Palatability and Predator Avoidance Behavior of Salamanders in Response to the Virginia Opossum (Didelphis Virginiana)

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Palatability and Predator Avoidance Behavior of Salamanders in Response to the Virginia Opossum (Didelphis virginiana)

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Abstract.—Our understanding of mammalian predation on salamanders is primarily restricted to small carnivorous species (*i.e.*, shrews). We conducted a series of investigations to determine whether the Virginia opossum (*Didelphis virginiana*) is a predator of various salamander species [*Desmognathus fuscus*, *Plethodon dorsalis*, and *Notophthalmus viridescens* (adult and eft)] from the eastern United States. All species, including toxic newt efts, were palatable to opossums. These results suggest opossums could be an active predator of salamanders. In a second study, each terrestrial salamander species exhibited avoidance responses to kairomones from opossums indicating that despite a significant risk of mortality each salamander has evolved mechanisms to minimize predation risk. Combined with overlapping habitat preferences, foraging behavior that places them in contact, and a dietary preference for animal matter, these data suggest opossums may be an important predator on terrestrial salamanders.

Introduction

Amphibians play an important ecological role in terrestrial and aquatic communities. As predators, amphibians regulate microinvertebrate communities and can lead to changes in species composition (Davic and Welsh, 2004; Walton and Steckler, 2005; Best and Welsh, 2014). The density of terrestrial amphibians, especially salamanders in the eastern United States, can be exceptionally high (Burton and Likens, 1975b; Davic and Welsh, 2004; Semlitsch *et al.*, 2014). Given their density, salamanders in these communities have been documented to affect soil structure, link aquatic and terrestrial food webs, and influence ecosystem processes, such as decomposition rates and thus nutrient cycling (Beard *et al.*, 2002; Davic and Welsh, 2004; Walton and Steckler, 2005). Furthermore, amphibians provide

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food for a variety of predators and are a critical link to tertiary consumers within food webs (Burton and Likens, 1975a; Davic and Welsh, 2004).

As prey amphibians provide an abundant, slow-growing, and long-lived resource. When coupled with their ability to consume smaller prey relative to other intra-guild members (*e.g.*, small birds, lizards, and snakes, shrews, mice), they may be one of the most valuable nutrient reserves available to predators within communities (Davic and Welsh, 2004). Amphibians are eaten by numerous predators, including invertebrates, such as insects and crustaceans, fishes, other amphibians, snakes, turtles, birds, and mammals (Petranka, 1998; Wells, 2007). Interactions between amphibians and some of these predator assemblages have received considerable attention. For example the relationship between amphibians and fish have been extensively studied (*e.g.*, Kats *et al.*, 1988; Hecnar and M'Closkey, 1997; Kiesecker and Blaustein, 1998; Smith *et al.*, 1999; Gillespie, 2001; Vredenburg, 2004; Wells, 2007; Gall and Mathis, 2010). Similarly, snakes and some birds are also major predators of amphibians (*see* review in Wells, 2007) with some species evolving specialized adaptions associated with predation of a particular amphibian species (Edgren, 1955; Brodie and Brodie, 1990; Mori *et al.*, 2011).

Though the relationship between amphibians and some predators has been heavily explored, amphibian predation by mammals has not. Dietary analysis has found many mammalian generalists include amphibians in their diet (Wells, 2007). Yet the role of many of these species in actual stalk and subjugation of amphibians, along with the associated defenses exhibited by the amphibians, remains largely unexplored (but *see* Hurlbert, 1970; Cochran and Redmer, 1992); small mammalian predators, such as shrews, are an exception (Brodie *et al.*, 1979; Formanowicz and Brodie, 1979; Brodie and Formanowicz, 1981). For example many medium-sized members of the Carnivora (*e.g.*, mongoose, weasels, skunk, badger, and otter) consume a diversity of prey that can include amphibians depending on local availability (Wells, 2007). The raccoon (*Procyon lotor*) has a reputation as an important amphibian predator, yet dietary studies indicate a relatively small proportion of their food is derived from amphibians (Stuewer, 1943). Furthermore, preliminary observations of raccoon interactions with salamanders indicate they are ineffective predators on many species (BGG, pers. obs.). In contrast the European Polecat (*Mustela putorius*) and Badger (*Meles meles*) have been documented to forage extensively on amphibians, even exclusively consuming amphibians during certain periods of the year (Henry, 1983, 1984; Weber, 1989a, b).

One species of mammal that has the potential to interact extensively with amphibians is the Virginia opossum (*Didelphis virginiana*). The Virginia opossum is the only extant marsupial found north of Mexico. The opossum's current range encompasses areas east of the Rocky Mountains (Gardner, 1982). In addition the opossum was introduced to western North America in the late 1800s and now ranges from California to British Columbia (Gardner, 1982). The combined native and introduced range of the opossum in North America overlaps with the most salamander-rich ecosystems in the world. This range overlap, when combined with similar habitat preferences between many salamanders and opossums (moist woodland), leads to the potential for extensive interaction between these species. This is compounded by the opossum's unselective omnivorous diet (Reynolds, 1945; McManus, 1970), distinct preference for animal matter (McManus, 1970), and proclivity for adaptations to deal with toxic and dangerous prey (Voss and Jansa, 2012).

The goal of this study was to evaluate the role of the Virginia opossum as a potential predator of salamanders. Opossums have been recorded to consume salamanders in previous experiments and are even known to eat toxic salamanders, such as the red spotted newt (*Notophthalmus viridescens*) (McManus, 1970). In feeding trials opossums were presented three different species of salamanders [*Plethodon dorsalis, Desmognathus fuscus, Notophthalmus viridescens* (both eft and adult stages)] and the propensity and ability to consume each species were observed. These salamander species were chosen because they encompass two of the most diverse salamander genera (*Plethodon* and *Desmognathus*) in eastern North America and are palatable to other species, including birds (Brodie and Brodie, 1980). *Notophthalmus* possess a neurotoxin in the granular glands in the skin that may provide the salamanders with a sufficient chemical defense to thwart predation by mammals (Brodie, 1968). The eft stage advertises toxicity by performing an unken display where the newt will arch its back while lifting its legs, neck and tail to display a brightly color underside (Brodie, 1977). Additionally, adult newts provide a potentially interesting comparison due to their lower toxicity relative to efts (Brodie, 1968; Spicer *et al.*, 2018) and an aquatic stage that should rarely encounter terrestrial predators. In a second set of experiments, these species were tested for their ability to detect mammalian predators by exposing them to kairomones from nonpredatory white-tailed deer (*Odocoileus virginianus*) and opossum to determine if these salamanders recognize opossums as a predator and respond with predator-avoidance behavior.

METHODS

PALATABILITY OF AMPHIBIAN SPECIES TO OPOSSUMS

Opossums (N = 5) were caught using live traps from a manicured lawn near Hanover, Indiana (38.7142°N, 85.4736°W). The opossums were transported to the Hanover College campus where they were housed in steel mammal cages (64 × 46 × 37 cm, L × W × H) with a plexiglass door containing a small hole for video recording. A large towel was placed on the bottom of the cage and two ceramic bowls were positioned at the front of the cage for food and water. The cages were maintained at 24 C on a 12 h light:dark cycle. The opossums were fed commercially available worms (*Lumbricus terrestris*) twice daily and had continual access to water. After 24 h the fecal samples produced by the opossums were collected and frozen for later experimentation (*see* below); the initial fecal samples were discarded to ensure the diet of the opossums prior to capture did not influence the results of the second experiment. Opossums were not fed for 24 h prior to the start of a palatability trial.

Adult *Desmognathus fuscus* and *Plethodon dorsalis* were collected from Hanover College's campus and housed in petri dishes with damp paper towels. Adult and eft *Notophthalmus viridescens* were collected near Madison, Indiana, and housed in 37 L aquaria with 5 cm of water (adult) or 9 L aquaria filled with damp *Sphagnum* sp. (eft) until testing. At approximately 2130 h, each of the opossum's food bowls were removed, cleaned, and a small amount of water was added to ensure the salamanders did not desiccate throughout the duration of the trial. Three salamanders of a single species were then added to the food bowl and a video camera (Cannon Vixia HF G20) was positioned on a tripod at the hole in the plexiglass door to record any interactions with the opossum at its food bowl. At 0900 h the following morning, the bowls and cages were searched for remaining salamanders and the video footage was evaluated to confirm consumption of any missing salamanders. A minimum of 3 d separated palatability trials with a single opossum and no opossum was tested with more than two species of salamander; each salamander species was tested with one male and one female opossum. Salamanders were presented live (*Desmognathus*, *Notophthalmus* adult), or if the salamanders could escape the food dish, they were first euthanized (*Plethodon, Notophthalmus* eft) by freezing (Lillywhite *et al.*, 2017). Three opossums were tested with two different salmanders [*Desmognathus* followed by adult newt (two opossums); *Plethodon* followed by eft (one opossum)]; the remaining two opossums were tested with a single salamander. All animal care protocols and sample size limitations using vertebrates are based on the *Guide for the Care and Use of Laboratory Animals* published by the National Research Council (2011).

BEHAVIORAL RESPONSES OF AMPHIBIANS TO OPOSSUM KAIROMONES

Treatment preparation.—We exposed *Plethodon dorsalis, Desmognathus fuscus*, and aquatic adult *Notophthalmus viridescens* to chemical solutions from a blank control (decholorinated water), nonpredator (white-tailed deer), and a predator (opossum). We also exposed the terrestrial eft stage of *Notophthalmus viridescens* to chemical stimuli from a control and predator (opossum) in field trials. Deer feces, likely deposited within the previous 24 h, were collected from Hanover College's campus and frozen for later use. Opossum fecal samples were collected from the captive opossums before the initiation of the palatability trials. The fecal solutions were prepared by homogenizing water with the appropriate type of feces in a 9:1 ratio (9 ml water:1 g feces) in separate blenders. The resulting solutions were then gravity filtered to remove solid particles, transferred to 50 ml centrifuge tubes in 40 ml aliquots, and frozen for later use. In addition dechlorinated water was processed and frozen in the same manner to serve as the control in all experiments. These solutions were used throughout the experiments described below.

Plethodon dorsalis.—Adult *Plethodon dorsalis* ($N = 64$) were collected from Hanover College campus and housed in 14 cm diameter petri dishes lined with moistened unbleached paper towels. The salamanders were held in an environmental chamber at 16 C on a 12 h light:dark cycle and fed adult and larval fruit flies (*Drosophila melanogaster*) ad libitum. The paper towel substrate was cleaned weekly. Salamanders were housed in the lab for 3 wk prior to testing to allow individuals to acclimate to artificial housing and establish territories within their petri dishes.

Three days prior to the start of the experiment all petri dishes were cleaned and the paper towel was replaced. At the start of the experiment a salamander was transferred into a temporary holding container. Two milliliters of control solution was then pipetted into the salamander's home dish and the dish was rotated to spread the solution evenly across the substrate. The salamander was then transferred back into its home container and a 5 min prestimulus period began. We then recorded the number of discrete movements, amount of time the salamander spent on container edge (head and body pressed against the side of the petri dish), and the number of escape movements (any discrete movement with at least two feet touching the side of the petri dish). After the prestimulus period, a poststimulus period was initiated where the process was repeated with the exception that a randomly chosen treatment (control, deer kairomones, and opossum kairomones) was added to the home dish. The change in behavior was calculated for each response variable by subtracting the prestimulus value from the poststimulus value. A One-Way ANOVA was used to compare the change in the number of moves and time on container edge by salamanders exposed to the three treatments. Due to failure to meet assumptions of normality and homoscedasticity, a Kruskal-Wallis test was used to compare the number of escape movements by salamanders exposed to the three treatments. Posthoc comparisons (Parametric: Tukey; Nonparametric: Dunn's) were used to evaluate between-treatment differences in cases with overall F-test significance ($\alpha = 0.05$).

Desmognathus fuscus.—*Desmognathus fuscus* ($N = 72$) were collected from Hanover College campus and housed individually in a plastic container ($22 \times 14.5 \times 8$ cm; $L \times W \times H$) with an

FIG. 1.—Photo showing an experimental arena for kairomone trials with *Desmognathus fuscus*. The paper towels were randomly assigned to the control treatment (dechloronated water) and either a control, white-tailed deer, or opossum kairomone treatment. The numbers correspond to the score given to salamanders based on their position within the enclosure relative to the treatment stimulus at 15 min intervals for 5 h. Scores were recorded as follows: on top of the paper towel on the treatment side (–2), on the wall of the treatment side or under the paper towel on the treatment side (–1), in the central neutral zone (0), on top of the paper towel of the control side (+1), and on the wall of the container or under the paper towel on the control side (+2)

unbleached paper towel substrate (22 × 14 cm) and sparsely dispersed *Sphagnum*. The salamanders were housed for 3 wk to acclimate the animals to their home chamber. During this acclimation they were fed small red worms (*Eisenia fetida*) once a week. Prior to the start of the experiment, a salamander was moved to a holding container, their home container was cleaned, and two pieces of paper towel (10 × 14 cm) were placed on either end of the container such that a 2 cm gap was positioned between them. Each container was then randomly assigned a treatment [either water (control), deer, or opossum]. Four milliliters of the appropriate treatment solution was then applied to a randomly chosen side while the remaining paper towel was moistened with control water (*i.e.*, a double control was possible). All salamanders were then placed into the center of their home containers between the two paper towels and the trial began. Each salamander's initial side choice was recorded every minute until all salamanders had selected a side. In addition, we recorded their side choice as well as position on that side (*e.g.*, on the wall or under the paper towel) at 15 min intervals for 5 h.

In order to accurately assess the salamander's avoidance of the kairomones, an avoidance score was tabulated based on salamander position in the container relative to the treatment solution (Fig. 1). The scores ranged from –2 (indicating the least amount of avoidance) to

+2 (indicating the greatest avoidance). An individual scoring a –2 was located on top of the paper towel on the treatment side. A salamander scoring –1 was located either on the wall of the treatment side or under the paper towel on the treatment side as this indicates some avoidance. Any salamander in the central neutral zone on the 15 min interval was given a score of zero. An individual scoring a +1 was located on top of the paper towel of the control side, and an individual scoring a +2 would be located on the wall of the container or under the paper towel on the control side. A mean avoidance score for each individual over the 5 h was calculated at the end of the experiment using their respective scores they received at each 15 min interval. The collective mean avoidance score of individuals within a treatment group (water control, deer kairomones, and opossum kairomones) was then compared using a One-Way ANOVA. In addition, the number of observations in which the salamanders were on the wall of the container in each of the treatments were compared with a Kruskal-Wallis test.

Eastern newt adult.—Adult eastern newts (N = 50) were collected near Madison, Indiana, separated by sex, and housed in 37 L aquaria in groups of approximately ten individuals. The newts were fed small pieces of commercially available red worms (*Eisenia fetida*) twice weekly for 3 wk prior to experimentation. The experimental chamber consisted of a 9 L aquaria filled with 1 L of water. A randomly chosen sex was selected and a newt was haphazardly removed from its holding tank and transferred to the 9 L test chamber. A 20 min acclimation period was initiated. At the conclusion of the acclimation period, 50 ml of control solution was injected down the side of the aquaria on the side opposite the newt to minimize disturbance. A 10 min prestimulus period was then initiated where the latency to move, number of discrete moves, and total time moving were recorded. After the prestimulus period, 50 ml of a randomly chosen treatment solution (control, deer, or opossum) was injected down the side of the aquaria opposite the newt and a 10 min poststimulus period was initiated. The same behavioral responses were again recorded. At the conclusion of a trial, the newt was moved to a holding tank and never retested. The experimental chamber was then rinsed thoroughly with warm tap-water and the process repeated. At the conclusion of testing, we subtracted the poststimulus response from the prestimulus response to determine the change in activity between the prestimulus and poststimulus period (excluding latency). We compared the latency to move between the three treatments using a One-Way ANOVA. Because differences in antipredator behavior between male and female newts have been documented (Rohr *et al.*, 2002), we conducted Two-Way ANOVA's on the change in number of moves and the change in time moving with treatment and sex as the two main effect factors.

Eastern newt eft.—Eastern newt efts were collected from Jefferson National Forest in Giles and Craig County, Virginia. The efts were placed in plastic bags filled with damp leaf litter until testing (<1 h). A single eft was removed from the bag by its tail and placed on a flat rock in the shade. Approximately 5 ml of a randomly chosen treatment solution (control or opossum) was then poured on top of the dorsal surface of the eft. After 3 s the dorsal surface of the eft was tapped with a clean scoop spatula three times to induce the unken reflex. A stopwatch was started and the time until the eft took a step (time in unken) was recorded. In addition we recorded the intensity of the unken display by counting the number of feet lifted off the substrate, as well as the angle (0°, <45°, >45°, 90°, >90°) of the head and tail relative to the substrate (Neuman-Lee *et al.*, 2015). Once the trial was complete we recorded the total length and snout-to-vent length of the eft, released it at the site of capture, and rinsed the rock with water. The control and opossum treatments were conducted on different rocks (similar structure and composition) to ensure there was no cross

TABLE 1.—Body region, response to stimulation, and assigned value for newts exposed to a simulated predation even. Values were tabulated to assign an unken score to each individual indicating the degree of intensity for the unken display

Body region	Behavioral measure	Assigned score
Feet off substrate	0	0
	1	1
	2	1
	3	2
	4	2
Head or tail	on substrate	0
	<45	1
	45	2
	>45	3
	90	4
	>90	5

contamination of the treatments. The recorded variables were then given a point value based on the intensity of the response, with a high intensity unken receiving a high point value and a low intensity unken having a low point value (Table 1). The scores for each variable were then combined to form an unken score for each eft. We compared the time in unken and the unken score between efts exposed to either control or opossum solutions with a *t*-test.

RESULTS

PALATABILITY OF AMPHIBIAN SPECIES TO OPOSSUMS

Video analysis confirmed that each species of salamander was eaten by opossums, indicating all three species are palatable to opossums (Table 2). In addition the toxic eft stage of the Eastern newt was also palatable to opossums (Table 2). Opossums approached the bowl within 2 h of the start of the trial and generally investigated the contents with their mouth and nose. After identifying a live salamander, they attempted to bite the salamander after which they pulled it out of the bowl and used their paws to manipulate and subjugate the prey until it could be successfully consumed; this entire process took a few seconds. All Northern Zigzag salamanders and adult newts were successfully consumed by the opossums (Table 2). All newt efts were consumed by one opossum, whereas a second opossum ate one eft, masticated a second, and manipulated but did not damage a third eft; the sequence in which this occurred is unknown because the opossum moved the camera's focal point away from the cage (Table 2). *Desmognathus* appeared difficult to catch due to their rapid escape movements, yet two of the three salamanders in each trial were eaten (Table 2).

BEHAVIORAL RESPONSES OF AMPHIBIANS TO OPOSSUM KAIROMONES

Plethodon dorsalis.—There was a significant difference in the number of moves ($F_{[2,61]} = 15.2$, $P < 0.001$), number of escape movements ($H = 12.0$, $P = 0.002$), and time on container edge ($F_{[2,61]} = 3.3$, $P = 0.044$) by Northern Zigzag salamanders when exposed to dechlorinated water (control) and kairomones from nonpredatory deer and predatory opossums. Salamanders moved less (Fig. 2a), increased the number of escape movements (Fig. 2b), and spent more time on the edge of the container (Fig. 2c) in response to

TABLE 2.—Species of salamanders provided to the Virginia Opossum to test palatability. ID# indicates the opossum in which the salamanders were tested (F = female, M = male). Video = trials in which salamanders were videotaped being consumed. Trials in which the video camera malfunctioned and video confirmation of consumption was not possible are indicated with (malf.)

Species	ID#	Total fed	# Siezed	# escaped/ dropped	# Consumed (video)	Total consumed (video + presumed)
Desmognathus fuscus	1F	3	3	1	1	2
	1M	3	3	2	0	2
Plethodon dorsalis	3F	3	3	0	3	3
	2M	3	3	0	3	3
N. viridescens (adult)	1F	3	malf.	malf.	0	3
	1M	3	3	0	3	3
N. viridescens (eft)	2F	3	3	1	3	3
	3F	3	malf.	malf.	0	1

opossum kairomones as compared to the control solution. In each case responses to deer kairomones were intermediate between the control and the opossum treatments (Fig. 2).

Desmognathus fuscsus.—There was a significant difference in the mean avoidance score between *Desmognathus* exposed to the three treatments ($F_{[2,65]} = 3.4$, $P = 0.04$, Fig. 3). *Desmognathus fuscus* exposed to opossum kairomones had a higher avoidance score (*i.e.,* greater avoidance) relative to individuals exposed to deer kairomones or a water control (Fig. 3). *Desmognathus* were observed on the wall of the container more frequently when exposed to opossum kairomones (median = 12.5 observations per trial) relative to white-tailed deer (median = 6 observations) or control (median = 7 observations); however, this trend was not significant ($H = 2.5$, $P = 0.29$).

Eastern newt adult.—There was no significant difference in the latency to move ($H = 0.08$, $P = 0.96$) by adult newts when exposed to a dechlorinated water control and kairomones from nonpredatory white-tailed deer and predatory opossums. In addition there was no significant interaction (# moves: $F_{[2,39]} = 0.37$, $P = 0.70$; time moving: $F_{[2,39]} = 1.44$, $P = 0.25$) or significant individual main effects of sex (# moves: $F_{[1,39]} = 0.31$, $P = 0.58$; time moving: $F_{[1,39]} = 0.12$, $P = 0.73$) or treatment (# moves: $F_{[2,39]} = 0.21$, $P = 0.81$; time moving: $F_{[2,39]} = 0.28$, $P = 0.76$) for the number of moves or time spent moving.

Eastern newt eft.—Newt efts exposed to kairomones from predatory opossums produced an unken display with significantly greater intensity than newt efts exposed only to a dechlorinated water control ($t = -2.47$, $P = 0.02$, Fig. 4). There was no significant difference in the time in unken between newt efts exposed to a dechlorinated water control (mean ± SE = 41.7 ± 14.7 s) or kairomones from opossums (mean = 34.1 ± 11.6 s; $t = 0.41$, $P = 0.69$).

DISCUSSION

We found under laboratory conditions, the Virginia opossum consumed three species of salamanders, including *Plethodon dorsalis, Desmognathus fuscus,* and *Notophthalmus viridescens* adult and efts. These salamanders belong to three of the most abundant salamander genera in the United States, and these results indicate opossums may be important predators of this group of vertebrates in the wild. Opossums possess several attributes that likely make them effective predators on salamanders. First, opossums are opportunistic omnivores that have been documented to consume a wide variety of food (McManus, 1970). While fruit and

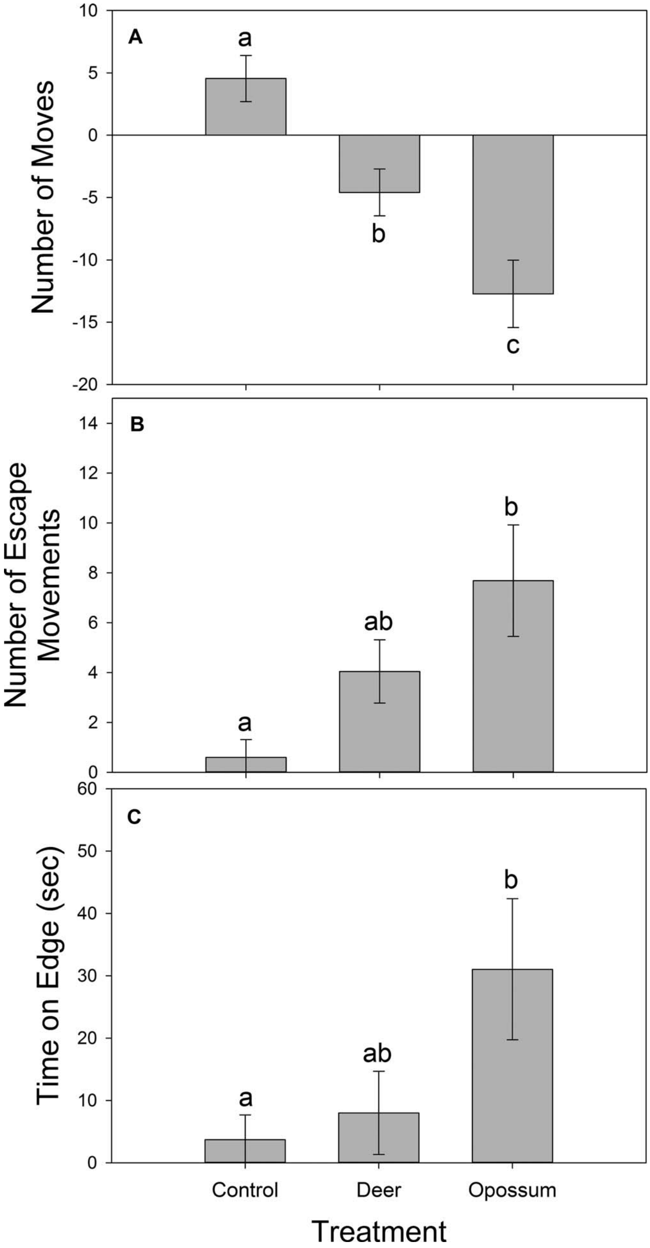

FIG. 2.—Mean (±SE) number of moves (**a**), number of escape movements (**b**), and time on container edge (**c**) by *Plethodon dorsalis* when exposed to a dechlorinated water control and fecal solutions from nonpredatory white-tailed deer (*Odocoileus virginianus*) and predatory Virginia opossums (*Didelphis virginiana*). Salamanders reduced activity ($F_{[2,61]} = 15.2$, $P < 0.001$), exhibited more escape movements ($H = 12.0$, $P = 0.002$), and increased time on container edge ($F_{[2,61]} = 3.3$, $P = 0.04$) when exposed to opossum fecal solutions. Different letters indicate significant differences between treatments ($P < 0.05$)

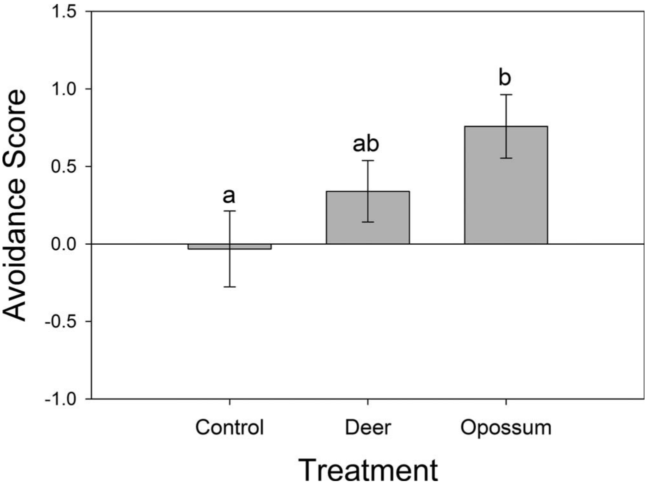

FIG. 3.—Mean (±SE) avoidance score of *Desmognathus fuscus* exposed to a dechlorinated water control and kairomones from nonpredatory white-tailed deer (*Odocoileus virginianus*) and predatory Virginia opossums (*Didelphis virginiana*). Salamanders exhibited greater avoidance of the opossum kairomones relative to the deer and control solutions ($F_{[2,65]} = 3.4$, $P = 0.04$). Different letters indicate significant differences between treatments ($P < 0.05$)

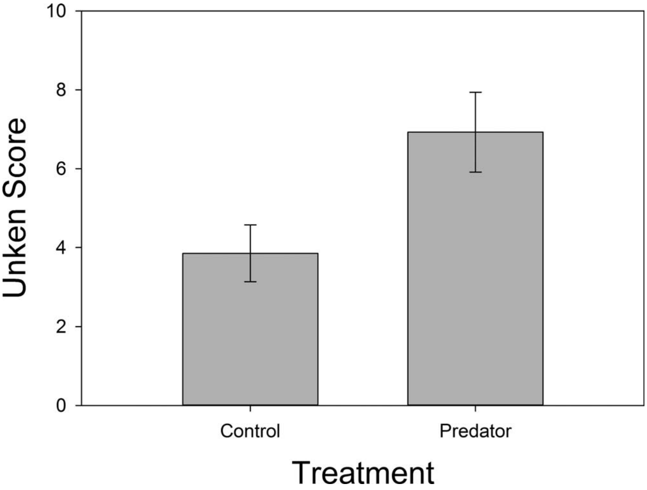

FIG. 4.—Mean (±SE) unken score for Eastern newt efts (*Notophthalmus viridescens*) exposed to dechlorinated water (control) or kairomones from predatory opossums (*Didelphis virginiana*). Efts responded to kairomones from opossums with significantly more intense unken displays relative to a control ($N = 14$ per treatment, $t = -2.47$, $P = 0.02$). Unken score was calculated based on a point system assigned to the angle of the head and tail relative to horizontal and the number of feet off the substrate after the eft went into the unken display

vegetable matter is readily consumed by captive opossums, a distinct preference exists for animal food, especially that of vertebrates (McManus, 1970). Outwardly, opossums do not appear to be active predators, yet active stalk, attack, and subjugation of even large vertebrate prey (*e.g.*, young rabbits) has been documented (McManus, 1970). In our laboratory trials, opossums also successfully attacked salamanders with rapid escape behaviors (*e.g.*, *Desmognathus*). Attacks were made either directly with the mouth (followed by grasping with the paws to prevent escape), or by first grasping with their paws followed immediately by several rapid bites to incapacitate the salamander. The opossum's front paws are highly dexterous, and they can manipulate and consume live salamanders without dropping them. When foraging in the wild, opossums walk with the head lowered, actively sniffing the leaf litter (McManus, 1970). This likely exposes them to salamanders that are active on the surface on damp nights. In addition the habitat preferences and range of opossums and many salamander species largely overlap. Salamanders are abundant in moist deciduous forests in the eastern United States (Petranka, 1998), and while opossums can be unselective with respect to habitat characteristics (McManus, 1970), preference data suggest moist forested habitats are preferred (McManus, 1970). This habitat overlap, combined with a range that encompasses some of the most dense salamander habitat in the world, suggests the potential for interaction between these species is likely high.

While all of the salamanders were palatable to opossums, each species of salamander responded to chemical stimuli from opossums with predator avoidance behavior. Terrestrial salamanders possess several behaviors/adaptations that are used to avoid or escape a predator, the most common being skin secretions and immobility (Brodie, 1977). In addition small plethodontids often coil their body and remain immobile to avoid predation (Brodie, 1977). In response to opossum kairomones, *Plethodon dorsalis* decreased general activity, while movements that did occur involved escaping the enclosure. Conversely, members of the genus *Desmognathus* do not use immobility, tail displays, or other defensive postures (Brodie, 1977), but predominantly use flight and spatial avoidance behaviors (Brodie, 1977). In trials with *Desmognathus*, individuals exposed to opossum kairomones had a higher mean avoidance score, suggesting they recognize the opossum as dangerous and actively avoid areas that contain their kairomones. The only salamander that did not respond to the opossum kairomones with predator avoidance behavior were adult Eastern newts. Adult newts are aquatic and while they have been documented responding to kairomones from aquatic predators (Chapman *et al.*, 2017), the potential for interaction between terrestrial opossums and the aquatic adults is likely limited. However, the juvenile eft stage is terrestrial and spends between 1–10 y on land before migrating back to a pond and undergoing a second transformation (Petranka, 1998). Eastern newt efts respond to a predator's attack with a static defensive posture called an unken display. In this display the eft arches its back and lifts its head, legs, and tail to expose its brightly colored dorsal and ventral surface. This display serves as a warning to the predator that further attack will lead to ingestion of the potent neurotoxin, tetrodotoxin, which is present in the skin of the eft (Brodie, 1968). While previous studies have indicated this toxicity is sufficient to repel raccoons (Hurlbert, 1970; Cochran and Redmer, 1992), in our study, efts were palatable to opossums. When exposed to opossum kairomones and a simulated predation event, efts exhibited a more extreme unken display compared to efts exposed to only water. Opossums are well-documented to consume a wide variety of other dangerous prey including venomous snakes (*see* review in Voss and Jansa, 2012), toads that contain toxic bufodienolides (McManus, 1970; Garrett and Boyer, 1993), and newts (McManus, 1970; this study). While the unken display may be an adaptive approach to dealing with avian predators, it is unlikely to work effectively against a relatively large mammal that has well-documented resistance to toxic compounds, such as opossums (Jansa and Voss, 2011; Voss and Jansa, 2012) and which relies heavily on chemosensory cues while foraging at night (McManus, 1970). Future research should focus on nonstatic behavioral changes exhibited by efts in response to these cues.

Recently, Crane *et al.* (2011) conducted a study on interactions between armadillo (*Dasypus novemcinctus*) and the Ozark zigzag salamander (*Plethodon angusticlavius*). When exposed to armadillo kairomones Ozark zigzag salamanders increased escape behavior and oxygen consumption (Crane *et al.*, 2011). These responses are similar to those exhibited in our study, suggesting amphibians exhibit broad-scale recognition of mammalian predators. Even more interesting, Ozark zigzag salamanders responded to airborne cues from armadillo by remaining flat against the substrate (Crane *et al.*, 2011). These results suggest salamanders are capable of recognizing mammalian cues in two different contexts indicative of both high and low risk situations and modify behavior to reduce their predation risk accordingly.

As a group meso-mammals are likely an important, yet understudied, group of predators on amphibians. While considerable attention has been directed toward interactions with small mammals such as shrews (Brodie *et al.*, 1979; Formanowicz and Brodie, 1979; Brodie and Formanowicz, 1981; Robinson and Brodie, 1982), little research has investigated interactions with larger mammals such as opossum, raccoon, badger, armadillo, otter, and skunk. Despite this many meso-mammals have been documented consuming anuran and caudate amphibians [opossum (McManus, 1970); raccoon (Schaaf, 1970; Azevedo *et al.*, 2006; Stokes *et al.*, 2011); armadillo (Fitch *et al.*, 1952; Sikes *et al.*, 1990); skunk (Hamilton, 1936; Wright, 1966; Azevedo *et al.*, 2006); badger (Azevedo *et al.*, 2006; Gutiérrez-González *et al.*, 2016); otter (Stokes *et al.*, 2015)]. These observations, coupled with the results presented here, suggest mammals, and especially opossums, may be an important predator on amphibians and that these species have evolved mechanisms to reduce predation risk.

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