Short Communication

An investigation of prey discrimination by neonate Red-Bellied Mudsnakes (*Farancia abacura*).

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Introduction

Snakes of the genus *Farancia* are found throughout the southeastern United States but we know little about their ecology and biology. Indeed, there are few studies focusing on either the Rainbow Snake (*Farancia erytrogramma*) or the Red-Bellied Mudsnake (*Farancia abacura*). Of the studies that have occurred, most have taken place at the Savannah River Site in southeastern South Carolina (e.g., Semlitsch et al. 1988, Willson et al. 2006, Vogrinc et al. 2018) and we have limited information about geographic variation in their biology (e.g., Rodríguez-Robles 2002).

Due primarily to their secretive nature (Durso et al. 2011), most information we have pertaining to the ecology of these species is based on qualitative assessments and incidental observations. Although largely anecdotal, this information has revealed fascinating aspects of their natural history, including maternal attendance of eggs and a highly specific diet: rainbow snakes and mud snakes appear to feed primarily on American eels (*Anguilla rostrata*) and eel-like salamanders (*Siren* spp. and *Amphiuma* spp.), respectively (Ernst and Ernst 2003). However, our knowledge about even basic aspects of their biology may be subject to revision pending formal study or greater attention. For example, both species are considered highly aquatic, but a review revealed they are frequently found in terrestrial habitats far from water (Steen et al. 2013).

The purported specialized diet of *Farancia* warrants further study as there is little information to determine whether observed prey items reflect a preference or simply disproportionate availability. Because snakes flick their tongues in response to chemical stimuli (Burghardt 1970) - a behavior that transmits scents to the vomeronasal system (Schwenk 1995) - this behavior can be used to determine whether snakes discriminate among different experimental treatments (Cooper and Burghardt 1990). This type of bioassay has been used to investigate snake behavior

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for decades and results have formed the basis of many inferences about their foraging ecology (Austin and Gregory 1998, Goetz et al. 2018). Although we still have much to learn regarding whether assumptions associated with this methodology are relevant for all species and research questions (Cooper 1998), the rate of tongue flicks can be used to calculate an index of interest; relatively high rates are typically interpreted to reflect high relative interest (i.e., preference; Goetz et al. 2018). In this study, we conducted an experiment on a clutch of recently-hatched Red-Bellied Mudsnakes to assess whether they discriminate among scents of potential prey.

Materials and Methods

On 9–10 August 2018, a wild-caught Red-Bellied Mudsnake from southeastern Georgia laid 34 eggs after arriving at the Georgia Sea Turtle Center (GSTC) for treatment. Following an approximate 41-day incubation period, 28 hatchlings successfully emerged. We conducted our experiment with all 28 hatchlings on 1 October 2018, when snakes were between 11-13 days old. All hatchlings shed their skins once before our experiment but had never been exposed to or consumed prey.

Prior to initiating experimental trials, snakes were transferred from a container in which they were communally housed into individual plastic containers containing a damp paper towel. Trials generally followed long-established protocols (Burghardt 1967, Goetz et al. 2018) for studies of this type. Specifically, lids were removed from each snake's container and snakes were exposed to one of the three scents. Scents included deionized water (our negative control), River Frog *(Lithobates heckscheri*; represented by one individual frog) and Lesser Siren (*Siren intermedia*; represented by five individuals). All amphibians were captured within swamps located in the region. Prior to each trial, a 15-cm long cotton-tipped applicator was either dipped in the deionized water (odorless control) or dipped in the deionized water and then run along the body of either the River Frog or one or more of the Lesser Sirens. We used a repeated-measures randomized design in which all snakes were presented with all three scents over the course of the study. This resulted in 84 individual experimental trials comprising three rounds (all 28 snakes were exposed to one scent in each round). All trials occurred within a single day.

We initiated trials as soon as snakes were not exhibiting defensive behaviors; once trials began we maintained the placement of cotton-tipped applicators approximately 2 cm in front of the snake's face for one minute while recording the number of times snakes flicked their tongue. Had snakes bitten the cotton-tipped applicator, this also would have been recorded and the trial would have ended. We then calculated a tongue-flick attack score (TFAS), as described in Cooper and Burghardt (1990). We counted tongue flicks whether the snakes were stationary or moving and this could impact tongue-flicking behavior; as a result, and due to potential intraspecific and interspecific variation in foraging behavior and activity, TFAS results may have limited utility for making comparisons with other studies (Cooper 1989, Cooper and Burghardt 1990).

We log-transformed TFAS data to meet assumptions of normal-distribution of residuals and homoscedasticity. Then, we fit data to a linear mixed-effects model with the fixed effect of scent (Lesser Siren, River Frog, odorless control) and random effect of individual. All analyses were performed in R (v3.5.1, R Core Team, 2016).

Results and Discussion

Presented stimuli elicited at least one tongue flick in all but 11 (four control; four River Frog; three Lesser Siren) of the 84 total individual trials and all snakes tongue-flicked in response to at least one stimulus. We recorded zero bites to the cotton-tipped applicators in response to any presented stimuli. Lesser Siren scent elicited 12.0 $\pm$ 2.1 (Mean $\pm$ 1 SE) tongue flicks; the strongest response to stimuli tested (Figure 1). The odorless control and River Frog scent elicited a weaker

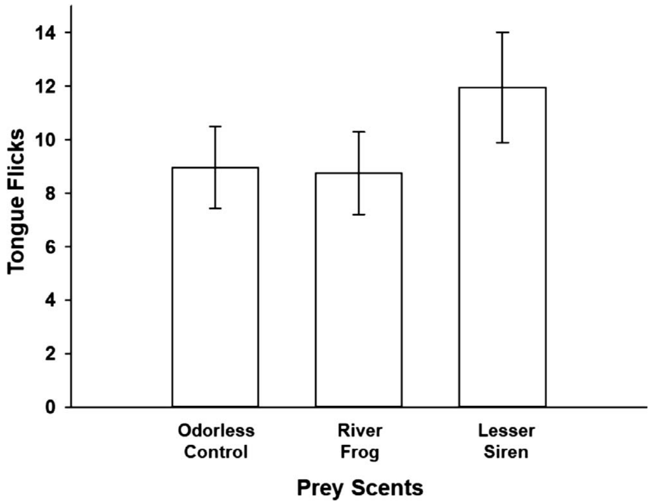

Figure 1. Tongue flicks (mean ± 1 SE) of digestively naïve hatchling Red-Bellied Mudsnakes (*Farancia abacura*; n = 28) in response to deionized water (odorless control) and scents from River Frogs (*Lithobates heckscheri*) and Lesser Sirens (*Siren intermedia*). Differences in responses were not significant.

response from snakes (9.0 ± 1.5 and 8.8 ± 1.5 tongue flicks, respectively). However, we observed considerable variation in response and the linear mixed-effects model revealed no statistically-significant difference among treatments ($F_{2,\ 54} = 0.90$, $P = 0.411$). Specifically, in pairwise comparisons, we did not generate evidence that Red-Bellied Mudsnakes distinguished between the odorless control and River Frog scent ($P = 0.798$) or Lesser Siren scent ($P = 0.316$) or between the two prey scents (River Frog–Lesser Siren, $P = 0.210$).

Red-Bellied Mudsnakes are considered amphibian-specialists but we failed to detect a significant difference among their responses to the scents of potential amphibian prey and an odorless control. This limits our ability to make inferences about Red-Bellied Mudsnake biology but perhaps these snakes forage in a manner such that they do not rely exclusively on chemosensory information to find and discriminate among prey. Many of the snakes in our trials were relatively active and moved around their containers; this may have led us to include exploratory tongue flicks in addition to those specifically directed at a potential prey item, which could obscure potential preferences (Cooper 1989, Cooper and Burghardt 1990). More information about how Red-Bellied Mudsnakes find and handle prey in aquatic and muddy habitats would be useful for interpreting this behavior. Finally, and critically, all the snakes within our study came from a single clutch; although there was intra-clutch variability in response to scents, we cannot assume samples were statistically independent. Ideally, we would have access to multiple Red-Bellied Mudsnake clutches to integrate into an experiment and control for clutch in analyses (e.g., Goetz et al. 2018) but wild clutches of this secretive species are rarely observed, and the species is not readily bred in captivity.

Although not a statistically significant difference, snakes flicked their tongue more often at Lesser Siren scents than at the other scents; this is notable because eel-like salamanders such as sirens are considered the primary prey of adult Red-Bellied Mudsnakes. However, given that snakes in our study did not significantly discriminate among prey types, perhaps this species does not select among prey and documented dietary habits of this species simply reflect relative prey abundance and availability. On the other hand, we investigated the responses of digestively-naïve neonates to quantify innate preferences and maybe this species experiences an ontogenetic dietary shift such that only adults exhibit a strong preference toward specific prey types. Finally, very small Red-Bellied Mudsnakes could prey primarily on larval amphibians, which might smell differently than the older individuals we used as scent donors in this study.

There is very little published on snakes within the *Farancia* genus; the few research papers that do exist focus largely on quantifying natural behaviors in a field setting and feeding behavior is rarely observed. Although we did not detect statistically-significant differences in the response of Red-Bellied Mudsnakes to potential prey scents, this study represents important new information for a poorly-known species that advances our understanding of their biology (Fanelli 2012, Parker et al. 2016). It is unusual to have access to a clutch of digestively-naïve snakes born from wild females; we hope future researchers similarly take advantage of these rare opportunities as they arise to learn more about snake biology.

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