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The Influence of Tail Autotomy on Avoidance of Chemical Alarm Cues in Northern Zigzag Salamanders, *Plethodon dorsalis*

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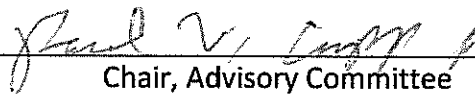
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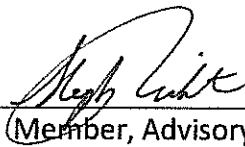
By

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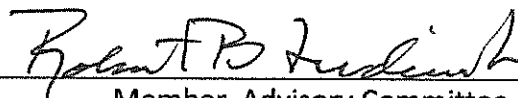
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The Influence of Tail Autotomy on Avoidance of Chemical Alarm Cues in Northern Zigzag
Salamanders, *Plethodon dorsalis*

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ABSTRACT

The morphology and behavior of most species are influenced by predator-prey interactions. To avoid microhabitats that pose an increased predation risk salamanders may use predator avoidance. Salamanders detect chemical alarm cues via the vomeronasal organ and their avoidance of the chemical cues is based upon the apparent associated risk. The objective of this study was to better understand predator avoidance by means of chemical alarm cues in northern zigzag salamanders, *Plethodon dorsalis*. Adult salamanders were placed into Petri dishes with a choice between treated substrate (filter paper treated with chemical cues) or untreated substrate (filter paper treated with distilled water). After a period of habituation, the salamander locations within the dishes were recorded every 3 minutes for 60 minutes. The dishes were then rotated 180° and tested for another 60 minutes. To test for the effects of tail autotomy and time on predator avoidance, responses of tail-autotomized salamanders to chemical cues were compared to responses of tail-intact salamanders on days ranging from 1 to 12 following tail autotomy. Tail-intact and tail-autotomized *P. dorsalis* avoided the chemical cues of injured conspecifics (whole body macerations) and those of eastern garter snakes, *Thamnophis sirtalis*. Tail autotomy combined with the time since tail autotomy did not influence predator avoidance of *P. dorsalis* to chemical alarm cues of injured conspecifics or those of *T. sirtalis*. Also, *P. dorsalis* did not avoid chemical cues of tail-intact or tail-autotomized ravine salamanders, *P. richmondi* (a related sympatric species) or whole body macerations of *P. richmondi*. *Plethodon dorsalis* was shown to avoid the chemical alarm cues of a rinse from the spotted salamander, *Ambystoma maculatum*. Chemical cue avoidance of autotomized conspecific tails approached significance in *P. dorsalis*. The results show no significant difference in the response of male and female salamanders to the chemical cues of *P. richmondi* or conspecific autotomized tails. *Plethodon dorsalis* may reduce the possibility of predation by avoiding microhabitats containing chemical alarm cues of injured conspecifics or those of predators. This research indicates that a reduction in

the anti-predator arsenal of a salamander does not play an influential role in predator avoidance, and the responses to chemical alarm cues are selective in minimizing the associated tradeoffs of reduced foraging and mating opportunities.

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CHAPTER 1

INTRODUCTION

Most organisms are prey to a variety of predators with predation influencing the evolution of morphological and behavioral adaptations of prey species. To avoid predation, salamander species may use anti-predator mechanisms or predator avoidance behaviors. Anti-predator mechanisms occur when salamanders share the same microhabitat as the predator and reduce the probability of successful predation upon predator contact (Brodie et al. 1991). Anti-predator mechanisms in terrestrial salamanders include chemical secretions, posturing, immobility, aposematic coloration, biting, vocalizations, and tail autotomy (Brodie 1977). Predator avoidance behaviors reduce predation in salamanders by decreasing the probability that a salamander will occupy the foraging microhabitat of a potential predator (Brodie et al. 1991). Predator avoidance results in a shift of microhabitat, decreased activity, and increased use of refuge use brought about by the detection of predator chemical cues (Lehtiniemi 2005, Mathis et al. 2003, Sih et al. 1992, Stauffer and Semlitsch 1993). The northern zigzag salamander, *Plethodon dorsalis*, uses mild skin secretions, immobility with coiling, cryptic coloration, and tail autotomy upon contact with predators. To reduce the probability of predator contact, *P. dorsalis* avoids substrates containing chemical odors of ringneck snakes, *Diadophis punctatus* (Cupp 1994). *Plethodon dorsalis* is able to avoid the odors of *D. punctatus* by the detection of chemical cues via the vomeronasal organ.

Chemical detection of predators plays an important role in the vitality of salamander populations. Chemical cues are picked up by the cilia of the nasalabial grooves during nose tapping and are carried to the vomeronasal organ (Dawley and Bass 1988). Along with *P. dorsalis*, the ravine salamander (*P. richmondi*) and the mountain dusky salamander (*Desmognathus ochrophaeus*) from the family Plethodontidae have been shown to avoid substrates marked with the scent of the ringneck snake, *Diadophis punctatus* (Cupp 1994). *Plethodon cinereus* has been shown to avoid chemical cues

from spotted salamanders, *Ambystoma maculatum*, and eastern garter snakes, *Thamnophis sirtalis*, presumably to reduce the risk of predation (McDarby et al. 1999, Madison et al. 1999a). Through the recognition of chemical alarm cues left on substrates, salamanders are able to avoid microhabitats that pose increased predation risk.

Salamanders rely on chemical cue detection for many life functions as well as predator recognition. Red-backed salamanders, *P. cinereus*, switch from a sit-and-wait strategy of foraging to chemical detection with the removal of visual cues (Placyk and Graves 2001). Along with foraging, *P. cinereus* uses scent marking in maintaining a territory (Wise et al. 2004). The seal salamander, *D. monticola*, may use the detection of chemicals on substrates to access resources or in mate recognition (Roudebush and Taylor 1987). *Desmognathus monticola* also uses chemical cues to avoid *D. quadramaculatus* and larger *D. monticola* because of possible predation (Roudebush and Taylor 1987).

With the cost of successful predation being so high, it would be advantageous for prey species to use innate as well as learned behaviors to avoid predation. In *Bufo americanus* (american toad), *B. bufo* (common toad), and *Rana temporaria* (common frog) tadpoles, innate chemo-sensory predator recognition has been shown to require no larval experience (Gallie et al. 2001, Laurila et al. 1997). Soon after hatching, larvae of the red-spotted newt, *Notophthalmus viridescens*, exhibited a response to predator chemical cues (Rohr et al. 2002). Small and large ravine salamanders, *P. richmondi*, showed differences in predator avoidance (although not significant) to chemical alarm cues released during tail autotomy (Hucko and Cupp 2001). While avoidance of chemical alarm cues may change with life stages, innate predator avoidance responses allow prey to avoid microhabitats that present a possible predation event without prior contact with the predator.

Salamander populations need to be able to assess predation risk because predator avoidance may interfere with foraging or reproduction opportunities (Madison et al. 1999b). The tadpoles of the *B. americanus* use chemical cues to balance conflicting demands between avoiding predators and maximizing foraging rates (Petranka 1989). One method of assessing predation risk is to account for predator diet via chemical detection. Sullivan et al. (2004) found that the plethodontids *P. cinereus*, *Eurycea bislineata* (northern two lined salamander), and *Desmognathus ochropheus* (mountain dusky salamander) avoided chemical cues left by predators fed sympatric heterospecifics based on microhabitat overlap. The use of predator diet to avoid predators feeding on closely related heterospecifics has also been observed in grey tree frogs, *Hyla versicolor* (Schoeppner and Relyea 2009). *Plethodon cinereus* has been shown to use diel rhythm and predator diet in assessing the predation risk from *T. sirtalis* (Madison et al. 1999b). By assessing predation risk, salamanders are able to increase the benefits of predator avoidance while decreasing the cost (Petranka 1989).

When predator avoidance has failed, salamander species may respond to encounters with predators using a wide variety of chemical defenses. Chemical glutinous skin secretions of some salamanders cause gagging, pawing, squeaking, and eye irritation in mammals (Brodie et al. 1979). Predators may be immobilized by glutinous skin secretions of some salamanders while allowing the salamander to escape (Evans and Brodie 1994). Toxicity and distastefulness of skin secretions were found to be inversely proportional to the adhesion strength of the secretions (Evans and Brodie 1994). Salamanders with mild skin secretions that pose little threat to predators may use immobility with high frequency upon detection by a predator. *Plethodon dorsalis* was shown to exhibit immobility in the field 66 out of 67 times with the removal of their cover object (Brodie 1977). Immobile salamanders were ignored by avian predators, while movement was found by Brodie (1977) to initiate an attack. By using immobility, an organism lessens the intensity and/or frequency of attacks by predators (Dodd 1989).

Along with cryptic coloration and mild skin secretions, immobility plays an important role in reducing predation in *P. dorsalis*.

If immobility has failed to prevent an attack, many salamander species use tail autotomy as a last line of defense against predation. Salamanders voluntarily autotomize their tails by cleaving the vertebra proximal to the stimulus via intense muscular contractions (Yurewicz and Wilbur 2004). The mountain dusky salamander, *Desmognathus ochrophaeus*, was observed by Brodie et al. (1989) to form a loop via biting its own tail, and the salamander would autotomize the tail when a garter snake bites it. The ability of larger predators to overcome the antipredator mechanisms of smaller prey species may influence the propensity for tail autotomy (Whiteman and Wissinger 1991). Ducey et al. (1993) noted the propensity for tail autotomy increases with a decrease in the noxiousness or toxicity of the salamander's secretions. The small size and mild noxiousness in *P. dorsalis* may lead to an increased use of tail autotomy as a last resort antipredator mechanism.

Salamanders in the family Salamandridae use highly toxic skin secretions as a major defense against predation and show no propensity for tail autotomy. In response to a predator, the fire salamander (*Salamandra salamandra*) is able to spray a high velocity fluid containing neurotoxins, causing death by respiratory paralysis (Brodie and Smatresk 1990). The skin toxin of the red-spotted newt, *Notophthalmus v. viridescens*, may cause neurological responses and death in predators (Brodie 1968). White mice injected with the toxin from *N. v. viridescens* lost coordination, experienced reduced body temperature, and had their lungs fill with fluid (Brodie 1968). The skin of the brightly colored juvenile terrestrial stage (red eft) of *N. v. viridescens* is ten times more potent in toxicity than the cryptic aquatic adult stage (Brodie 1968). The highly toxic red eft stage appears to show no predator avoidance and may be observed on top of leaf litter during daylight hours. *Plethodon dorsalis* mostly remains under rocks, logs, and leaf litter during daylight hours to reduce the likelihood of desiccation and predation.

Tail autotomy is used as a last resort antipredator mechanism due to its high cost (Cooper 2003). In future encounters with predators prior to tail regeneration, the tailless salamander may be more susceptible to predation due to a loss of an antipredator mechanism. However, the high frequency of tail breaks in natural populations suggests the effectiveness and use of tail autotomy as an antipredator mechanism (Vitt et al. 1977). Tail autotomy may also influence various behaviors exhibited by salamanders. Tailless *P. cinereus* exhibit an increase in the number of postcloacal presses in marking its territory and experiences increased aggression by tailed salamander intruders in the marked territory (Wise et al. 2004). In lizards, feeding behavior may be affected by tail autotomy. Some lizards show a reduction in the rate of feeding attempts after tail autotomy as a result of a smaller attack radius and reluctance to move from cover (Cooper 2003). Lizards suffering from recent tail autotomy respond more strongly to the chemical detection of snakes than tailed lizards within the first 10 days after tail loss (Downes and Shine 2001).

Tail autotomy of one individual salamander may influence the predator avoidance behavior exhibited by conspecifics. *Plethodon cinereus* avoids chemical cues from injured conspecifics and heterospecifics in order to evade areas of high potential predation risk (Sullivan et al. 2003). Ravine salamanders, *P. richmondi*, were shown to avoid areas containing the scent of conspecific autotomized tails, but did not avoid areas containing the scent of heterospecific autotomized tails of *P. dorsalis* (Hucko and Cupp 2001).

I investigated the predator avoidance behavior of *P. dorsalis*, a small terrestrial salamander found under rocks and logs in mesic forests. The preferred habitats of *P. dorsalis* contain rocky substrates that offer access to deep underground passages (Petranka 1998). Potential predators of *P. dorsalis* include screech owls (*Otus asio*), woodland birds, small snakes, shrews, and other small predators (Petranka 1998). Antipredator mechanisms employed by *P. dorsalis* consist of cryptic coloring, immobilization, mild noxious skin secretions, and tail autotomy.

Studies have focused on the relationship between predator avoidance and anti-predator behaviors in salamanders, but no study has focused on predator avoidance after tail autotomy. The first objective of this study was to examine the influence of tail autotomy on predator avoidance. The second objective of the study was to study the responses (and the effect of gender) of tail-intact *P. dorsalis* to various chemical cues of sympatric species. Following tail autotomy, it was predicted that *P. dorsalis* would increase the avoidance of substrates containing chemical cues of predators or injured conspecifics. Avoidance was not expected of *P. dorsalis* in responses to substrates marked with a rinse from the sympatric *P. richmondi* due to the sharing of cover objects. I predicted that *P. dorsalis* would avoid substrates treated with chemical cues from injured *P. richmondi* via risk assessment of the chemical alarm cues of the closely related sympatric species. It was also predicted that *P. dorsalis* would avoid substrates marked with chemical cues from a rinse of the much larger *A. maculatum* (a potential predator). Lastly, gender was not predicted to have an influence in the responses of *P. dorsalis* to chemical alarm cues in any of the above tests.

CHAPTER II

MATERIALS AND METHODS

Collection and Care of Study Animals

Adult *P. dorsalis* and *P. richmondi* were collected in February and March of 2011 from mesic hillsides from three sites north of Richmond, Madison County, Kentucky. The salamanders were transported to the laboratory in plastic bags containing moist soil and leaves. Each salamander was placed into a numbered Petri dish (15.0 x 1.5 cm) which had paper towel substrate moistened with aged water. The snout-vent and vent-tail lengths were measured and the weight of each individual was collected. The salamanders were maintained at 10°C and a 12-hr photoperiod. The salamanders were fed wingless fruit flies, *Drosophila melanogaster*, *ad libitum*; and the weight of each individual was periodically determined. Due to the risk of infectious disease introduction from research amphibians into native populations (Picco et al. 2007), the salamanders were not released back into their natural habitat.

Specimens of *T. sirtalis* and *A. maculatum* in the Eastern Kentucky University Biology Department served as the donors of chemical cues in this experiment. Two *T. sirtalis* were maintained at 27°C with a 12-hr photoperiod. Each snake was housed in a 50.8 L x 25.4 W x 30.5 cm H glass aquarium with cypress mulch as a substrate and maintained on an *ad libitum* diet of worms, fish, and salamanders. One *A. maculatum* was maintained on a diet of crickets and earthworms *ad libitum*.

Behaviors Tested

Chemical cue avoidance responses of *P. dorsalis* to the following were tested: (1) tail-intact salamanders to *T. sirtalis* rinse, (2) salamanders without tails to *T. sirtalis* rinse, (3) tail-intact salamanders to *P. dorsalis* macerations, and (4) salamanders without

tails to *P. dorsalis* macerations. Also, chemical cue avoidance responses of *P. dorsalis* to the following were tested: (5) rinse of *P. richmondi*, (6) tails of *P. richmondi*, (7) macerations of *P. richmondi*, (8) rinse of *A. maculatum*, and (9) tails of *P. dorsalis*.

Chemical Cue Collection

The rinse of *T. sirtalis* was prepared by feeding 13 *P. dorsalis* (total mass = 7.27 g) to a snake. The snake was transferred into a 50.8 L x 25.4 W x 30.5 cm H glass aquarium and the aquarium was covered with cheese cloth. After 96 hours, an additional six salamanders (total mass = 6.17 g) were fed to the snake. The salamanders were cooled prior to feeding and placed on a Petri dish lid within the aquarium. After 72 hours the snake was gently transferred into its home aquarium to avoid the release of musk. The aquarium was rinsed with 300 ml of distilled water, and the rinse was passed through a grade 102, medium flow qualitative filter paper in a plastic Buchner funnel to remove large solid particles. The rinse was then rapidly frozen in 5 ml cryovial tubes and stored at -20°C until use.

The body maceration rinses of conspecifics were prepared by homogenizing the entire body after decapitation in a blender with 60 ml of distilled water per 2.6 g of tissue. The rinses were filtered through a fine mesh strainer and prepared the morning of the testing.

The rinse of *P. richmondi* was prepared by placing paper towel substrates of six *P. richmondi* into 60 ml of distilled water. After a period of 0.5 hours, the paper towels were squeezed into the distilled water and the rinse was filtered through a fine mesh strainer. The rinses of autotomized tails were prepared following the procedures of Hucko and Cupp (2001) via inducing tail autotomy by grasping salamanders at 5 mm posterior to the end of the vent with forceps, applying light pressure, and allowing the salamander to release its tail. The tails of eight *P. richmondi* salamanders (total mass =

1.84 g) were crushed by mortar and pestle into 60 ml of distilled water. The tails of ten *P. dorsalis* salamanders, 1.46 g, were crushed by mortar pestle into 40 ml distilled water. The rinses were then filtered through a fine mesh strainer. The body maceration rinse of *P. richmondi* was prepared utilizing the same method as the preparation of the conspecific maceration rinse. The rinse of *A. maculatum* was prepared utilizing the same method as the preparation of the *P. richmondi* rinse.

Avoidance Trials Design

Adult salamanders were placed into Petri dishes with a choice between treated substrate (filter paper treated with chemical cues) or untreated substrate (filter paper treated with distilled water). Two filter paper semicircles were placed on opposite sides of 15.0 x 1.5 cm Petri dishes with a 3 mm gap between each semicircle. Distilled water (1.5 ml) was added to one semicircle of each Petri dish, and chemical cues (1.5 ml) were added to the semicircles of each Petri dish. The Petri dishes were arranged in a grid with five Petri dishes per row (Figure 1). Each Petri dish was spun for an undetermined amount of time so the observer was unaware of which side was the treatment.

Salamanders were transferred to the individual Petri dishes with minimum handling and a 15 mm collar of black paper was placed around each dish to visually isolate each animal. The lights in the room were turned off, and two 40 watt red lights were turned on. After the salamanders were transferred to their Petri dishes, they were given 15 minutes to habituate. Salamander locations within the Petri dishes were observed from a hole in a black curtain. The side of a Petri dish occupied by each salamander was recorded every 3 minutes for 1 hr including the initial position. If the salamander was straddling the 3 mm middle gap then the side estimated to contain the greatest body length was recorded as the side occupied. After the first hour of observation, the Petri dishes were rotated and the experiment was repeated for an additional 1 hr for a total of 42 observations.

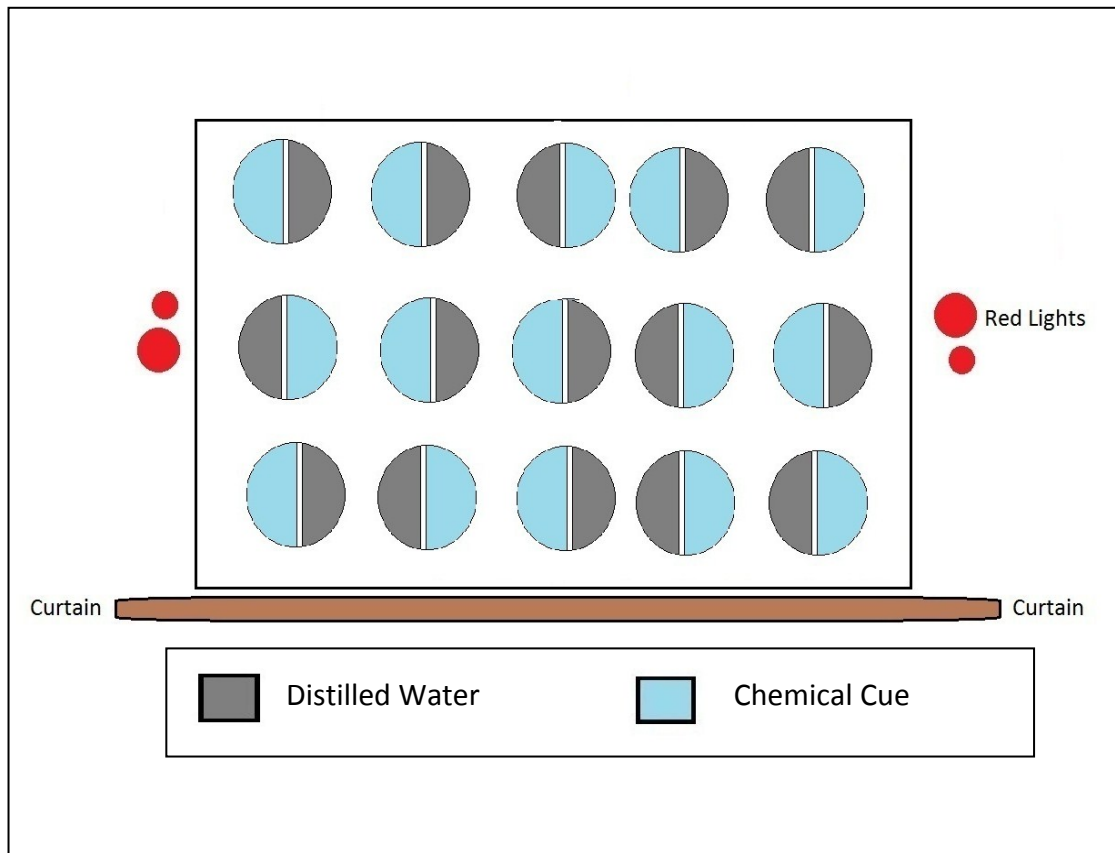


Figure 1. Experimental layout in testing the avoidance behavior of *Plethodon dorsalis* to chemical alarm cues by providing the salamanders with a choice between treated substrate (filter paper treated with chemical cues) or untreated substrate (filter paper treated with distilled water). Salamanders were placed into individual visually isolated Petri dishes under red light and laboratory ambient temperature.

Chemical Cues Avoidance Trials

The trials testing tail-intact salamander responses to a rinse of *T. sirtalis* consisted of 15 randomly chosen females with a meat snout-vent length of 39 mm. The trials testing tail-autotomized *P. dorsalis* to a rinse of *T. sirtalis* consisted of 15 randomly

females with a mean snout-vent length of 41 mm. Tail autotomy was induced via grasping the salamanders 5 mm posterior of the vent, applying light pressure, and allowing the salamander to release its tail. The trials were completed corresponding to days 1, 4, 8, and 12 following tail autotomy with new filter paper and chemical cues. All trials were performed between 1400 and 1900 hr, except for day one following tail autotomy at 2200 to 2400 hr. Temperature was between 20 and 23°C. All trials took place from April 2011 and May 2011.

The trials testing tail-intact salamander responses to maceration rinse of conspecifics consisted of 15 randomly chosen males with a mean snout-vent length of 42 mm. The trials testing tail-autotomized *P. dorsalis* responses to a maceration rinse of conspecifics consisted of 15 randomly chosen males with a mean snout-vent length of 43 mm. The trials were completed corresponding to days 1, 4, 7, and 12 following tail autotomy with new filter paper and chemical cues. The trials were performed from 1100 to 1800 hr with a temperature range of 19 to 24°C. All trials took place from April 2011 and May 2011.

Trials testing the chemical cue avoidance of *P. dorsalis* to rinse, autotomized tails, and body macerations of *P. richmondi* and autotomized tails of *P. dorsalis* consisted of ten randomly chosen males (not previously tested) with a mean snout-vent length of 42 mm and ten randomly chosen females (not previously tested) with a mean snout-vent length of 37 mm. All trials were completed between 1200 and 1800 hrs and between 19 to 22°C. Trials testing the chemical cue avoidance of *P. dorsalis* to the rinse of *A. maculatum* consisted of 13 randomly chosen males (previously not tested) with a mean snout-vent length of 42 mm and seven randomly chosen females (previously not tested) with a mean snout-vent length of 44 mm. The trials testing the chemical cue avoidance of *P. dorsalis* to the rinse of *A. maculatum* were completed from 1100 to 1500 hrs at 21°C. All trials took place from April 2011 and May 2011.

Data Analysis

There were a total of 300 behavioral trials analyzing *P. dorsalis* responses to chemical cues from predators, injured conspecifics, and healthy sympatric salamanders. One hundred and twenty trials were conducted to gauge their responses to chemical cues from eastern garter snakes based on tail status. Fifteen of 30 female salamanders were tail-intact and 15 were observed following tail autotomy. The salamanders were observed for 42 observations over 120 minutes, 1, 4, 8, and 12 days after tail autotomy of the 15 affected animals. A similar analysis was conducted on a collection of 30 male salamanders to gauge their responses to chemical cues from injured conspecifics. The salamanders were observed for 42 observations over 120 minutes, 1, 4, 7, and 12 days after tail autotomy of the 15 affected animals. The same 30 salamanders were used at each time interval, so a repeated-measures design was used. Ten male and ten female salamanders were exposed to chemical cues from *P. richmondi* and conspecific tails to gauge any variation in responses across gender. Finally, 20 salamanders were exposed to chemical rinse from *A. maculatum*. In all experiments, the individual salamander was considered the unit of analysis.

Based on the recommendations of Murray et al. (2004), no attempt was made to test, alter, or regulate the lighting regimen. The chemical rinses were applied immediately before each experiment, so the intensity of chemical cues was consistent in all experiments. The ambient temperature was similar across all experiments.

To examine for differential use of the two sides of the observation area, the analyses used a mixed design two-way ANOVA and independent sample t-tests. The dependent variable for avoidance behavior was the percentage of time spent on the untreated substrate. There were a few cases where the salamander straddled the area between substrates and could not be assigned to one substrate over the other for the particular observation.

Paired-samples t-tests were used to test for temporal effects (potential threat to validity) between the first 21 observations and the final 21 observations for each experiment. No temporal effects were found in the responses to *T. sirtalis* (P=0.593), the responses to *P. richmondi* (P=0.384, 0.867, and 0.957), the responses to *A. maculatum* (P=0.414), and the responses to conspecific tails (P=0.895). There was, however a significant temporal effect in the response to conspecific macerations (P=0.011). In spite of the one exception, the percent of time spent on the control substrate was calculated based on total time for all experiments. Table 1 shows the paired-sample t-tests for temporal effects.

Table 1. Temporal effects (paired-sample t-tests) of the first 21 observations versus the final 21 observations on the untreated substrates across all treatments.

Treatment	t-statistic	df	p-value (2-tailed)
Easter garter snake (<i>Thamnophis sirtalis</i>)	-0.536	119	0.593
Conspecific macerations	-2.596	119	0.011
Ravine salamander (<i>Plethodon richmondi</i>) rinse	-0.891	19	0.384
Ravine salamander autotomized tail	0.170	19	0.867
Ravine salamander macerations	0.054	19	0.957
Spotted salamander (<i>Ambystoma maculatum</i>)	-0.835	19	0.414
Conspecific autotomized tail	0.134	19	0.895

Animal Care

This research was approved by Eastern Kentucky University's Institutional Animal Care and Use Committee: IACUC Protocol Number 03-2010. Animals were collected with the appropriate permits from Kentucky Department of Fish and Wildlife Resources: Educational Wildlife Collecting, SC1011048.

CHAPTER III

RESULTS

In general, zigzag salamanders avoided chemical cues from predators and conspecific macerations. The responses to chemical cues from *T. sirtalis*, *A. maculatum*, and conspecific macerations were statistically significant (Table 2). The responses to conspecific autotomized tails approached significance. The responses of *P. dorsalis* to the chemical cues of *P. richmondi* rinse, autotomized tails, and macerations were not significant (Table 2).

Table 2. Mean proportion of time spent by *P. dorsalis* on untreated substrate (\pm SE) and subsequent one- sample t-tests of the hypothesis of a random (i.e., 50%) use of the untreated substrate.

Treatment	Mean Time on Untreated (\pm SE)	n	t-statistic	p-value (2-tailed)
Easter garter snake (<i>Thamnophis sirtalis</i>)	0.593 (0.033)	120	2.788	0.006
Conspecific macerations	0.610 (0.031)	120	3.482	0.001
Ravine salamander (<i>Plethodon richmondi</i>)	0.549 (0.060)	20	0.808	0.429
Ravine salamander autotomized tails	0.559 (0.057)	20	1.027	0.317
Ravine salamander macerations	0.564 (0.906)	20	0.709	0.487
Spotted salamander (<i>Ambystoma maculatum</i>)	0.751 (0.041)	20	6.121	0.000
Conspecific autotomized tails	0.637 (0.075)	20	1.824	0.084

Effects of Tail Autotomy on Avoidance of Predator Cues

While there was a statistically significant response to *T. sirtalis*, this section shows the results of experiments that analyzed the responses to predators based on tail status and time since tail autotomy. The sample comprised two groups: half with tails and half tested after tail autotomy. The time frames only apply to the tailless group, so the tailed group could be considered as a comparison. The same salamanders were used at each time interval, so a mixed design two-way ANOVA was used to test for differences in responses to predator cues based on tail status and time. For comparison, the time mean percentage of time spent on the untreated substrates across time and standard error of the means for both the tail-autotomized and tail-intact salamanders are shown in Table 3.

Table 3. Mean proportion of time spent by *P. dorsalis* on control substrate based on tail status and time since tail autotomy in responses to chemical cues of *Thamnophis sirtalis*.

	Days	Mean Time on untreated (\pm SE)	n
Tailed	1	0.491 (0.082)	15
	4	0.581 (0.089)	15
	8	0.702 (0.102)	15
	12	0.581 (0.089)	15
	Total	0.587 (0.045)	60
Untailed	1	0.654 (0.079)	15
	4	0.647 (0.096)	15
	8	0.490 (0.116)	15
	12	0.597 (0.105)	15
	Total	0.597 (0.049)	60
TOTAL	1	0.573 (0.058)	30
	4	0.614 (0.064)	30
	8	0.596 (0.078)	30
	12	0.589 (0.067)	30
	Total	0.593 (0.033)	120

The mixed design two-way ANOVA did not show statistically significant differences in the mean proportion of time spent on the untreated substrate based on either tail status ($F_{1,28}=0.009$, $p=0.927$), time since tail autotomy ($F_{3,84}=0.089$, $p=0.966$), or the interaction between tail status and time since tail autotomy ($F_{3,84}=1.896$, $p=0.136$). Mauchly's test confirmed that the data satisfied the assumption of sphericity of the standard errors. The mixed design ANOVA was appropriate due to the fact that the same salamanders were observed over the four time intervals (the repeated measure) for the group with tails and those without (the independent measure) (Field, 2009). The Bonferonni adjustment was used to control the type I error rate.

Effects of Tail Autotomy on Avoidance of Injured Conspecific Cues

This analysis parallels the previous experiment and compares responses to chemical cues from injured conspecific salamanders based on tail status and time since tail autotomy. For comparison, the mean percentage of time spent on the untreated substrates across time and standard error of the means for both the tail-autotomized and tail-intact salamanders are shown in Table 4. The mixed design two-way ANOVA did not show statistically significant differences in the mean proportion of time spent on the untreated substrate based on either tail status ($F_{1,28}=0.412$, $p=0.526$), time since tail autotomy ($F_{2,163,60.552}=0.071$, $p=0.942$), or the interaction between tail status and time since tail autotomy ($F_{2,163,60.552}=0.850$, $p=0.440$). Mauchly's test did reject the assumption of sphericity of the standard errors, so the above statistics are based on the Greenhouse-Geisser adjustment leading to the non-integer degrees of freedom and the adjusted p-values (Field, 2009). The Bonferonni adjustment was used to control the type I error rate.

Table 4. Mean proportion of time spent by *P. dorsalis* on untreated substrate based on tail status and time since tail autotomy in responses to chemical cues of injured conspecific salamanders.

	Days	Mean Time on Untreated (±SE)	N
Tailed	1	0.632 (0.063)	15
	4	0.629 (0.103)	15
	7	0.610 (0.080)	15
	12	0.479 (0.114)	15
	Total	0.587 (0.046)	60
Untailed	1	0.611 (0.049)	15
	4	0.594 (0.099)	15
	7	0.631 (0.996)	15
	12	0.692(0.097)	15
	Total	0.632 (0.044)	60
TOTAL	1	0.621 (0.039)	30
	4	0.611 (0.070)	30
	7	0.620 (0.063)	30
	12	0.586 (0.076)	30
	Total	0.610 (0.031)	120

Effects of Gender on Avoidance of Chemical Cues

Where the two previous analyses broke down responses to predators and conspecific macerations by tail status, this section reports the results of experiments by comparing the responses to chemical cues broken down by gender. The initial analyses used parametric tests. Due to the non-normality of the dependent variable, the section concludes with some non-parametric tests that confirm the results of the parametric tests.

No significant differences were found in the responses of *P. dorsalis* to chemical cues between males and females. The results of the independent samples t-tests comparing the responses of the males and females in the *P. richmondi* and conspecific autotomized tails treatments are shown in Table 5.

Table 5. Independent samples t-tests comparing the mean difference between the proportion of time spent by *P. dorsalis* on control substrate (\pm SE) for the male specimens compared to the female specimens in responses to chemical cues from *P. richmondi* and conspecific salamanders.

Treatment	Mean Difference on Untreated (\pm SE)	df	t-statistic	p-value (2-tailed)
Ravine salamander rinse (<i>Plethodon richmondi</i>)	0.064 (0.123)	18	0.522	0.608
Ravine salamander autotomized tails*	0.083 (0.116)	13.9	0.715	0.486
Ravine salamander	0.090 (0.185)	18	0.489	0.631
Conspecific autotomized tails	0.664 (0.153)	18	0.419	0.680

* The results for Levene's Test ($p=0.042$) for the equality of variances rejected the null hypothesis, so the statistics shown for this experiment are adjusted for unequal variances.

A fundamental assumption of the comparison of means with a t-test is that the data are normally distributed (Field 2009; Zar 2010). The Shapiro-Wilk tests for the responses to macerated ravine salamanders ($p=0.004$) and the responses to a conspecific autotomized tail ($p=0.018$) confirm that the data are not normally distributed.

The results of a Mann-Whitney nonparametric test for the mean differences between males and females across all treatments are consistent with the parametric tests (Table 6). Exact values of the significance are given due to the small sample size (Field 2009). In no case does the nonparametric test reject the null hypothesis that the mean differences are zero.

Table 6. Mann-Whitney nonparametric test comparing the mean difference between the proportion of time spent by *P. dorsalis* on the untreated substrate for the male specimens compared to the female specimens in responses to chemical cues from *P. richmondi* and conspecific salamanders.

Treatment	Mann-Whitney U statistic	p-value (2-tailed)
Ravine salamander (<i>Plethodon richmondi</i>)	46.000	0.782
Ravine salamander autotomized tails	46.500	0.809
Ravine salamander macerations	46.000	0.780
Conspecific autotomized tails	44.500	0.697

CHAPTER IV

DISCUSSION

The purpose of this study was to understand any possible effects that tail autotomy (an anti-predator mechanism) in the northern zigzag salamander, *Plethodon dorsalis*, might have on predator avoidance behaviors to predator chemical cues (eastern garter snake, *Thamnophis sirtalis*, rinse) or to chemical cues of injured conspecifics (whole body macerations) representing a predation event. The study was also aimed at understanding avoidance behavior in *P. dorsalis* in responses to chemical cues of sympatric salamanders; ravine salamander, *P. richmondi*, and the spotted salamander, *Ambystoma maculatum*.

Effects of Tail Autotomy on Chemical Cue Avoidance

Few previous studies have focused on the effects of tail autotomy on salamander behaviors. This is the first study known to focus on the effects of tail autotomy in a salamander on predator avoidance behavior based on chemical cue detection. *Plethodon dorsalis* was shown to avoid substrates treated with a rinse from *T. sirtalis*. Cupp (1994) showed that *P. dorsalis* avoided substrates marked with odors of the potential predatory ringneck snake, *Diadophis punctatus*. *Thamnophis sirtalis* shares habitat structure (under rocks and logs in moist soils) and likely preys upon *P. dorsalis*. When offered in the lab, *T. sirtalis* actively accepted *P. dorsalis* as a prey species. The chemical odors of *T. sirtalis* were avoided by red-backed salamanders, *P. cinereus*, regardless of diet during the day; however, avoidance at night was governed by predator diet (Madison et al. 1999b). Both tail-intact and tail-autotomized *P. dorsalis* were able to assess the risk in predation in responses to the substrates marked with predator odor. However, the results of the experiment showed that tail autotomy and

time since autotomy in *P. dorsalis* does not play a role in avoidance of chemical cues from *T. sirtalis*.

The results of the experiment showed that *P. dorsalis* avoided the chemical alarm cues of macerated conspecifics. Amphibians have been shown to avoid areas of injured conspecifics due to an imposed increased predation risk (Hucko and Cupp 2001, Rohr et al. 2002, Chivers et al. 1999). Tail-intact and tail-autotomized salamanders were shown to avoid the substrates containing the chemical cues of whole body macerations of conspecifics by spending a significantly greater proportion of time on the untreated substrates in the trials. However, tail autotomy and time since tail autotomy was not shown to have an effect on the avoidance of conspecific macerations in *P. dorsalis*. While the salamanders were shown to avoid the predatory and injured conspecific chemical cues, induced tail autotomy resulted in no behavioral changes in the time spent on substrates treated with distilled water versus the time spent on substrates that were treated with chemical cues. The study indicates that tail autotomy in the field does not have an effect on the perceived risk and/or responses to chemical cues.

Responses to Chemical Cues of *Plethodon richmondi*

Previous studies have shown avoidance behaviors of salamanders to the chemical cues of injured sympatric species or closely related species. *Plethodon dorsalis* and the ravine salamander, *P. richmondi*, are active from October thru March (Petranka 1998), and these sympatric species observed during this study were often found together under the same cover objects. No interspecific aggression was observed by Hoppe (2002) between *P. dorsalis* and *P. richmondi* in laboratory studies. In this study, *P. dorsalis* did not avoid substrates marked with the rinse of *P. richmondi*. To reduce the risk of predation, species may avoid areas that contain chemical cues of injured heterospecific species (Sullivan et al. 2003). However, *P. richmondi* has not been found to avoid substrates marked with the chemical cues of autotomized tails from *P.*

dorsalis (Hucko and Cupp 2001). This study tested for the reverse of Hucko and Cupp (2001), and the results indicated that *P. dorsalis* did not avoid the chemical cues from autotomized tails of *P. richmondi*. Whole body macerations may more accurately represent predation events than skin extracts (Sullivan et al. 2003); however, the chemical cues of *P. richmondi* whole body macerations were not avoided by *P. dorsalis*. The results indicate that *P. dorsalis* does not avoid the chemical cues from *P. richmondi* rinse, autotomized tails, or whole body macerations.

Responses to Chemical Cues of *Ambystoma maculatum*

The responses of *P. dorsalis* to the rinse of *A. maculatum* were statistically significant in this experiment. *Ambystoma maculatum* was observed under the same type of cover objects as *P. dorsalis* during the course of this study. However, *A. maculatum* and *P. dorsalis* were not observed sharing the same cover object. *Ambystoma maculatum* has been noted to attack and consume *P. cinereus* in the lab (Ducey et al. 1994), and *P. cinereus* was later found to avoid substrates that were marked with a rinse of *A. maculatum* (McDarby et al. 1999). The results of the study indicate that *P. dorsalis* may avoid cover objects that are occupied by the larger sympatric *A. maculatum*. The avoidance of substrates marked with the chemical cues from the rinse of *A. maculatum* infers a predatory relationship between these sympatric species.

Effects of Gender on Avoidance of Chemical Cues

In testing the responses of *P. dorsalis* to the chemical cues of *P. richmondi* and autotomized conspecific tails, this study showed no difference in the responses of the ten male and ten female salamanders across all four experiments. Gender was shown to have no significant effect on chemical cue avoidance in *P. cinereus* (McDarby et al.

1999, Madison et al. 1999a) and *P. richmondi* (Hucko and Cupp 2001). Dawley (1992) showed sexual dimorphism in the vomeronasal organ in *P. cinereus*. The presence of larger vomeronasal organs in males is thought to occur due to males seeking potentially receptive females during the breeding season (Dawley 1992). All salamanders of this study were sexually mature, either possessing a mental gland or being 32 mm SVL (Petranka 1998). Despite a potential presence of sexual dimorphism in the vomeronasal organ and overlap in the breeding season, no differences were discovered in the responses of *P. dorsalis* to chemical cues of *P. richmondi* or injured conspecifics based on gender.

This study failed to show differences in male and female *P. dorsalis* in responses to substrates marked with the chemical cues of *P. richmondi* or conspecific autotomized tails. However, the chemical cues of *P. richmondi* or conspecific autotomized tails were not found to be significantly avoided. Female sample size in the avoidance responses of *P. dorsalis* to the chemical cues of *A. maculatum* was too small (eight) for comparison with the responses of males. Both genders showed avoidance of substrates marked with chemical cues that are perceived as risk (female responses to *T. sirtalis* rinse and male responses to conspecific whole body maceration).

Responses to Chemical Cues of Conspecific Autotomized Tails

While avoidance by *P. dorsalis* to whole body macerations was observed, this study failed to show avoidance of chemical cues from autotomized conspecific tails. The responses of *P. dorsalis* to conspecific autotomized tails approached significance in this experiment. Hucko and Cupp (2001) showed avoidance in *P. richmondi* to substrates marked with the chemical alarm cues of autotomized conspecific tails. However, *P. cinereus* showed no significant responses to substrates with chemical cues of conspecifics that have been induced to tail autotomize (McDarby et al. 1999). The observation of tail-autotomized *P. dorsalis* in the field may be a result of intraspecific

aggression instead of instances of predator-prey interactions. The alarm cues of autotomized tails may be perceived as a lesser risk by *P. dorsalis* than whole body macerations.

CHAPTER V

SUMMARY AND CONCLUSIONS

My research showed the selectiveness of *P. dorsalis* in the avoidance of substrates marked with chemical alarm cues. The first step of detecting cues in predator avoidance is followed by assessing risk. Due to the reduction in foraging and mating opportunities associated with predator avoidance, salamanders need to be selective in their responses to detected cues. Once the predation risk is assessed, a corresponding adjustment in behavior or habitat use may occur. This research did show avoidance in responses to predator and injured conspecific chemical cues by *P. dorsalis*. However, alarm cues arising from an injured sympatric and closely related species did not invoke predator avoidance. It is through the use of risk assessment that *P. dorsalis* may maximize the gain of predator avoidance while reducing the associated tradeoffs.

This research failed to show a difference in responses of tail-intact and tail-autotomized salamanders in percent time spent on untreated substrates (marked with distilled water) and treated substrates (marked with chemical cues). An opportunity for future research is in the possible effects of tail autotomy on predator avoidance in salamanders on salamander locomotion (percent displaying movement) and nose tapping rates in responses to chemical cues. Future research may focus on the potential effects of tail autotomy on the predator avoidance behavior in responses to both visual and chemical cues (e.g. time spent by *P. dorsalis* on untreated and treated substrates with the absence/presence of contained but visually present *A. maculatum*).

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