect predators; this has been observed across an array of species. Past studies have found that the precise shape of the predator stimulus is not as important as movements, orientation, and elevation of the stimulus (Fine 1999; Cooper et al. 2007). Neonate hognose snakes (*Heterodon platirhinos*) remained immobile longer when in the presence of a direct gaze rather than an averted gaze (Burghardt and Greene 1988). Cryptic frogs (*Craugastor* spp.) rarely attempted escape when approached horizontally but jumped more frequently when a stimulus was presented diagonally or from directly above (Cooper et al. 2007). White-footed mice (*Peromyscus leucopus*) and meadow voles (*Microtus pennsylvanicus*) only exhibited flight responses to aerial silhouettes that were moving compared to the same silhouettes that were not moving (Bildstein and Althoff 1979).

Not all species can assess predation-risk levels. Some species demonstrate a generalized predator recognition with individuals responding to both dangerous and non-dangerous predators equally. This is known as the predator recognition continuum hypothesis (Ferrari et al. 2007). Natterjack toads (*Bufo calamita*) demonstrate this type of behavior and will avoid all snake odors rather than just those of snakes that prey upon toads (Gonzalo et al. 2008). Velvet geckos (*Oedura lesueurii*) exhibit similar behavior and will display antipredatory behaviors to both harmless and dangerous snake species (Webb et al. 2009; Webb et al. 2010). Young corn snakes (*Pantherophis guttatus*) also do not respond differently to predatory and to nonpredatory odors (Weldon et al. 1990).

Studies that evaluate the suite of cues that an organism might utilize to detect and avoid predators can provide greater insight into prey perception than those evaluating a single cue. Frequently, differences in responses to chemical and visual cues depend on ambient lighting. For example, in low light conditions the prey animal should rely on chemical stimuli more than visual stimuli and vice versa (Amo et al. 2006; Mathis and Vincent 2000). Studies comparing the relative importance of visual and chemical stimuli have reported contrasting results. For example, wall lizards (*Podarcis muralis*) elicit greater antipredator responses to chemical cues rather than visual cues (Amo et al. 2006). The eastern indigo snake (*Drymarchon couperi*) relies more on visual cues than chemical cues during predator recognition (Saviola et al. 2011).

When a predator is detected, prey are likely to be distracted from carrying out normal activities or to be dissuaded from exploiting resources such as food or shelter (Lima and Dill 1990). Evidence for the importance of predator avoidance has been observed across a wide array of taxa and conditions. For instance, white tailed deer (*Odocoileus virginianus*) and woodchucks (*Marmonta monax*) will avoid predator odors by foraging in other areas, whereas novel odors, such as humans, do not affect their feeding behavior (Kats and Dill 1998). Thus, evaluating prey foraging behavior in the presence of predator stimuli provides a powerful opportunity for assessing threat perception by prey species. It is common for predator perception experiments to examine trade-offs between predation risk and resource acquisition to understand an organism's priorities and compare threat sensitivity levels to different possible predators.

Cottonmouths (*Agkistrodon piscivorous*) are semi-aquatic pit vipers found throughout the southeastern United States in many low elevation aquatic habitats (Burkett

1966; Gloyd and Conant 1990). Cottonmouths prey on small mammals, fish, amphibians, lizards, birds, other snakes, and some insects (Burkett 1966). Predators of cottonmouths include large birds (hawks, egrets, storks, eagles, and horned owls), alligators, snapping turtles, other snakes (king snakes and indigo snakes), large predatory fish (gars, bullheads, largemouth bass), various carnivorous and omnivorous mammals (opossums, raccoons, mink, canines, felines, and domestic swine), and humans (Burkett 1966; Knight and Erickson 1976; Ernst 1992). Cottonmouths exhibit a very specific defensive behavior toward king snakes known as the ophiophage defensive response (Carpenter and Gillingham 1975; Weldon and Burghardt 1979; Gutzke et al. 1993; Hoss and Clark 2014). No previous studies have examined cottonmouth responses to natural predators other than kingsnakes.

In addition to predator avoidance, cottonmouths exhibit a suite of defensive displays such as biting, mouth gaping, musk release, and tail vibration during encounters with predators, making them an important model organism for studying anti-predatory behavior (Greene 1988; Glaudas et al. 2005). A variety of experiments using mock predatory encounters with human predators have thoroughly characterized cottonmouth antipredatory behaviors and elucidated sources of variation. When initially approached by humans, or other large predators, cottonmouths typically either retreat or stand their ground while exhibiting an alert demeanor with head elevated or laying on its coiled body (Gutzke et al. 1993; Gibbons and Dorcas 2002). Active antipredatory behaviors are initiated when a predatory threat increases. For instance, physical contact with the predator elicits defensive displays, and responses escalate to biting and musk secretion when the snake is grasped (Gibbons and Dorcas 2002). Antipredatory responses vary

ontogenetically, with smaller individuals being more aggressive than adult conspecifics, likely because smaller individuals experience greater predation risk than larger individuals that are more capable of defending themselves (Roth and Johnson 2004; Glaudas et al. 2005).

I examined differences in anti-predator responses displayed by the western cottonmouth when subjected to visual and chemical cues representing different potential predators. The goal of my study was to determine whether cottonmouths exhibit threat sensitivity when exposed to visual and chemical cues from predators and non-predators. I predicted that avoidance behaviors such as seeking shelter, reduced movement, reduced foraging, and elevated tongue-flick rates would be performed when presented with a predator odor. In accordance with the threat sensitivity hypothesis, I hypothesized that cottonmouths should elevate their antipredator responses when they feel more threatened. I predicted that mobile, visual, aerial cues would elicit animated behavioral response such as coiling, gaping, and tail vibration, which has been observed in past studies where snakes were confronted with human predators (Gibbons and Dorcas 2002; Roth and Johnson 2004; Glaudas et al 2005). I also predicted that stationary terrestrial models would not elicit aggressive defensive behaviors, as has been shown in rattlesnakes (Scudder and Chiszar 1977).

## METHODS

### Study Animals

I conducted my experiments using 19 juvenile cottonmouths that were born in the lab to five gravid females from a single population in southwestern Missouri. All individuals were approximately 21 months of age with mean length and mass of  $37.50 \pm 0.60$  cm (SVL) and  $65.50 \pm 2.25$  g, respectively, when testing began. Snakes were individually housed in the Missouri State University vivarium from birth and individually housed in  $42 \times 30 \times 22.5$  cm polycarbonate enclosures with newspaper substrate, a shelter made of varying sizes of bisected PVC pipe, and a clear plastic water bowl. Snakes were maintained on a 12L:12D photoperiod at an ambient temperature of 24–25 °C and were fed two to three small (1.50 g–2.62 g) live minnows (mostly *Pimephales* sp.) on a 10-day feeding cycle. Water was provided ad libitum. Experimental procedures in this study involving animals were approved by the Missouri State University Institutional Animal Care and Use Committee (March 2015, Protocol 15-022).

### General Experimental Design

I conducted experiments to evaluate behavioral responses of cottonmouths to chemical and visual stimuli. For the chemical experiment I assessed feeding responses in the presence of predator and non-predator chemical cues. Because the survival risk posed by predation threat is likely to distract prey from carrying out other essential tasks, the presentation of predator stimuli during feeding events provides a logical way to assess threat perception by prey (Lima and Dill 1990; Churilla 2015). To evaluate responses to

chemical cues from predators, I presented cottonmouths with chemical stimuli from two mammalian snake predators: domestic dogs (*Canis familiaris*) and domestic swine (*Sus scrofa*). Canids are historically important snake predators throughout the range of the cottonmouth (Gans and Huey 1988; Ernst 1992), whereas domestic swine are opportunistic omnivores that consume snakes (Gans and Huey 1988; Wilcox and van Vuren 2009) but have only appeared in southwestern Missouri recently (Hartin et al. 2007). I expect that innate predator responses may be different between the two predator stimuli because of the different history of interactions. Ringed crayfish (*Orconectes neglectus*) were used as a non-predator cue because crayfish are neither a predator nor a prey item for cottonmouths. Dechlorinated tap water served as an odorless, blank control.

I evaluated responses to visual stimuli to evaluate threat perception by cottonmouths from terrestrial and aerial predators. In each context, I compared snake behavioral responses to stimuli representing a predator, a non-predator, and an inanimate object that served as a visual control. For the aerial trials I used silhouette models of a red-tailed hawk (*Buteo jamaicensis*) (36 × 88 cm) and mallard duck (*Anas platyrhynchos*) (36 × 82 cm) and a rectangular piece of acrylic plastic (34 × 78 cm) as a non-animal control stimulus. Silhouettes of each species were traced from to-scale profiles at the Springfield Conservation Nature Center (Springfield, MO). The silhouettes were painted flat black and the red-tail hawk model was scaled-down to the mallard duck model dimensions to control for effects of model size (Fine 1999). The red-tailed hawk is an abundant opportunistic carnivore and is a known snake predator (Fitch 1963; Knight and Erickson 1976; Ernst 1992). The mallard duck was chosen as a non-predator model because of its association with wetlands, relative body size similarity and distinctly

different shape to red-tailed hawks, and omnivorous diet composed of plants and invertebrate animals. Mobile aerial models of animals have reportedly elicited anti-predatory responses in lizards and small mammals (Bildstein and Althoff 1979).

For the terrestrial trials I presented cottonmouths with single taxidermed specimens of a mink (*Neovison vison*) (35 × 11 × 16 cm) or a muskrat (*Ondatra zibethicus*) (22 × 12 × 18 cm). These species were chosen because they inhabit aquatic habitats and are similar in size posture and size. The taxidermy specimens used in this study were specifically chosen because they were both mounted in a standing position with head elevated and a similar body posture. The mink was chosen as a predator model because they are carnivores that include snakes in their diets (Schwartz and Schwartz 2001). The muskrat was chosen as a non-predator because it is primarily herbivorous and does not prey upon reptiles. A log similar in size (38 × 10 × 12 cm) and color to the animal models was chosen as a non-animal control stimulus.

### **Experiment 1: Behavioral Response to Chemical Stimuli**

**Chemical Stimulus Collection and Preparation.** I obtained chemical stimuli from ringed crayfish, domestic dogs, and swine to prepare stimulus solutions for behavioral trials. Canine saliva samples were collected from three domestic dogs by rolling a cotton swab on the inside of each donor's cheek to produce a sample of ~1 ml. Domestic swine urine was purchased (Boarmasters, Chubbuck, ID) and ~1 ml of urine was used per sample. Crayfish stimulus solutions were prepared by collecting water from separate 25 × 10 × 8 cm plastic enclosures that each contained a single crayfish. In an attempt to standardize cue concentrations, I placed each crayfish into a dry container and

added de-chlorinated tap water in a 10:1 water-to-crayfish mass ratio. After 24 hr, I extracted ~1 mL water samples from each container on individual cotton swabs. All 1-ml samples of each stimulus were diluted with 15 ml of dechlorinated tap water and placed in 28.5 × 95 mm polystyrene tubes. Samples were frozen at -18 °C and defrosted to room temperature 4 hr prior to use.

**Testing Protocol.** Chemical stimulus trials were conducted from April to July 2015. To maximize snake feeding responses, I simulated a naturalistic crepuscular foraging situation by conducting trials between 17:00 and 21:00 in low light conditions. The desired light levels were achieved using a portable 50W lamp to cast dim, indirect light on the arenas from 1 m distance in an otherwise dark room. Trials were performed every 10–12 days to standardize snake hunger levels. I minimized acclimation concerns by conducting trials in each experimental subject's home enclosure. A few days prior to trials, I taped newspaper to the floor of the enclosure to prevent snakes from using it as a shelter. Each enclosure contained a clear plastic water bowl and a bisected PVC pipe shelter on opposite ends (Fig. 1). To facilitate assessment of movement behaviors, a midline was drawn across the paper using a red pencil (Bevelander et al. 2006). All trials were video recorded, without myself being present in the testing room, to minimize disturbance to the snakes. Video documents were then analyzed at a later date.

Chemical response trials began with each snake concealed within its shelter. Any snake that was not already in this location was gently coaxed beneath its shelter. Then the shelter and snake were covered with an inverted plastic cup while stimuli were added to the enclosure. The treatment was then placed in a petri dish, uncovered, on the opposite side of the arena (Bevelander et al. 2006). To ensure snakes would encounter cues from

the minnows and the treatment simultaneously, an acrylic platform elevated the water bowl ~ 1 cm above a petri dish containing the treatment stimulus solution. Tap water and two live minnows were then placed in the water bowl. Once the treatment and minnows were in place, I removed the plastic cup restraining the snake, covered the arena with an acrylic sheet, and activated the video camera to record the trials. So that multiple trials could be simultaneously recorded, I grouped four cages into a rectangular array within the field of vision of the video camera. I placed cardboard barriers between enclosures to obscure visual stimuli from adjacent trials. Petri dishes were sanitized using Virkon™ disinfectant (Neogen Corporation) to remove residual odors before use in subsequent trials. Both the order of snake testing and the order of treatments administered to each snake were randomized.

During each 1-h foraging trial I evaluated a series of time-dependent variables including latency to emerge from shelter, cross the midpoint of the enclosure, time to strike at prey, and prey handling time. A snake was considered emerged from the shelter when its head became visible. I also quantified the number of tongue flicks each snake performed until they captured a fish and calculated a mean tongue flick rate. Individuals that did not leave their shelters were retested under the same conditions at a later date. Individuals that did not leave the shelter for three or more of the trials was omitted from data analyses. For trials in which the subject left the shelter but no feeding occurred, the subjects were evaluated for 48-h for signs of illness or ecdysis. If neither was observed, and the snakes fed without the presence of a stimulus, the trial was included in the analysis. Snakes that failed to feed during a trial but then soon entered a pre-shed condition were retested following ecdysis.

## **Experiment 2: Behavioral Response to Visual Stimuli**

I conducted trials assessing responses to visual stimuli from July to August 2015. Trials were conducted between 10:00 and 15:00 h, under bright illumination and 3–4 days after feeding to avoid any recent effects of feeding on locomotion or behavior. I used a 151-L black stock tank ( $68.5 \times 96.5 \times 43$  cm) with a clear acrylic wall positioned vertically across the middle of the tank (Fig. 2) as a testing arena. The snakes were removed from their home enclosures and placed under an inverted black plastic box ( $40 \times 15.5 \times 24.5$  cm) on one side of the arena, where they were acclimated for 10 min. The subject's PVC pipe shelter was placed on the snake side of the arena as a refuge.

For the terrestrial trials the stimulus was placed on the other side of the clear barrier approximately 0.50 m away from the acclimation box, and the acclimation box was then removed from the subject. Aerial models were attached to a pulley system and were suspended 1.5 m above the test arena (Cooper et al. 2007). The pulley system was designed so that the head of the silhouette always pointed in the direction it was flying. The direction of the stimulus changed each time the stimulus flew over the arena (Figure 2). To standardize movement, the individual operating the pulley system would count 4 s across with a 2 s turn around during which the stimulus was out of sight. All trials were carried out for 60 s and were video recorded for later analysis. Following each trial, the arena and acclimation box were cleaned using Virkon™ disinfectant to minimize any chemicals left by the previous subject.

I evaluated snake responses using time-dependent variables during visual trials. These measures included time spent in the shelter, motionless, and in a defensive posture. Defensive posturing was defined as the snake being coiled with its head held in an

s-shaped curve and elevated above the body and toward the stimulus. I also recorded several binary variables during trials. These measures included if the subject performed gaping, tail vibration, or defensive posturing, and if the subject maintained eye contact with the stimulus (Gibbons and Dorcas 2002; Roth and Johnson 2004). If gaping, tail vibration, or both displays were exhibited I categorized it as a defensive display. I grouped gaping and tail vibration together because previous studies have shown that both behaviors are strongly associated with one another (Glaudas et al. 2005; Glaudas and Winne 2007).

### **Statistical Analyses**

I used one-way repeated measures ANOVAs to evaluate treatment differences for all time-dependent variables and tongue-flick rates (Roth and Johnson 2004). Data for both predator chemical cues were included together in the analyses. Prior to analyses, I evaluated data for normality and homogeneity of variance, and transformed data when necessary. All data was log transformed except for time spent in shelter for chemical trials, which was square root transformed. For both visual and chemical analyses, the factors were stimulus and snake identification, with snake identification included as a nested factor under stimulus because the design used repeated measures. Stimulus was set as a fixed factor and snake identification as a random factor. Following significant ANOVAs, pairwise comparisons were analyzed using Tukey's range test. For visual experiment data, I also performed Chi square tests of association for defensive posturing, eye contact with stimulus, and whether tail vibration or gaping was performed to assess binary behavioral data across treatments. Analyses were conducting using Minitab 17

(Minitab Statistical Software). All means are reported  $\pm 1$  SE and all statistical tests were evaluated using a Type I error rate of 0.05.

Effect sizes were calculated to make inferences about "biological significance." This approach allows trends to be recognized when sample sizes are small or p-values are nonsignificant and facilitates comparisons with other studies (Nakagawa 2004; Dugan and Hayes 2012). For Chi square tests, I computed Cramer's V as a measure of effect size. Following Cramer (1999), I considered effect sizes 0–0.1, weak association if between 0.1–0.2, moderate association if between 0.2–0.4, somewhat strong association if between 0.4–0.6, strong association if between 0.6–0.8, and very strong association if between 0.8–1.0. Time-dependent ANOVA effect sizes were estimated with  $\eta^2$  for which values of 0.01, 0.06, and 0.14 were regarded as small, medium, and large, respectively (Cohen, 1988).

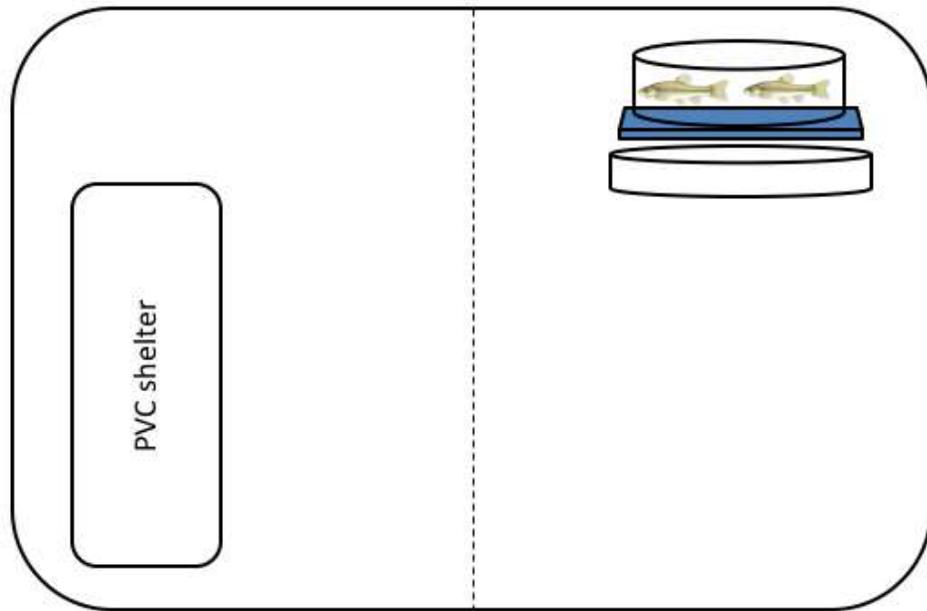


Fig. 1. Chemical stimuli testing arena ( $42 \times 30 \times 22.5$  cm). Dotted line down the center represents the midpoint of the arena. The top dish represents the water bowl where the minnows were held. The rectangle represents the apparatus that held the minnows over the treatment stimulus. The bottom dish represents the petri dish which held the treatment stimulus underneath of the water bowl.

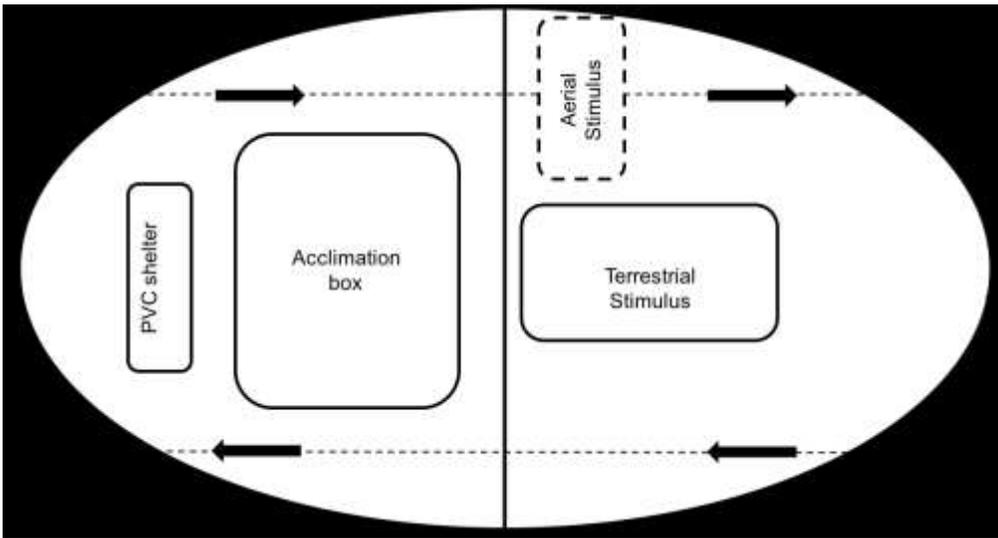


Fig. 2. Visual stimuli testing arena ( $68.5 \times 96.5 \times 43$  cm). The line down the middle represents the Plexiglas™ divider that separated the snake from the stimulus. The dotted line with arrows represents the route of the aerial stimulus (represented by the box with dashed border) during an aerial trial. The direction of the stimulus changed every time the stimulus flew over the arena but the head always remained in the direction the silhouette was flying. Only one stimulus was presented at a time, terrestrial or aerial. The acclimation box was removed once the stimulus was presented.

## RESULTS

### Behavioral Response to Chemical Stimuli

Whether or not the individual snake ate was not significantly different among treatments ( $\chi^2 = 0.69$ ,  $P = 0.71$ ,  $DF = 2$ , Cramer's  $V = 0.09$ ; Fig. 3). Latency to strike fish ( $F_{2,34} = 0.02$ ,  $P = 0.98$ ,  $\eta^2 = 0.006$ ; Fig. 4), latency to cross midpoint ( $F_{2,41} = 0.15$ ,  $P = 0.86$ ,  $\eta^2 = 0.022$ ; Fig. 5), and handling time ( $F_{2,33} = 0.24$ ,  $P = 0.79$ ,  $\eta^2 = 0.179$ ; Fig. 6) were also not significantly different. Effect sizes were weak for whether or not the individual ate, latency to strike fish, and latency to cross midpoint. Handling time had a relatively large effect size. The mean tongue flick rate was significantly greater for predator cues than both non-predator and control cues with a large effect size ( $F_{2,51} = 3.65$ ,  $P = 0.03$ ,  $\eta^2 = 0.324$ ; Fig. 7).

### Behavioral Response to Visual Stimuli: Aerial

The hawk silhouette elicited defensive displays (tail vibrations and gapes) more often than the duck silhouette or acrylic rectangle. A somewhat strong stimulus effect was observed for defensive displays amongst treatments ( $\chi^2 = 12.003$ ,  $DF = 2$ ,  $P = 0.002$ ,  $V = 0.56$ ; Fig. 8). Defensive posturing was significantly influenced by the presence of an avian silhouette, whether it be the hawk or duck, when compared to the clear rectangle ( $\chi^2 = 8.205$ ,  $DF = 2$ ,  $P = 0.02$ ;  $V = 0.379$ ; Fig. 9). Snakes also maintained eye contact with with avian silhouettes significantly more often than with the clear rectangle ( $\chi^2 = 27.502$ ,  $P = 0.0001$ ,  $DF = 2$ ;  $V = 0.694$ ; Fig. 10). There was no significant difference among stimuli in time spent in the shelter ( $F_{2,54} = 0.62$ ,  $P = 0.54$ ,  $\eta^2 = 0.02$ ; Fig. 11) or time spent immobile ( $F_{2,54} = 0.70$ ,  $P = 0.50$ ,  $\eta^2 = 0.02$ ; Fig. 12). However, the difference

in time spent in a defensive posture was marginally non-significant with a medium effect size ( $F_{2,54} = 2.91$ ,  $P = 0.06$ ,  $\eta^2 = 0.09$ ; Fig. 13) with a significantly greater response to the duck silhouette than the clear rectangle (Tukey Test,  $P < 0.05$ ).

### **Behavioral Response to Visual Stimuli: Terrestrial**

The number of snakes performing defensive postures was not significantly influenced by the type of stimulus ( $\chi^2 = 2.462$ ,  $P = 0.29$ ,  $DF = 2$ ;  $V = 0.207$ ; Fig. 14). However, a significantly greater number of snakes maintained eye contact with animal models than with the log ( $\chi^2 = 12.238$ ,  $P = 0.002$ ,  $DF = 2$ ;  $V = 0.463$ ; Fig. 15) with nearly identical responses to the mink and muskrat models. There were no significant differences in snake responses to stimuli for time spent in the shelter ( $F_{2,54} = 0.70$ ,  $P = 0.50$ ,  $\eta^2 = 0.02$ ; Fig. 16), time spent immobile ( $F_{2,54} = 1.12$ ,  $P = 0.34$ ,  $\eta^2 = 0.02$ ; Fig. 17), or time spent in a defensive posture ( $F_{2,54} = 1.13$ ,  $P = 0.33$ ,  $\eta^2 = 0.04$ ; Fig. 18).

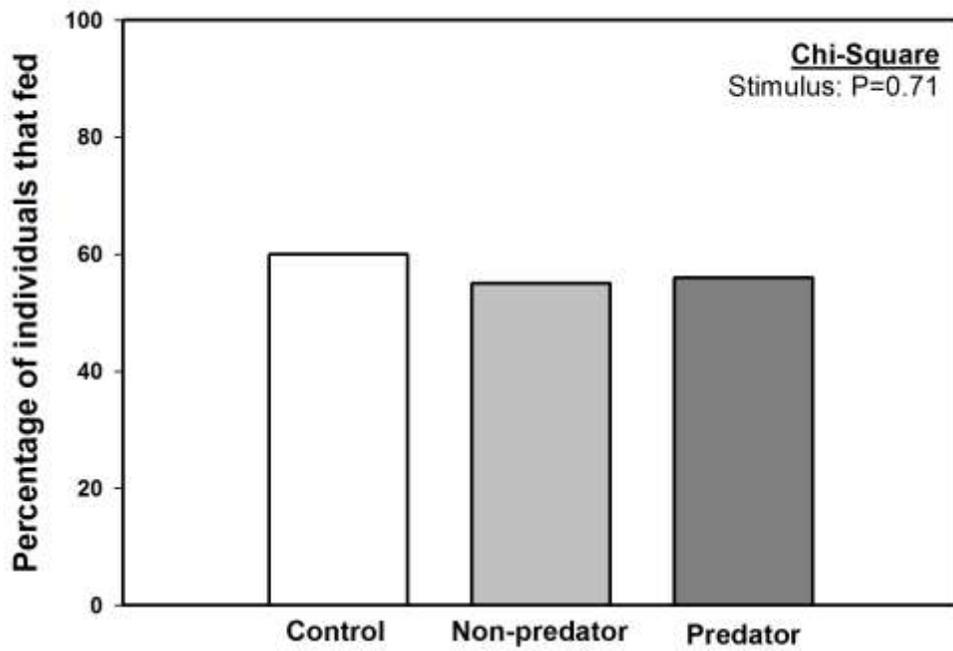


Fig. 3. The percentage of individuals that consumed at least one fish, during the 60-min trial, in the presence of each stimulus.

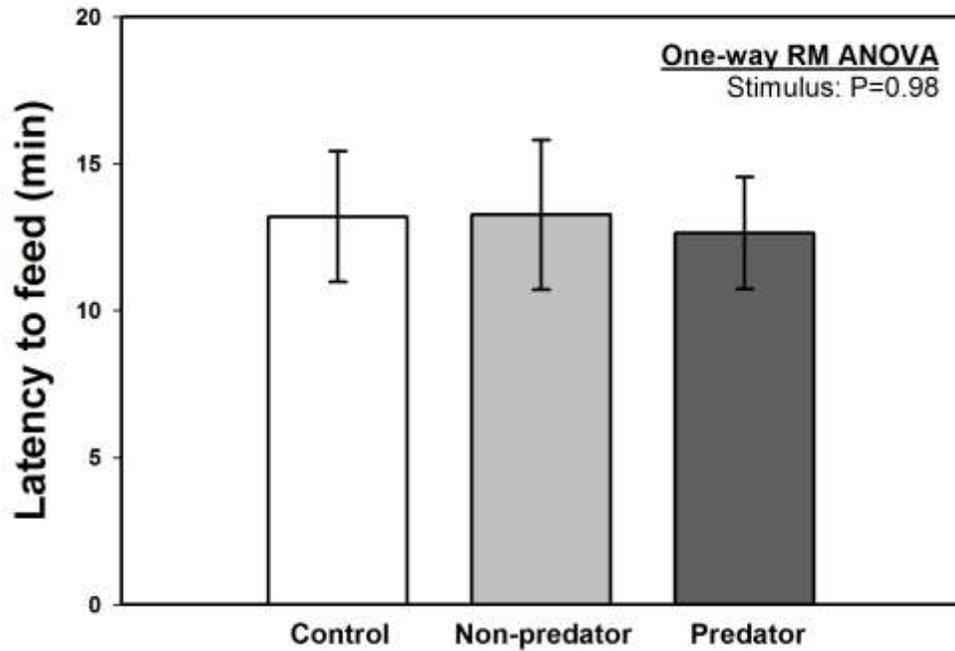


Fig. 4. Mean ( $\pm$  SE) latency to feed on one fish for each treatment in trials where feeding occurred. The control had a sample size of 9 individuals. The non-predator stimulus used was crayfish ( $N = 9$ ). The predator stimulus consisted either of domestic swine or canine stimuli, combined during statistical analysis ( $N = 20$ ).

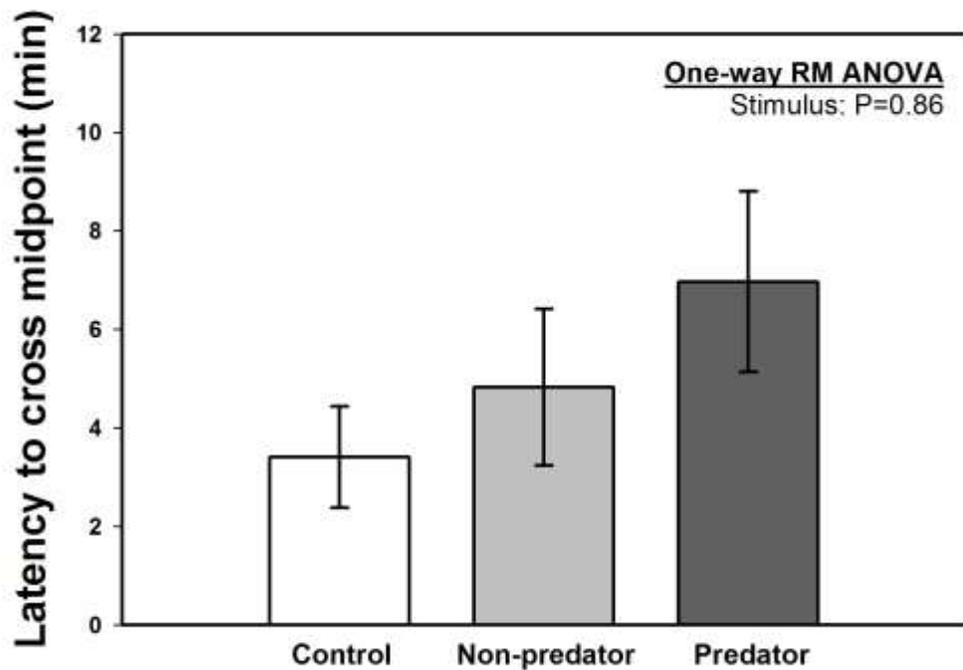


Fig. 5. Mean ( $\pm$  SE) latency to cross midpoint of arena. Sample sizes were not equal among conditions because some snakes did not cross the midpoint. The control had a sample size of 13 individuals. The non-predator stimulus used was crayfish ( $N = 14$ ). The predator stimulus consisted of a pooled sample of responses to domestic swine and canine stimuli, presented separately, but combined during statistical analysis ( $N = 30$ ).

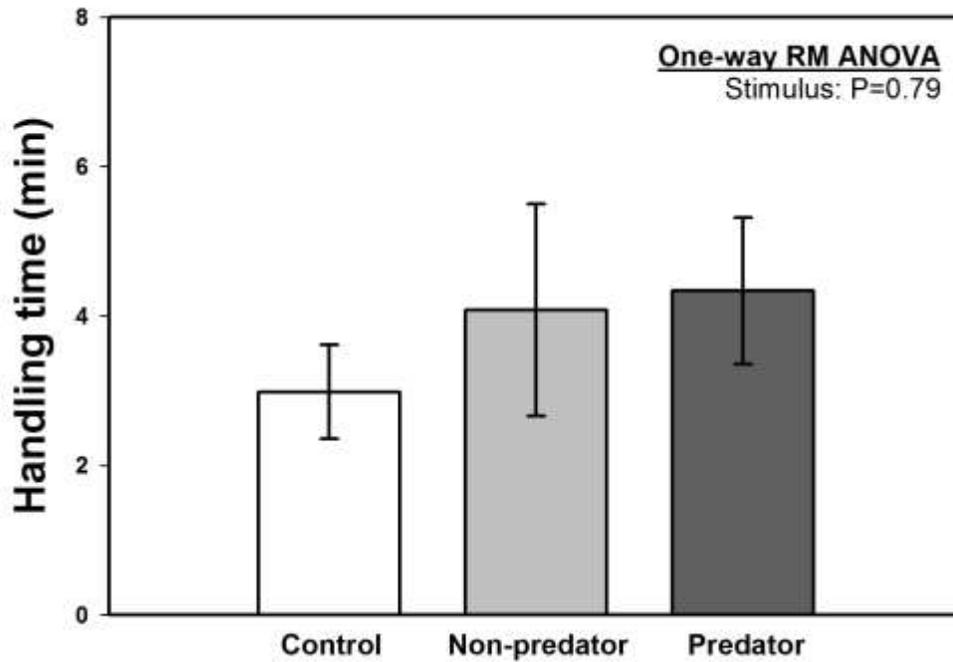


Fig. 6. Mean ( $\pm$  SE) handling time for consumption of one fish for each treatment. Sample sizes were not equal among conditions because some snakes did not feed in all trials. The control had a sample size of 12 individuals. The non-predator stimulus used was crayfish (N = 8). The predator stimulus consisted of a pooled sample of responses to domestic swine and canine stimuli, presented separately, but combined during statistical analysis (N = 20).

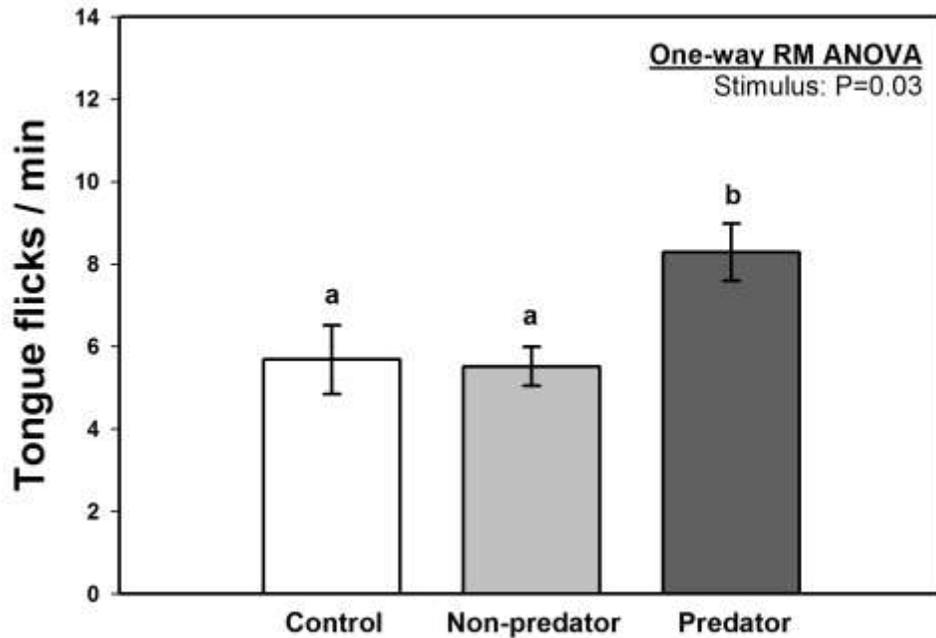


Fig. 7. Mean ( $\pm$  SE) tongue flicks per minute for control predator, and non-predator treatments. The control had a sample size of 12 individuals. The non-predator stimulus used was crayfish (N = 15). The predator stimulus consisted of domestic swine and canine stimuli, presented separately, but combined during statistical analysis (N = 31). Responses labeled with different letters are significantly different.

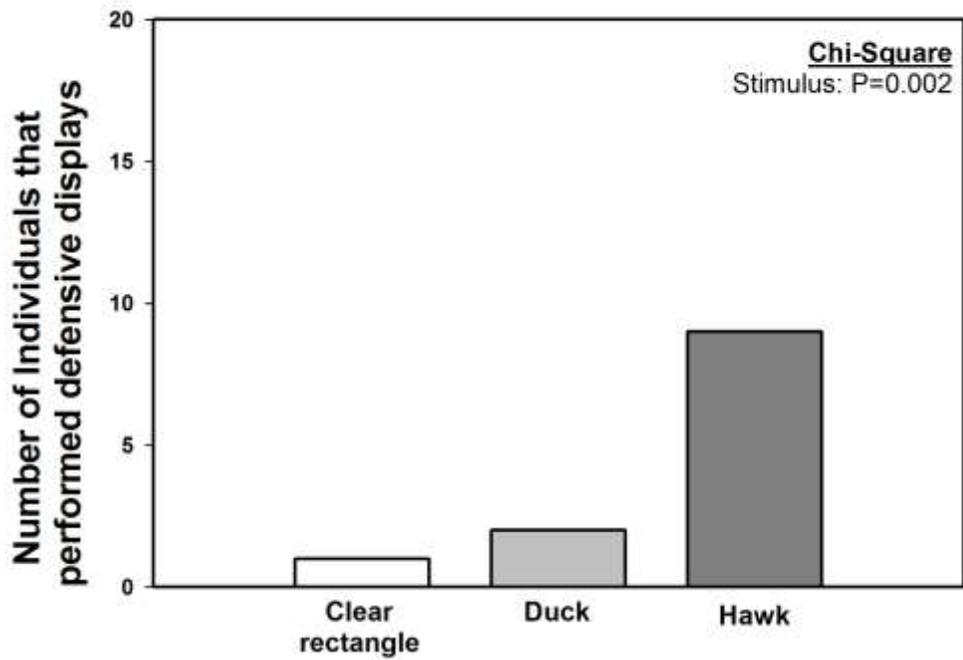


Fig. 8. The number of individuals that performed a defensive display of tail vibration, gaping, or both in response to visual aerial stimuli (N = 19).

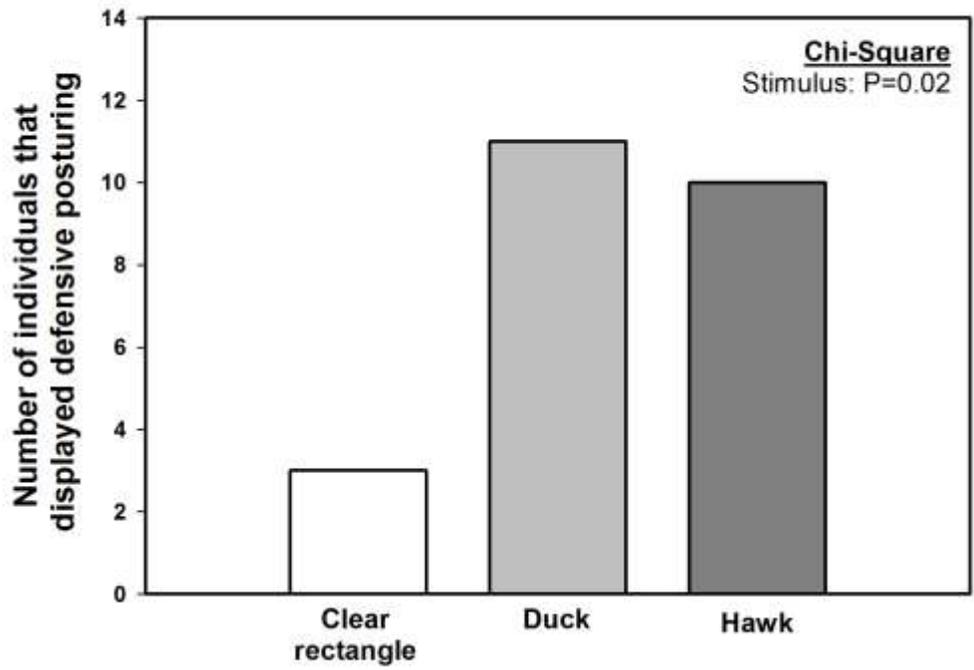


Fig. 9. The number of individuals that displayed defensive posturing for each stimulus (N = 19).

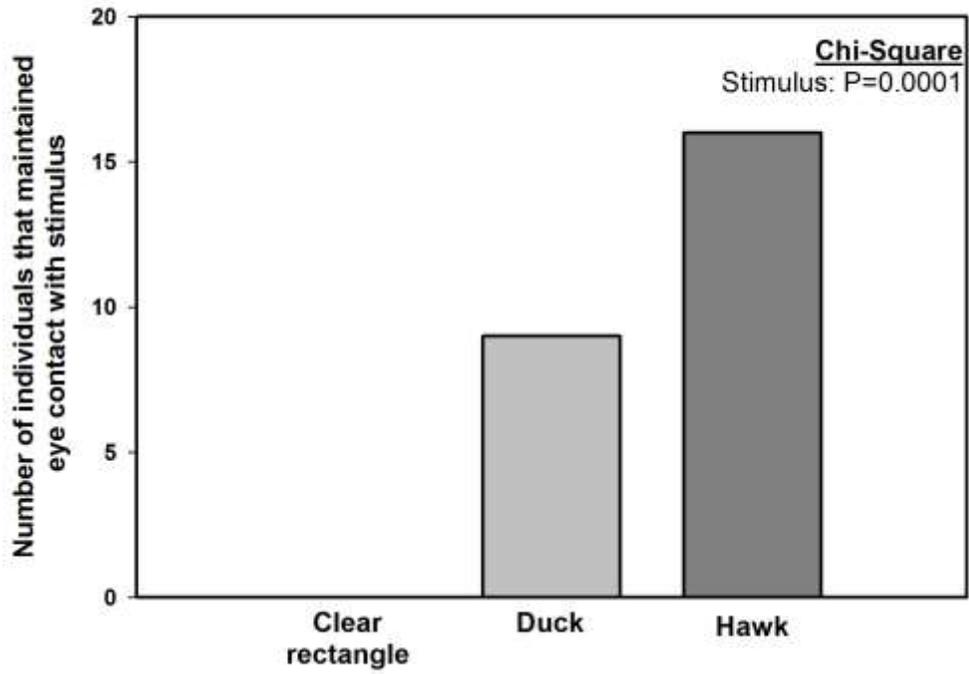


Fig. 10. The number of individuals that maintained eye contact with each stimulus (N = 19).

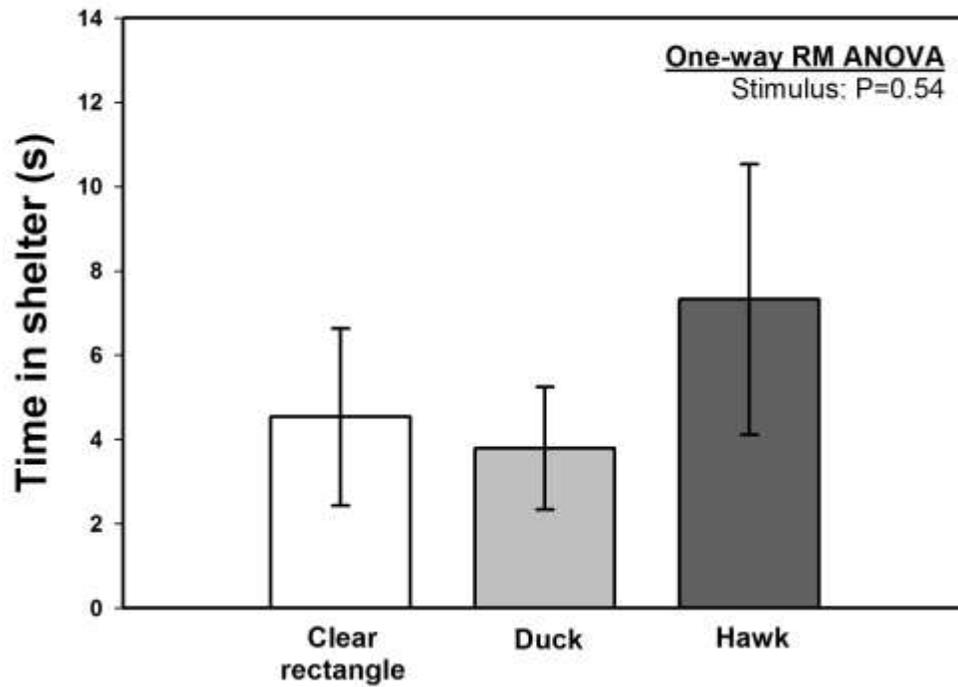


Fig. 11. Mean ( $\pm$  SE) time spent in shelter, in seconds, for each stimulus (N = 19).

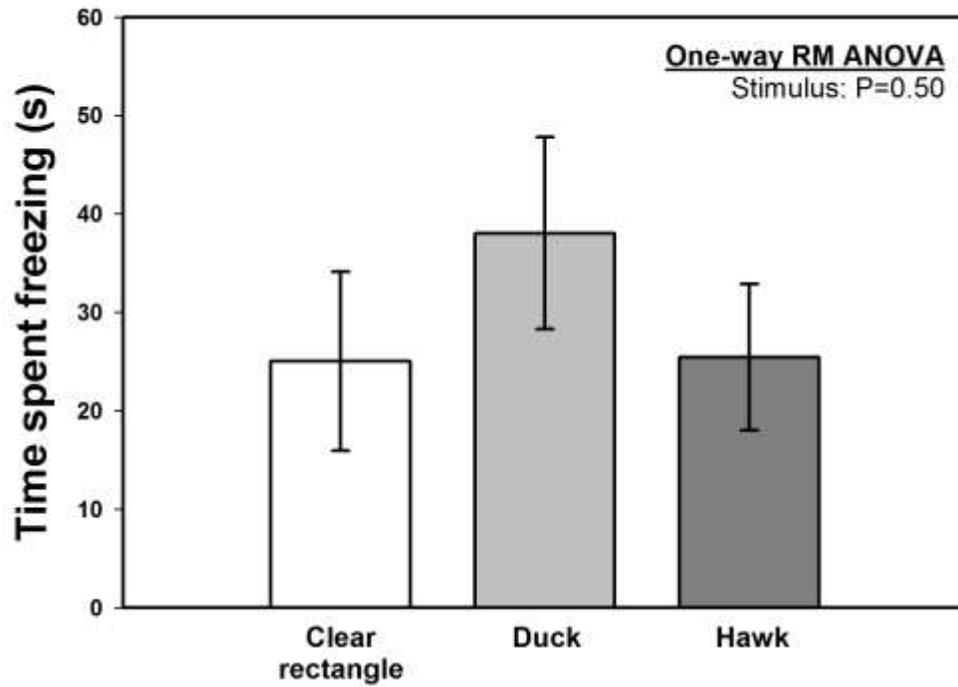


Fig. 12. Mean ( $\pm$  SE) time spent motionless, in seconds, for each stimulus (N = 19).

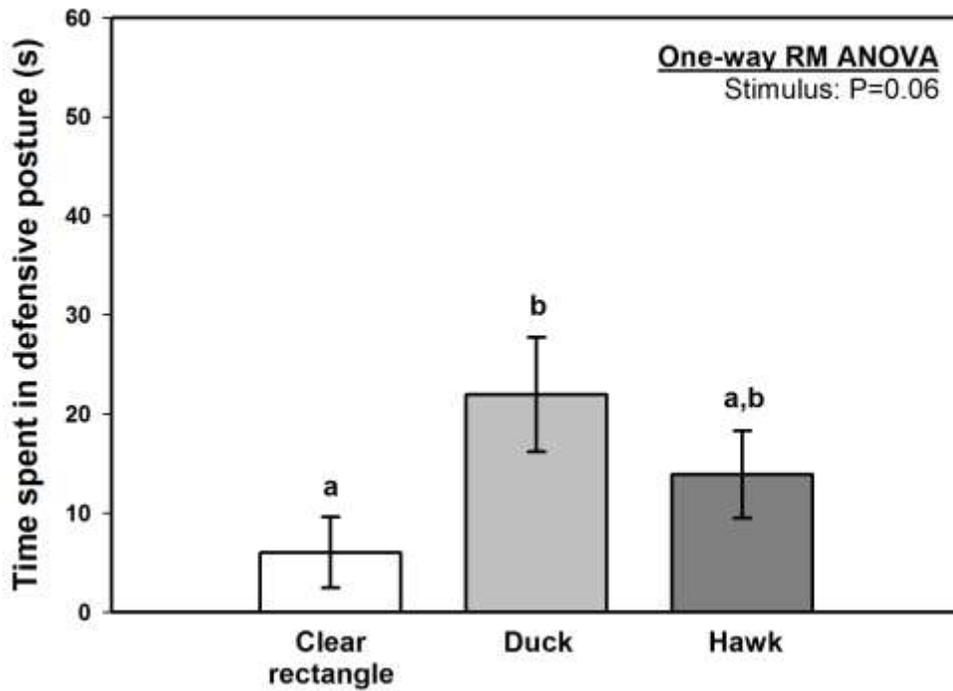


Fig. 13. Mean ( $\pm$  SE) time spent in defensive posturing, in seconds, for each stimulus (N = 19). Treatment responses bearing the same letters are not significantly different.

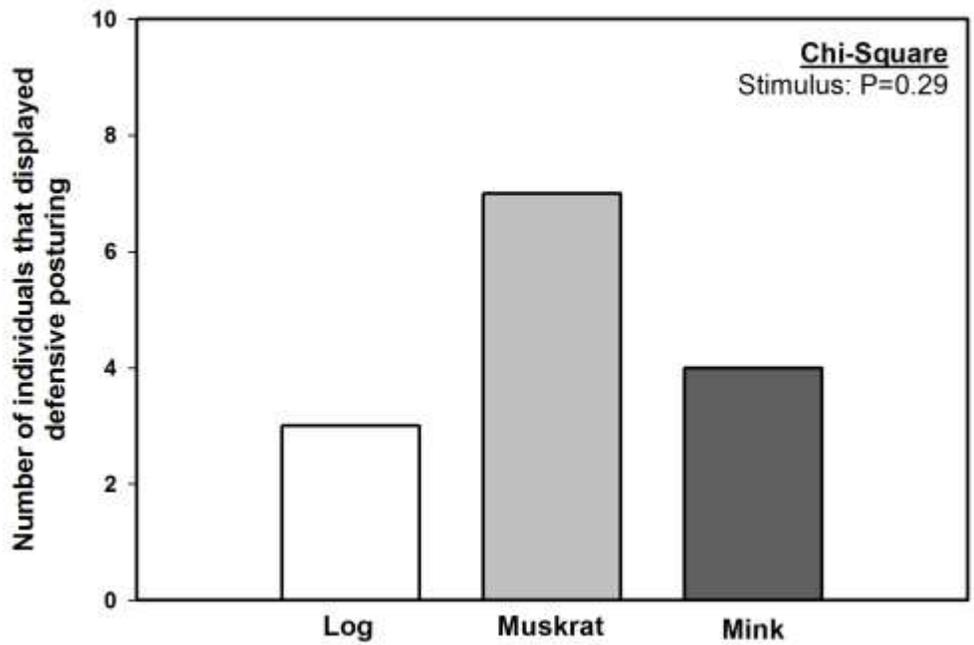


Fig. 14. The number of individuals that displayed defensive posturing for each stimulus (N = 19).

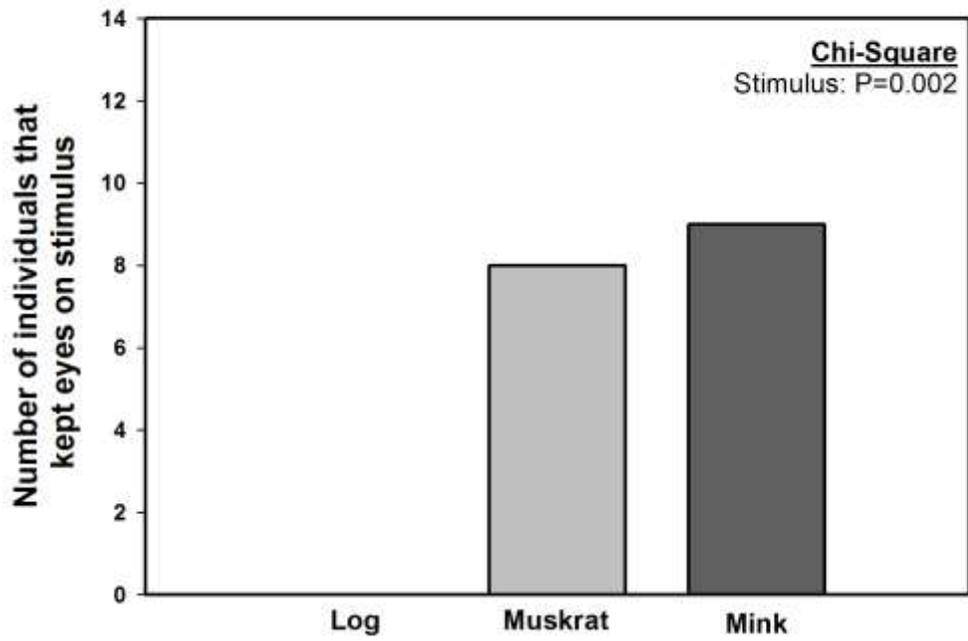


Fig. 15. The number of individuals that kept their eyes directed toward each stimulus (N = 19).

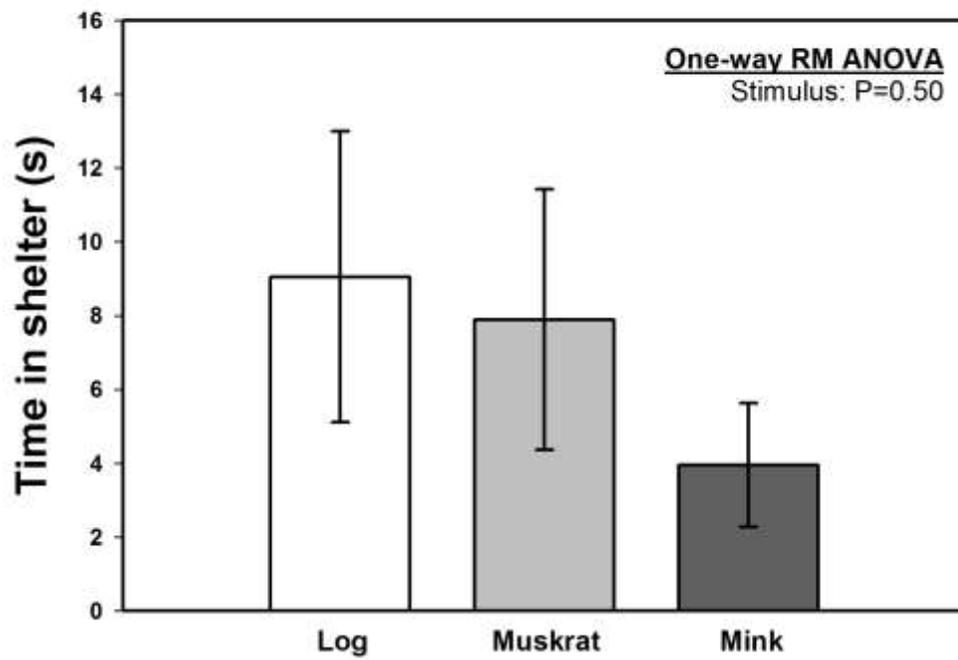


Fig. 16. Mean ( $\pm$  SE) time spent in shelter, in seconds, for each stimulus (N = 19).

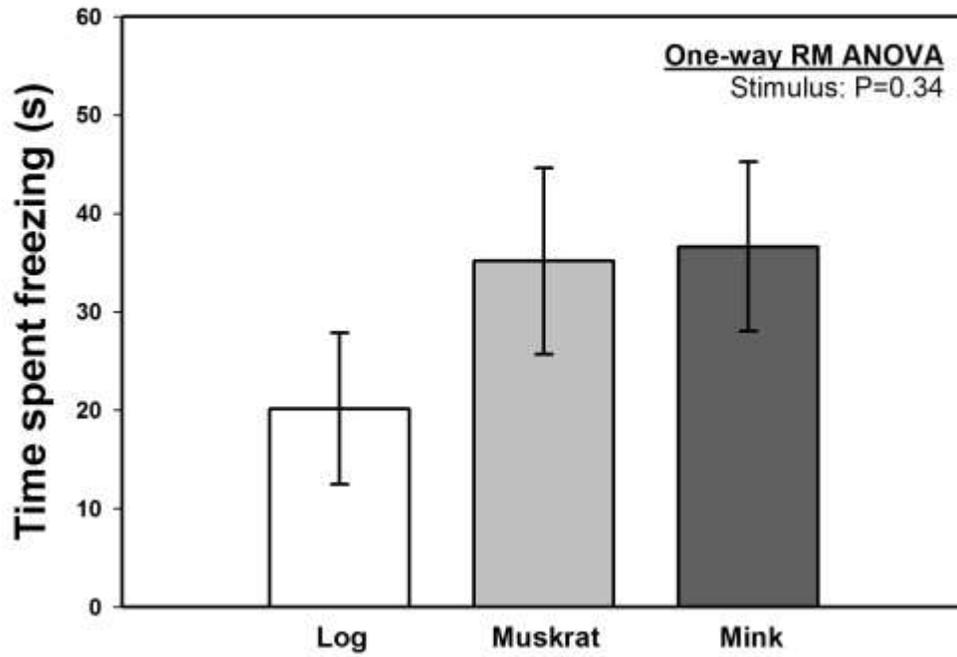


Fig. 17. Mean ( $\pm$  SE) time spent motionless, in seconds, for each stimulus (N = 19).

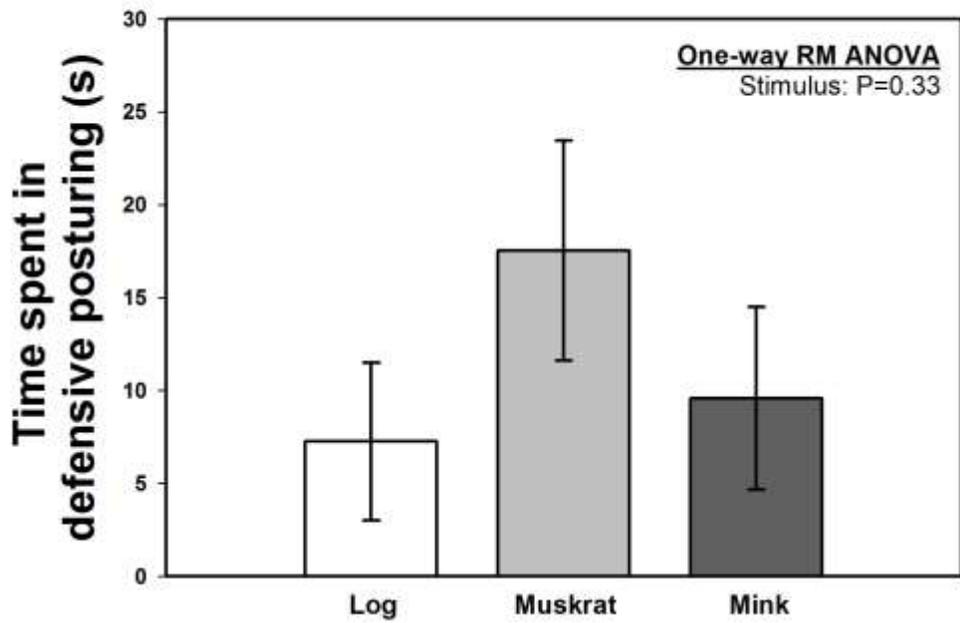


Fig. 18. Mean ( $\pm$  SE) time spent in defensive posturing, in seconds, for each stimulus (N = 19).

## DISCUSSION

My results provide evidence that cottonmouths discriminated between predator and non-predator stimuli. However, snake responses differed between chemical and visual stimuli with cottonmouths being able to distinguish between predator and non-predator stimuli in chemical trials and mobile aerial trials. In feeding trials, cottonmouths exposed to predator chemical cues exhibited elevated tongue-flick rates, which is consistent with heightened alertness associated with predator detection (Gove and Burghardt 1983). Alertness was elevated for stationary mammal models, where the cottonmouths responded similarly to both the predator and non-predator animal models. Mobile aerial models elicited the most animated antipredator behavioral responses and represented the only stimulus that elicited tail vibration and mouth gaping. Collectively these results are consistent with the threat sensitivity hypothesis (Helfman 1989) in that visual contact with a predator poses greater risk than exposure to chemical cues from a visually undetected predator.

Snakes did not reduce movement or feeding in chemical predator chemical cue trials, which is contrary to other herpetofaunal studies of predator recognition (Gall and Mathis 2010; Crane et al. 2012; Churilla 2015). The differences observed could be explained by the type of predator. It would make sense for the snakes to reduce movement if they are being stalked by a cryptic ambush predator. However, if the predator is an active forager, such as swine or canine, and there is no visible evidence that the predator is still in the vicinity, the perceived threat may be insufficient to reduce movement or feeding.

Parallel foraging experiments using the same test subjects as those used in my experiments, found that snakes exhibited prolonged approach movement, decreased movement, and elevated tongue-flick rates when exposed to musk gland secretions instead of a predator odor (Churilla 2015). These responses are more consistent with heightened vigilance associated with predator detection and suggest that the snakes were perceiving greater risk when exposed to musk than a predator cue. Cloacal musk typically is not secreted by cottonmouths unless a predatory encounter has occurred (Gibbons and Dorcas 2002; Roth and Johnson 2004) and has been suggested to function as an intraspecific alarm cue in pitvipers (Graves and Duvall 1989; Churilla 2015). Following the threat sensitivity hypothesis, I expect that cottonmouths exposed to musk would perceive greater threat than those exposed to a predator odor because the former cue would indicate that a conspecific in close proximity has encountered a predator, whereas a predator cue may only indicate a recent predator presence.

Prey responses to chemical cues of predators should vary with the level of threat perceived (Mathis and Vincent 2000; Cabido et al. 2004; Ferrari et al. 2007). In my study, domestic swine and canine predator cues both elicited elevated tongue-flick rates, which is generally interpreted as an indication of predator or prey recognition in squamates (Schwenk 1995). The similarity of responses by cottonmouths to each cue is interesting given the differences in their exposure to each predator. Canines represent potentially important snake predators throughout the cottonmouth's geographic range. However, the length of historical exposure of cottonmouths to swine has been very brief; approximately 20 years (Hartin et al. 2007). One possible explanation for the domestic swine recognition is that cottonmouths may be generalizing the recognition of a

mammalian cue. The generalization of antipredator responses to unfamiliar species related to known predators describes the predator recognition continuum hypothesis which is supported by studies of numerous vertebrates (Griffin et al. 2001; Ferrari et al. 2007; Crane et al. 2012). Prey species may benefit from generalizing predator recognition in environments where predator diversity is high, whereas there may be a benefit to more precisely recognizing predator species in environments containing many non-predators. Given their exposure to many mammalian species across a wide geographical range, cottonmouths may benefit from generalized recognition of potential predators.

In my study, cottonmouths were significantly more likely to exhibit defensive postures to both the red-tailed hawk and mallard duck model silhouettes than a transparent rectangle control. However, the hawk model elicited significantly more defensive displays and eye contact than the duck silhouette and the effect sizes were moderate to strong, indicating substantial differences in the cottonmouths ability to distinguish predator and non-predator stimuli. Because my design controlled for both size and color of the models, it appears that snakes were evaluating models solely on their shape. This result contrasts with those reported for free-ranging spiny lizards (*Sceloporus occidentalis*), which responded similarly to models of an important raptor predator and a circle of equal area (Fine 1999). Additionally, Texas horned lizards (*Phrynosoma cornutum*) increased escape responses to an aerial raptor model when its shadow passed over their bodies relative to the shadow moving nearby (Cooper and Sherbrooke 2010). Unfortunately, that study only used a single stimulus so predator discrimination based on model characteristics was not tested. Studies on predatory risk perception in iguanas have reported distinct responses to different predator types (Ito and Mori 2012) and

documented specific visual stimuli associated with the magnitude of antipredator responses (Burger et al. 1991; Burger et al. 1993). The factors affecting antipredator behavior in squamate reptiles is clearly complex and in need of further study.

Stationary terrestrial models of mammals did not elicit escape behavior or defensive displays from cottonmouths. These responses are consistent with those of rattlesnakes (*Crotalus viridis viridis* and *Sistrurus catenatus tergeminus*) presented with stationary mammalian carnivore models that also failed to elicit any defensive responses (Scudder and Chiszar 1977). The consistency of results between both studies suggests that movement is necessary to elicit defensive responses from pitvipers. However, responses differed among stimuli in that cottonmouths frequently maintained eye contact with the mammal models but never did so with the log. This difference was both statistically significant and involved a large effect size. Although snakes may not have felt particularly threatened by the stationary stimuli, it appears that the mammal models were perceived differently than the log. Several previous studies have shown that eyes are important in visual predatory risk assessment in lizards. For example, Green anoles (*Anolis carolinensis*) remained immobile longer in the presence of a model hawk with eyes compared to a model without eyes (Gallup 1973). One possible explanation for why the cottonmouths could not distinguish between the mink and muskrat threat is that unlike the mallard duck and hawk, the mink and muskrat were similar in shape. Therefore, it may be hard for the snake to distinguish between a predator and non-predator threat in the absence of additional cues.

My results are consistent with previous snake studies that show threat-sensitivity to the risk posed by a predator. In the words of Pope (1958), “snakes are first cowards,

then bluffers, then last of all warriors”. More recent experimental studies formally quantifying cottonmouth antipredator responses have validated Pope’s statement, showing that snakes initially attempt to flee but then employ antipredator displays and, finally, aggressive defense as threat levels escalate (Gibbons and Dorcas 2002; Roth and Johnson 2004). This characterization of cottonmouth antipredatory behavior can be used to interpret cottonmouth responses in my study. The stationary terrestrial predator models elicited no response, other than increased eye contact, suggesting that no immediate threat was perceived, likely because they were stationary. Gaping and tail vibration are exhibited when cottonmouths perceive an imminent predatory threat to deter the predator from attacking. I only observed this type of behavior when snakes were presented with a mobile aerial predator. When threat escalates further, such as actual contact with the predator, striking and biting typically occur as a last attempt to stop the predator. This type of behavior was not observed in my study, which was expected because none of the predator models made physical contact with the cottonmouths.

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