

Territorial Behavior in the Plethodontid Salamander *Desmognathus conanti*

Senior Honors Thesis Project Funding Proposal

Introduction

The global decline of amphibian populations has been an issue of great concern within the ecological community over the past several decades (Blaustein and Wake 1996, Stuart et al. 2004, Beebee and Griffiths 2005). The IUCN's 2004 Global Amphibian Assessment reported that one third of the world's amphibian species have now undergone severe population declines or extinctions, with over 5,700 species affected globally (IUCN Red List 2004). This widespread phenomenon has been attributed to a multitude of factors, including diseases, climate change, pollution, and the introduction of invasive species (Blaustein and Wake 1996, Stuart et al. 2004, Beebee and Griffiths 2005). Threats to amphibian populations are of particular interest because amphibians are crucial members of their ecological communities. The majority of amphibians are semi-aquatic, meaning that they occupy different habitats at different life stages and consequently facilitate an important exchange of biomass and energy between aquatic and terrestrial communities. Additionally, their vulnerability to a diversity of environmental stressors throughout their life cycles makes them an important bioindicator species, meaning that their presence and abundance can be used to assess the overall health of an ecosystem.

Although many different factors and their complex interactions with one another contribute to global amphibian decline, the destruction and alteration of habitat via anthropogenic activity is thought to be the primary threat to species today (Gardner, Barlow, and Peres 2007). Given this reality, efforts to conserve existing amphibian populations must focus on conservation of their habitat. Our knowledge of the various environmental factors influencing amphibian population distributions is far from complete for the majority of species. In order for conservation efforts to be effectively targeted, we must come to better understand the various determinants of species abundance and occurrence (Van Buskirk 2005, Gardner et al. 2007). As territoriality mediates both inter- and intraspecific competition in salamanders (Hairston 1987, Hairston 1996, Jaeger and Forester 1993), thereby shaping their wild distributions and regulating their communities, studies of how and why salamander territoriality manifests are essential to conservation efforts. While territorial behavior has been widely observed in terrestrial plethodontids, similar studies in semi-aquatic plethodontid species are scarce. Additionally, while interspecific territoriality in salamanders has been well documented, intraspecific variation in territorial behavior is relatively poorly understood.

In this study, I intend to assess the territoriality of the spotted dusky salamander (*Desmognathus conanti*), which, to the best of my knowledge, has never before been examined. The model of territoriality in plethodontid salamanders that I intend to use in my evaluation is based primarily on studies conducted with the redback salamander (*Plethodon cinereus*) (Rollinson and Hackett 2015). According to these studies, a salamander is only to be understood as territorial if it exhibits site fidelity, site defense (a high probability of expelling intruders), and a mechanism of territorial advertisement (Gergits 1982, Mathis et al. 1995). In order to assess whether *D. conanti* exhibits these behaviors, I will conduct "agnostic" and "pheromone" trials using wild-caught spotted salamanders from Emory University's Lullwater preserve and the Wesley woods research area.

Methods

Field Collections

This study will be conducted in Lullwater preserve, a 135-acre oak-pine-hickory forest on Emory University's campus in metro Atlanta, and Wesley woods research area, a specially designated tract of forest near Emory's campus. A total of 168 spotted dusky salamanders will be collected to be used over the course of this study. In order to account for seasonal variations in activity or behavior, 42 sexually mature individuals will be collected from Lullwater and Wesley woods at four different points, with collections in April, September, October, and November. For every collection, 14 individuals will be captured from each of three distinct streams. One of the streams is located in Wesley woods, and the other two are in Lullwater preserve. These streams reside at approximately the same low elevation (~280m), and their flora composition (predominantly Chinese privet [*Ligustrum sinense*] and English ivy [*Hedera helix*]) and hydroperiod are homogenous. Presence of a variety of Plethodontid salamanders has been established for these streams by previous studies (Orser and Shure 1967, Committee on the Environment 2002) and supported by my personal encounters with them during recreational searching. For each wild-collected individual, I will record the GPS coordinates of the location of its capture. All individual salamanders will be given an identification code linked to information about their sex, approximate age, weight, body size, and any other information that may be relevant to the individual's identity or behavior.

Animal Husbandry

Before they are moved into their lab enclosures, all individuals will be sexed, weighed, and measured (snout-vent length). Additionally, all individuals will be assigned an identification code and checked over for any sign of injury. The salamanders will be kept individually in clear plastic enclosures with ventilation holes. Each enclosure will be lined with moistened paper towels and contain a water dish and a wooden brick propped up on one end as a cover object. Enclosures will be covered by 50% shade cloth throughout the day and night in order to simulate the low-light conditions of their natural habitat. All individuals will be kept for 14 days prior to testing in order to minimize the influence of capture stress on their behavior. Captive individuals will be maintained on a diet of live fly larvae (*Calliphora vomitoria*) and pinhead crickets. The salamanders will be fed and watered every other day and will otherwise remain undisturbed. Paper towels will be replaced as needed, however, only half of the paper towels may be replaced at a time to avoid complete erasure of the animal's pheromone signature.

Agnostic Trials

In order to assess the aggression and expulsion of intruders exhibited by spotted dusky salamanders, agnostic trials will be conducted using 30 paired individuals from each of the 4 collection cohorts. All trials will be conducted between 7:00 and 8:00 in the evening, as wild salamanders are most active in the evening. Within each cohort of 30 individuals, every salamander will be paired with an individual of the same sex and similar body size (n = 15 pairs). Within each pairing, individuals will be randomly assigned the role of "resident" or "intruder". Each salamander will be tested only once, either as a resident or as an intruder, as participation in past trials may influence future behavior.

Each agnostic trial will consist of a timed encounter between paired resident and intruder individuals. In each encounter, the intruder will be removed from its original enclosure and placed into the enclosure of the corresponding resident. Immediately after it is placed into the

resident's enclosure, the intruder will be covered by a clear habituation lid (overturned petri dish) for three minutes. The resident will similarly be removed from its enclosure, handled, replaced, and habituated at the same time as the intruder individual in order to standardize handling stress. The open enclosure will be covered by a clear pane of glass so that the animals may be observed while contained. After both individuals have been habituated, their behavior and any ensuing interaction will be observed for 15 minutes.

For every interaction, I will record the frequency of occurrence of several behaviors that have been shown to indicate aggression in plethodontids. These behaviors include assuming the "all trunks raised" (ATR) body position, various escape behaviors, biting, tail-whipping, head-butting, and chasing (Rollinson and Hackett 2015). Trails will be terminated if either animal is observed repeatedly moving away from the aggressor or fleeing onto the enclosure's walls or ceiling. All trials will be video recorded for reference in further analysis.

Pheromone Trials

In order to assess the capacity for territorial advertisement of spotted dusky salamanders, choice tests will be conducted to determine whether individuals demonstrate a preference for their own chemical markings versus the markings of conspecifics. A specially designed enclosure will be used in these trials, situated as one "origin" chamber connected by similar tunnels to two identical chambers marked by differing pheromonal signaures. One of these two "pheromone" enclosures will be distinguished by a paper towel substrate possessing the chemical signature of a conspecific individual, and the other will contain substrate from the trial participant's own enclosure. The conspecific-marked chamber and the home-marked chamber will both contain a never-before-used cover object (block of wood) and water dish.

For these choice tests, each of the 12 salamanders from the four collection cohorts that are randomly selected to participate in the pheromone trials will be removed from their enclosures into the "origin" chamber of the trial enclosure. After being covered by a habituation lid for three minutes, the individual will be allowed to explore the trial enclosure, eventually "choosing" one of the two connected chambers. For each trial, the number of times that the individual touches its nasolabial cirri to the enclosure's substrate will be recorded. This behavior, called nose-tapping, is used by the spotted dusky salamander to convey chemosensory information to its vomeronasal organ; frequency of nose-tapping may reflect the intensity of the trial individual's interest in the chemical information (odors + pheromones) contained on the substrate.

Data Analysis

In order to assess whether *D. conanti* are indeed territorial, I will conduct a chi-squared test of independence to assess whether territorial behaviors are exhibited in a statistically significant proportion of the agnostic trial interactions. Separate tests will also be conducted to determine whether the occurrence of certain behaviors are correlated with salamander body size, weight, age, and sex. For my analysis of the results of the pheromone trials, I will assess whether the salamanders are choosing one chamber over the other in a statistically significant proportion of the trails (>0.5). Additionally, I will conduct two-sample t-tests assessing whether the frequency of nose-tapping behavior of salamanders in each of the two differently-marked pheromone trial chambers is correlated with salamander body size, weight, age, and sex.

Budget

VWR International

Item	Description	Quantity	Cost (each)
Top Fin ^R Fish Net – 6 inch	(PetSMART.com) For capturing wild salamanders in the field.	2	\$3.99
ScotchBlue TM painter's tape, 0.94 in x 60 yd	(Target.com) For marking salamander enclosures.	2	\$3.29
Sharpie ^R permanent marker, fine tip, 2ct - black	(Target.com) For marking salamander enclosures.	1	\$2.00
Multi-purpose latex gloves – 30ct – up&up TM	(Target.com) For handling salamanders in accordance with sterile technique.	2	\$3.59
IRIS 26.9 Qt plastic storage bin – 6 pack (color: Clear)	(Target.com) Enclosures for animals, for use in lab and for transporting animals from the field to lab. 42 of these enclosures will be used as lab housing or transportational containers for the salamanders. The remaining six bins will be used to fashion two multi-chambered enclosures for the pheromone trials.	8	\$64.99
Zoo Med TM Repti Rock reptile water dish – x small	(PetSMART.com) Water dishes for each individual enclosure.	45	\$5.99
2 in. x 4 in. x 10 ft. standard and better kiln-dried heat treated spruce-pine-fir lumber (model# 161659)	(homedepot.com) I intend to cut this lumber into several 2 in. x 4 in. x 6 in. planks, which will serve as cover objects for the salamander enclosures. In the wild, spotted dusky salamanders spend the majority of the daytime concealed beneath rocks.	3	\$5.76
Make-a-size paper towels – 12 giant rolls – up&up TM	(Target.com) For use as a moistened substrate in animal enclosures and for cleaning enclosures.	1	\$12.39
All Living Things ^R reptile habitat thermometer & hygrometer	(PetSMART.com) For monitoring the temperature and humidity of individual salamander enclosures.	45	\$20.99
6 ft. x 15 ft. evergreen sun screen shade cloth	(homedepot.com) For regulating photoperiod of captive salamanders.	1	\$19.19
Sterilite 20 gal latching storage tote	(Target.com) Enclosure for pinhead cricket breeding colony.	1	\$15.00
Pinhead crickets (1000 crickets in a cup)	(rainbowmealworms.net) Feeder insects for the salamanders. This is the website that I have always ordered feeder insects for my pets from, and I have always had wonderful experiences with them. I intend to start a breeding colony of these crickets so that I do not have to continually order	1	\$30.00

	them. I have experience with starting breeding arthropod colonies through my work as an intern with the amphibian foundation.		
“Spikes” fly larvae (<i>Calliphora vomitoria</i>) (250 larvae)	(rainbowmealworms.net) Feeder insects for the salamanders. It is important for them to have a diverse diet, as they would in the wild. As these will eventually mature into adult flies, I will need to re-order them for each new salamander cohort. The larvae come packaged with all of the food that they will need.	4	\$10.00
Rainbow cricket feed	(rainbowmealworms.net) For feeding pinhead cricket colony.	3	\$5.00
VWR ^R specimen forceps, serrated (catalog #82027-440)	(us.vwr.com) For transporting feeder insects into salamander enclosures.	1	\$14.92
Spray bottle - up&up tm	(Target.com) For maintaining humidity of enclosures and substrate moisture.	1	\$3.79
VWR ^R E-series balance (catalog #10204-996)	(us.vwr.com) For weighing salamanders. *note: I would be more than happy to use any technical balances that the ENVS department may be willing to lend me for this project*	1	\$995.00
16 in. x 20 in. x 0.125 in. clear glass (model# 91620)	(homedepot.com) For placement over top of enclosures so that individuals may be clearly observed during trials.	2	\$5.68
		Total:	\$2931.69

Certifications/Approvals

References

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