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Effects of Sex and Reproductive Condition on Behavior of Southern Red-Backed Salamanders: Activity, Agonistic Behavior and Escape Velocity

Megan N. Mosier
Missouri State University, Mosier753@live.missouristate.edu

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EFFECTS OF SEX AND REPRODUCTIVE CONDITION ON BEHAVIOR OF SOUTHERN RED-BACKED SALAMANDERS: ACTIVITY, AGONISTIC BEHAVIOR AND ESCAPE VELOCITY

A Master's Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science, Biology

By

Megan Mosier

August 2022

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EFFECTS OF SEX AND REPRODUCTIVE CONDITION ON BEHAVIOR OF SOUTHERN RED-BACKED SALAMANDERS: ACTIVITY, AGONISTIC BEHAVIOR AND ESCAPE VELOCITY

Biology

Missouri State University, August 2022

Master of Science

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ABSTRACT

Producing and carrying eggs is energetically costly and therefore can affect behavior. Female Southern Red-backed Salamanders (*Plethodon serratus*) in the Ozarks reproduce biennially, and so during the reproductive season some females are gravid and some are non-gravid. In this study, we compared the exploratory and aggressive behaviors of gravid females, non-gravid females, and males, as well as the escape velocities of each sex class. In exploratory trials, sex did not affect distance travelled, but gravid females and males showed shorter latencies to move than non-gravid females during the winter. During the aggression trials, individuals were subjected to a stress treatment (simulated attack) or a control (no attack) and allowed to recover for 5 min. Individuals were then placed into chambers containing chemical secretions from another salamander, and agonistic and movement data were recorded. For all behaviors, salamanders in all three groups decreased their activity when they were in the stress treatment. Gravid females showed a faster latency to move and higher levels of threat behavior than non-gravid females or males. Sex class did not significantly affect the escape velocities of the salamanders. Overall, gravid females moved sooner than non-gravid females and males and showed higher levels of aggressive behavior.

KEYWORDS: *Plethodon serratus*, gravid, behavior, exploration, aggression, sprint speed, sex differences, reproductive condition

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A Master's Thesis
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August 2022

Approved:

Alicia Mathis, Ph.D., Thesis Committee Chair
Day Ligon, Ph.D., Committee Member
Brian Greene, Ph.D., Committee Member
Julie Masterson, Ph.D., Dean of the Graduate College

In the interest of academic freedom and the principle of free speech, approval of this thesis indicates the format is acceptable and meets the academic criteria for the discipline as determined by the faculty that constitute the thesis committee. The content and views expressed in this thesis are those of the student-scholar and are not endorsed by Missouri State University, its Graduate College, or its employees.

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I dedicate this thesis to Joe Hardesty, who was always excited to ask me if I was still studying worms. You are missed.

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INTRODUCTION

Changes in behavior and energetic requirements associated with reproduction can impose significant fitness costs. Advertisement and courtship rituals often require large expenditures of energy and can expose individuals to greater predation risks (Hughes et al., 2012, Grafe et al. 1992). In some species, migration to breeding sites increases energy expenditures (Ryser, 1989), compromises the immune system's response to diseases (Venesky et al., 2020), and exposes individuals to increased predation risk (Rittenhouse et al., 2009). For females, the production of gametes can require substantial energy input (Hayward & Gillooly, 2011) leading to increased metabolic rates (Angilletta & Sears, 2000; Fitzpatrick, 1971), significantly reducing growth and survival (Cox & Calsbeek, 2010). In addition, added weight of carrying eggs or embryos can hinder locomotion, which can hinder escape from predators (Husak, 2006). These costs of reproduction can lead to changes in behavior of the individuals in breeding condition.

Behavioral changes by gravid females have both associated benefits and costs. For example, increased mass or bulk ("load") can impair sprint speed (Shine, 2003), so gravid females in some species compensate for the increased predation risk by remaining closer to refugia (Braña ,1993; Dominguez-Lopez et al., 2018). However, limiting foraging areas can have other costs: pregnant Common Wall Lizards (*Podarcis muralis*) remained close to refugia and relied more heavily on crypsis rather than speed, but at a cost of significantly lowered body temperatures (Braña, 1993). Oftentimes, gravid females can mitigate some of the costs of behavioral changes associated with predator avoidance by assessing immediate predation risk and changing their behavior only when threat levels are high (threat-sensitive predator avoidance: Helfman, 1989). For example, gravid Spanish Pygmy Newts (*Triturus pygmaeus*), forage in the water column in the absence of predatory Eastern Mosquitofish (*Gambusia holbrooki*), but remained at the bottom of the water column when mosquitofish were present; the fitness cost of this change in foraging behavior was direct: females oviposited fewer eggs in the presence of mosquitofish (Cabrera-Guzmán et al., 2019). Other changes in behavior associated with decreased locomotor performance include allowing predators to have a closer approach distance (Kissner et al., 1997) and increasing aggression toward predators (Mullich, 2007). Not all changes in behavior are related to predation risks. Pregnant female Western Terrestrial Garter Snakes (*Thamnophis elegans*), for example, raised their body temperatures by increasing time basking, but their focus on basking resulted in reduced time spent feeding (Gregory et al., 1999).

Increased aggression by gravid females during interactions with conspecifics occurs across a variety of taxa (Svare et al., 1986; Mello et al., 1999; Mathiron et al., 2019) and most likely indicates a need to secure resources necessary for egg production or compensation for a lowered ability to escape due to egg load. In a non-social parasitoid wasp, *Eupelmus vuilleti,* injections of juvenile hormone increased the number of mature eggs and increased the number of aggressive behaviors displayed and success in competition for hosts for egg deposition (Mathiron et al., 2019). Similarly, pregnant Rockland-Swiss Albino Mice (*Mus musculus*), show heightened levels of aggression towards conspecific intruders, but this pregnancy-induced aggression decreased following hysterectomy, and did not increase to normal levels despite injections of progesterone (Svare et al., 1986). Heightened frequency and intensity of aggression by gravid female American Lobsters (*Homerus americanus*), is apparently due to a compromised ability to escape due to a larger egg load (Mello et al., 1999).

For terrestrial woodland salamanders (Plethodontidae), the gravid period (i.e., carrying enlarged follicles) typically lasts for several months (Herbeck and Semlitsch, 2000). In some species of the genus *Plethodon*, both males and gravid and nongravid females are territorial, defending feeding patches under and around cover objects on the forest floor (e.g., Mathis et al., 1998). Exploring areas that are more distant from an established territory increases the risk of losing territorial resources or coming into conflict with a conspecific. Females of the southern red-backed salamander (*Plethodon serratus*), and the Ozark Zig-zag Salamander (*P. angusticlavius*), were more cautious than conspecific males about leaving their territories (Lynn et al., 2019), but no comparisons were made between gravid and nongravid females.

Carrying eggs has been shown to affect sprint speed and metabolic rates in salamanders. Gravid female Smallmouth Salamanders (*Ambystoma texanum*) have resting O_2 consumption rates that are double that of males or post-gravid females of the species (Finkler & Cullum, 2002; Finkler, 2006), and male and post-gravid female Spotted Salamanders (*A. maculatum*) are able to sustain terrestrial locomotion for longer periods of time than gravid females (Finkler et al., 2003). However, no similar studies have tested the effects of reproductive status on metabolic rates of plethodontid salamanders.

For salamanders, the effects of reproductive condition have only been studied in Red-backed Salamanders (*P. cinereus*); gravid female territory residents spent more time in submissive behaviors toward non-gravid intruders than non-gravid female residents (Horne, 1988), but there was no difference in aggressive behavior between the two reproductive classes. In addition, aggressive behavior by male residents was consistent whether the intruder was a gravid, non-gravid, or male conspecific (Thomas et al., 1989). Brooding female *P. cinereus* exhibit heightened aggression to defend both eggs and the nesting cavity (Bachmann, 1984). My research focuses on a comparison of behaviors of gravid versus non-gravid females and males of *P. serratus*, the Southern Red-backed Salamander. These terrestrial salamanders inhabit the damp leaf litter of mesic and moderately dry areas of hardwood forests in four disjunct populations associated with the Appalachian Mountains, the Ouachita Mountains, the Ozark Plateau, and the Southern Tertiary Uplands of central Louisiana (Petranka, 1998).

Courtship for *P. serratus* occurs in late winter or early spring after cool rains. Females typically produce clutches of 4–10 eggs, which are deposited underground during the spring, and guard the eggs until hatching occurs in August (Herbeck & Semlitsch, 2000). Researchers can easily determine the presence of enlarged follicles by shining a pen light through the wall of the body ("candling") (Gillette & Peterson, 2001) (Figure 1). Some aspects of its life history make *P. serratus* a good candidate for studying the effects of gravidity on a variety of behaviors, including exploratory behavior, sprint speed, and aggression. Both males and females exhibit territorial behavior (Lynn et al., 2019) and it breeds biennially in the Ozarks (Herbeck & Semlitsch, 2000) at the northern extent of its range. Biennial reproduction suggests an energetic cost to being gravid (Highton, 1972). About half of the adult females from my study population were gravid during the 2018 breeding season (pers. obs.).

In this study, I examined the effects of gravidity on exploratory behaviors, sprint speed, and aggression in the Ozark population of *P. serratus*. This species was chosen because of its simultaneous availability of both gravid and nongravid females and because it commonly achieves high local abundance (Herbeck & Semlitsch, 2000; personal observations). Because energetic investment in eggs by gravid females varies seasonally due to growth of follicles (Herbeck & Semlitsch, 2000), I predicted that behavior of gravid females would differ between the early (November/December) and late (May) phases of the reproductive season. I hypothesized that gravid females would exhibit less exploratory behaviors and travel shorter distances. In addition, I predicted that gravid females would exhibit more aggression in response to the physical stress of a simulated predator attack. Lastly, I predicted that gravid females would have a slower escape speed than non-gravid females or males.

METHODS

Collection and Housing

Adult *Plethodon serratus* were collected from Reis Biological Station in Crawford County, Missouri, in fall 2018 and spring 2019 and were transported to Missouri State University. Each salamander was sexed and snout-vent length (SVL) was measured. Sex was determined using the candling method (Gillette & Peterson, 2001) in which the salamander is placed inside a clear plastic bag with a small amount of water, and a pen light is shined through the ventral surface of the body cavity. Males are identified as having visible testes (dark black lines), gravid females are identified by visible yolk-filled eggs (yellowish orbs) and non-gravid females are identified as being of adult size (about 32 mm SVL: Herbeck & Semlitsch, 2000) with no testes and no yolk-filled eggs.

In the lab, each salamander was housed in a petri dish (15 cm diameter) that was lined with dampened filter paper. Dishes were stored in an environmental chamber at 15°C on a 12Light:12Dark cycle, and salamanders were fed 10 *Drosophila hydei* once weekly. Filter paper was changed every 7–14 d as needed.

Winter exploratory behavior trials took place 14 November–11 December 2018, which falls within the courtship season. The agonistic behavior trials and escape speed trials were conducted in the spring during the gravid period. Spring exploratory trials took place 03–16 May. Escape velocity was tested 20–21 May 2019. Agonistic behavior trials took place 24 May–02 June 2019.

Exploratory Behavior: Effects of Reproductive Category and Season

This experiment tested the effects of two factors on exploratory behavior: Sex category (male vs. gravid female vs. non-gravid female) and season (fall vs. winter). Salamanders were selected for testing using a randomized block design. Each salamander was placed in an 8.5-cm habituation dish lined with filter paper and allowed 5 d to establish a territory. These individuals were kept in the environmental chamber at 15°C. Testing occurred on the fifth day. Exploratory behavior was tested by means of an Experimental Ring Design (ERD) arena (Lynn et al., 2019). The ERD consisted of five concentric metal rings (height = 1.5 cm) placed on top of a flat surface lined with dampened paper towels. The largest ring had a diameter of 48.5 cm, and the smallest had a diameter of 18.5 cm. The covered dish containing a salamander with an established territory was then placed in the center circle; this dish became Zone 1, with succeeding zones labeled 1– 6 (Figure 2). After a 10-min habituation period, the cover was removed from the dish and movements of the salamander were recorded during a 15-min observation period.

Behaviors recorded included latency to move, latency to cross a barrier into a new zone, number of reversals into an inner zone, and the outermost zone entered by the salamander. Crossing a barrier occurred when the salamander's pelvic girdle passed over the ring as it entered into another zone (Lynn et al., 2019). The ERD was washed and the paper-towel substrate replaced between trials to remove any chemical cues left by the salamander during the trial.

Because data were not normally distributed (Shapiro-Wilk test, R Studio version 3.0.2), they were transformed using the align-rank procedure (Higgins & Tashtoush, 1994). Means for gravid females, non-gravid females, and males were compared using a two-way ANOVA with "sex" and "season" as factors. Each behavior was analyzed separately. All analyses were conducted in R Studio version 3.0.2.

Agonistic Behavior of Intruders: Effects of Reproductive Category and Stress

In this experiment I tested the effect of two factors on agonistic behavior of territorial intruders: reproductive category (male vs. non-gravid female vs. gravid female) and stress treatment (low vs. high stress). Salamanders were assigned to be either territory residents or intruders using a randomized block design. “Intruders”, which were the focal salamanders in this study, were subjected to either a "high stress" or a "low stress" treatment. Those assigned to the "high stress" treatment were grasped with forceps for 2 min around the pelvic girdle to simulate a predator attack (Watson et al., 2004), while those assigned to the "low stress" treatment were left in their home dish untouched for 2 min. Residents and intruders in each trial were matched for sex category and SVL (within 4.0 mm).

Territorial “residents” were removed from their home chambers and placed in a holding dish. Immediately after the application of the stress treatment, intruders were placed beneath an opaque cover object in the center of a resident’s dish, which had been marked with the resident’s advertisement chemicals. The intruder was released from the cover dish after 5 min. and its behavior was recorded for 10 min. Behaviors recorded were: Latency to Move (LTM); amount time spent in the aggressive All Trunk Raised posture (ATR), in which the salamander's ventral surface is raised up off of the substrate; Flat, in which the entire ventral surface of the salamander is in contact with the substrate; and Edge, in which the salamander is pressed into the side of the dish (Jaeger, 1984; Mathis, 1990).

As in the exploratory behavior data analysis, data in this study were not normally distributed. Therefore, statistical analyses were the same as in the previous study.

Escape Velocity: Effects of Reproductive Category

A camera (GoPro Hero 6 black, 29.97 frames/second, GoPro, Inc.) was suspended 33 cm above the trial platform, which was covered with a damp paper towel substrate. The smallest ERD ring was placed in the center of the platform. A ruler was placed at the corner of the platform for scale. An individual salamander in one of the three randomly selected reproductive categories was placed in the center of the ring and covered with a habituation dish for 1 min. After 1 min, the dish was removed and the recording session began. A predator attack was simulated by grasping the salamander around the pelvic girdle and then immediately releasing it, after which the salamander always rapidly moved around the chamber in an apparent attempt to escape. The recording session ended once the salamander came to a complete stop.

Video clips were uploaded to a computer and then trimmed and converted to MP4 files using an open-source video editing program (Avidemux 2.7). The edited clips were then opened individually in SAVRA, an open-source video analysis program designed specifically to measure sprint speed by digitizing the video frame by frame (Donihue & Kazez, 2014). Data were collected by clicking on the tip of the salamander's snout in each frame. The program, designed to convert the pixels in each frame to *x*- and *y*-coordinates, was used to calculate maximum velocity of the salamander.

Data in this experiment were normally distributed (Shapiro-Wilk test), (R Studio version 3.0.2). Maximum velocities of the three sex categories were compared using an analysis of covariance (ANCOVA) with SVL included as a covariate to account for variation in body size.

Institutional Approval

This study was approved by the Institutional Animal Care and Use Committee on 01 October 2018 and received Approval #17-012.0 (See Appendix).

RESULTS

Exploratory Behavior: Effects of Reproductive Category and Season

Latencies to move were significantly faster during spring than during winter (df = 1; F = 39.677; $p \leq 0.001$) (Figure 3). Although there was no significant main effect of reproductive category on LTM (df = 2; F = 1.906; p = 0.153), there was a significant interaction between reproductive category and season (df = 2; F = 3.586; p = 0.0309) (Figure 3), with gravid females and males beginning to move more quickly than non-gravid females during the winter.

Distance travelled was significantly further during spring than winter (df = 1; F = 46.047; $p \leq 0.001$) (Figure 4). There was no significant main effect of sex category (df = 2; F = 2.540; p = 0.083) on distance travelled and no significant interaction between sex classification and season (df = 2; F = 2.540; p = 0.083) (Figure 4). Although not statistically significant, gravid females showed a tendency ($p < 0.1$) to move further, particularly during spring.

Agonistic Behavior: Effects of Reproductive Category and Stress

There was a significant effect of stress treatment on latency to move, with stressed salamanders taking longer to move than non-stressed salamanders (df = 1; F = 9.756; p = 0.003; Figure 5). Additionally, gravid females exhibited shorter latencies to move than non-gravid females or males (df = 2; F = 4.814; p = 0.011) (Figure 5). Stressed individuals also spent less time in ATR than non-stressed individuals (df = 1; F = 25.460; $p \leq 0.001$; Figure 6), and gravid females spent more time in ATR than non-gravid females or males (df = 2; F = 6.537; p = 0.00237; Figure 6).

Stressed salamanders spent significantly more time in both submissive behaviors Flat (df = 1; F = 4.495; p = 0.0372; Figure 7) and Edge (df = 1; F = 4.237; p = 0.0429; Figure 8) than non-stressed individuals. However, there was no significant effect of sex category on either submissive behavior (Flat: df = 2; F = 2.487; p = 0.090; Edge: df = 2; F = 0.5090; p = 0.603) and no significant interaction (Flat: df = 2; F = 1.248; p = 0.2926; Edge: df = 2; F = 0.933; 0.398) effects for either behavior.

Escape Velocity: Effects of Reproductive Category

There was no difference in the SVL-adjusted maximum escape velocities of gravid females, non-gravid females, and males (df = 1; F = 0.424; P = 0.518; Figure 9).

DISCUSSION

Exploratory Behavior: Effects of Reproductive Category and Season

Season influenced the effects of sex category on exploratory behavior, with gravid females and males showing a shorter latency to initiate movements early in the breeding season (winter trials) than nongravid females. Courtship season for this species falls during the winter months, and during this time, many females begin to develop eggs (Camp, 1988). This may have contributed to the need to quickly begin exploration of the new environment.

There was no significant effect of sex classification on distance moved in the exploratory trials. Other studies of this species using the same methodology reported different results, with males moving further than females (mid-spring trials: Lynn et al., 2019; autumn trials: Reeder, 2013). However, those studies did not distinguish between gravid and nongravid females.

Overall, season influenced exploratory behavior of individuals in all sex categories, with salamanders being faster to initiate movement and moving further distances in the spring than in the winter trials. It is difficult to know whether this difference is due to time of year per se or due to ambient temperature conditions. Although we did not attempt to approximate "natural" temperature regimes in the laboratory, temperatures were warmer during spring trials than during the winter trials, and locomotion in salamanders is positively correlated with temperature (Marvin, 2003), as is typical for ectotherms (Abram et al., 2017). Photoperiod also affects the activity levels of salamanders (e.g., Whitford & Hutchison, 1965; Wood & Orr, 1969). However, both the salamanders tested in winter and those tested in the spring were subjected to the same photoperiod while kept in the lab, so differing day lengths during the two trial periods did not influence activity levels. In the late spring, there is a relatively short amount of time left in the season before terrestrial salamanders retreat to underground burrows for the summer. In comparison to earlier in the activity season, in late spring, the "intruder" salamanders in my study may have had less to gain by remaining in the territory of another individual where they risked engaging in an aggressive contest over ownership.

Agonistic Behavior: Effects of Reproductive Category and Stress

Although gravid females showed shorter latencies to move during exploratory trials, at least during the winter, they were quicker to move when a potential competitor (resident salamander) was present. Gravid females also showed more aggressive posturing (ATR) than nongravid or male intruders. Lynn et al (2019) reported female intruders (reproductive status not taken into account) of this species and of *P. angusticlavius* were also more aggressive than male intruders against same-sex residents (Lynn et al., 2019). The authors suggested that acquiring territories may be particularly important for females because of the high energetic costs of developing and brooding for female plethodontid salamanders (Ng & Wilbur, 1995; Cox & Calsbeek, 2010). This benefit may be even more critical for females that are gravid; females of *P. cinereus* that had large energetic investments in their offspring during egg formation/brooding experienced higher offspring survival (Wise & Jaeger, 2021). This benefit appears to outweigh the high short-term energetic costs (Bortosky & Mathis, 2016) of performing aggressive behaviors such as ATR. In some species from other taxa, such as Eastern Mosquitofish (*Gambusia holbrooki*), aggression also increases in the later stages of gravidity despite gravid females having less energy available (Seebacher et al., 2013). The greater willingness of gravid females to challenge residents was present even when the individuals had experienced the threat of a simulated predator attack.

Although gravid females were more aggressive than males and non-gravid females when stressed, all sex categories spent less time engaging in aggressive postures and more time in submissive behaviors (flat and edge) than when they were not stressed. The aggressive posture, All Trunk Raised (ATR), is a typical vertebrate "look big" threat display (Jaeger, 1984), which would potentially draw attention from a potential predator. Because it is energetically costly (Bortosky & Mathis, 2016), it would also be more difficult to maintain by individuals who are already experiencing stress. Similarly, individuals of *P. angusticlavius* that were subjected to simulated predator attacks also showed decreased threat posturing and increased submissive behaviors (Watson, 2001). Decreased aggression between conspecifics is a widespread response to exposure to predators (e.g., Lange & Leimar, 2001; Zhang et al., 2003; Meuthen et al., 2016). *Plethodon serratus* also respond to physical stress and stress cues from conspecifics by reducing movement and feeding (Watson et al., 2004).

Escape Velocity: Effects of Reproductive Category

Carrying eggs has been reported to inhibit locomotor performance in other taxa. Gravid females are typically slower than nongravid females (e.g., Brown & Shine, 2004; Husak, 2006; Noren et al., 2011). I hypothesized that gravid salamanders in my study would also exhibit slower sprint speeds (maximum escape velocity) than nongravid females or males, but this was not the case. Speed was generally positively correlated with body length (SVL), and gravid females tended to be the largest individuals, but there was no difference in in sprint speed after adjusting for differences in body size. In a similar case, speed was not always affected by reproductive condition for the Australian Scincid Lizards *Lampropholis guichenoti*. Instead, there were population differences in which gravid females in one population were slower than nongravid females, but the opposite was true in a neighboring population (Qualls & Shine, 1997). Gravid females from my study population may compensate for their greater load by investing more heavily in terms of energetics to fuel short-term burst speeds than other sex categories. In addition, individuals may not have had the motivation to sprint at maximum velocity in a laboratory setting. Further study is required to determine trade-offs.

REFERENCES

Abram, P. K., Boivin, G., Moiroux, J. & Brodeur, J. (2017). Behavioural effects of temperature on ectothermic animals: unifying thermal physiology and behavioural plasticity. ‒Biol. Rev. 94:1859–1876.

Angilletta, M. J. & Sears, M.W. (2000). The metabolic cost of reproduction in an oviparous lizard. — Func. Ecol. 14:39–45.

Bachmann, M. D. (1984). Defensive behavior of brooding female Red-Backed Salamanders (*Plethodon cinereus*). — Herpetologica 40(4):436–443.

Bortosky, R. & Mathis, A. (2016). Honest signaling in aggressive contests between Ozark zigzag salamanders (*Plethodon angusticlavius*). — Copeia, 104(1):60–66.

Braña, F. (1993). Shifts in body temperature and escape behaviour of female *Podarcis muralis* during pregnancy. — Oikos 66:216–222.

Brown, G.P & Shine, R. (2004). Effects of reproduction on the antipredator tactics of snakes (*Tropidonophis mairii Colubridae*). ‒ Behav. Ecol. Sociobiol. 56:257–262.

Cabrera-Guzmán, E., Díaz-Paniagua, C., & Gomez-Mestre, I. (2019). Invasive mosquitofish (*Gambusia holbrooki*) affect egg-laying and behaviour of Spanish pygmy newts (*Triturus pygmaeus*). — Amphibia-Reptilia 40(1):103–112.

Camp, C. D. (1988). Aspects of the Life History of the Southern Red-back Salamander *Plethodon serratus Grobman* in the Southeastern United States. — Am. Midw. Nat. 119(1):93–100.

Cox, R. M. & R. Calsbeek. (2010). Severe costs of reproduction persist in Anolis lizards despite the evolution of a single-egg clutch. — Evolution 64(5):1321–1330.

Dominguez-Lopez, M. E., Kacoliris, F. P., & Simoy, M. V. (2018). Escape behavior in gravid and non-gravid females of *Gonatodes albogularis* (Squamata: Sphaerodactylidae). Phyllomedusa 17(1):73–81.

Donihue, C. & Kazez, B. (2014). Illustrating a free, open-source method for quantifying locomotor performance with sprinting Aegean wall lizards. — PeerJ PrePrints, 2, p.e701v1.

Finkler, M. S. (2006). Effects of temperature, sex, and gravidity on the metabolism of small-mouthed salamanders, *Ambystoma texanum*, during the reproductive season. — J. Herp. 40(1):103–106.

Finkler, M. S. & Cullum, K. A. (2002). Sex-related differences in metabolic rate and energy reserves in spring-breeding small-mouthed salamanders (*Ambystoma texanum*). — Copeia 2002(3):824–829.

Finkler, M. S., Sugalski, M. T., & Claussen D. L. (2003). Sex-related differences in metabolic rate and locomotor performance in breeding spotted salamanders (*Ambystoma maculatum*). — Copeia 2003(4): 887–893.

Fitzpatrick, L. C. (1971). Influence of sex and reproductive condition on metabolic rates in the Allegheny Mountain Salamander *Desmognathus ochrophaeus*. — Comp. Biochem. Phys. A: Phys. 40(3):603–608.

Gillette, J. R. & Peterson, M. G. (2001). The benefits of transparency: candling as a simple method for determining sex in red-backed salamanders (*Plethodon cinereus*). — Herp. Rev. 32(4): 233.

Grafe, T. U., Schmuck, R., & Linsenmair, K. E. (1992). Reproductive energetics of the African Reed Frogs, *Hyperolius viridiflavus* and *Hyperolius marmoratus*. — Phys. Zool. 65(1)153–171.

Gregory, P. T., Crampton, L. H., & Skebo, K. M. (1999). Conflicts and interactions among reproduction, thermoregulation and feeding in viviparous reptiles: are gravid snakes anorexic? — J. Zool., Lond. 248:231–241.

Hayward, A. & Gillooly, J. F. (2011). The cost of sex: quantifying energetic investment in gamete production by males and females. — PLoS One 6(1): e16557.

Helfman, GS. (1989). Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. — Behav. Ecol. Sociobiol. 24:47–58.

Herbeck, L. A. & Semlitsch, R. D. (2000). Life History and Ecology of the Southern Redback Salamander, *Plethodon serratus*, in Missouri. — J. Herp. 34(3):341–347.

Higgins, J. J., & Tashtoush, S. (1994). An aligned rank transform test for interaction. — Nonlinear World 1(2): 201–211.

Highton, R. (1972). Distributional interactions among eastern North American salamanders of the genus Plethodon. — Virg. Polytech. Inst. St. Uni. 4:139–188.

Horne, E. A. (1988). Aggressive behavior of female red-backed salamanders. — Herpetologica 44(2):203–209.

Hughes, N. K., Kelley, J. L., & Banks, P. B. (2012). Dangerous liaisons: the predation risks of receiving social signals. — Ecol. Lett. 15:1326–1339.

Husak, J. F. (2006). Do female collared lizards change field use of maximal sprint speed capacity when gravid? — Oecologia 150:339–343.

Jaeger, R.G. (1984). Agonistic behavior of the red-backed salamander. – Copeia 1984:309–314.

Kissner, K. J., Forbes, M. R., & Secoy, D. M. (1997). Rattling behavior of prairie rattlesnakes (*Crotalus viridis viridis*, Viperidae) in relation to sex, reproductive status, body size and body temperature. — Ethology 103:1042–1050.

Lange, H. & Leimar, O. (2001). The influence of predation risk on threat display in great tits. – Behav. Ecol. 12:375–380.

Lynn, C.S., Dalton, B., & Mathis, A. (2019). Territorial behaviour in southern red-backed and Ozark zigzag salamanders: effects of sex, species, and ownership. — Behaviour 156: 1017–1037.

Marvin, G.A. (2003). Aquatic and terrestrial locomotor performance in a semiaquatic Plethodontid Salamander (*Pseudotriton ruber*): influence of acute temperature. Thermal acclimation and body size. – Copeia 2003:704–713.

Mathiron, A. G. E., Earley, R. L., & Goubault, M. (2019). Juvenile hormone manipulation affects female reproductive status and aggressiveness in a non-social parasitoid wasp. — Gen. Comp. Endocrin. 274:80–86.

Mathis, A. (1990). Territoriality in a terrestrial salamander: the influence of resource quality and body size. — Behaviour, 112(3-4), pp.162–175.

Mathis, A., Deckard, K., & Duer, C. (1998). Laboratory evidence for territorial behavior by the southern red-backed salamander, *Plethodon serratus*: influence of residency status and pheromonal advertisement. — Sw. Nat. 43(1):1–5.

Mello, J. J., Cromarty, S. I., & Kass-Simon, G. (1999). Increased aggressiveness in gravid American Lobsters, *Homarus americanus*. — Agg. Behav. 25:451–472.

Meuthen, D., Baldauf, S.A., Bakker, T.C., & Thünken, T. (2016). Predator-induced neophobia in juvenile cichlids. — Oecologia, 181(4):947–958.

Mullich, D. E. (2007). Variation in Defensive Behaviors of the Western Cottonmouth (*Agkistrodon Piscivorus*) as a Result of Temperature, Size, and Reproductive Condition. — MS Thesis. Missouri State University, Springfield.

Ng, M.Y. & Wilbur, H.M. (1995). The cost of brooding in *Plethodon cinereus*. — Herpetologica 1-8.

Noren, S.R., Redfern, J.V., & Edwards, E. F. (2011). Pregnancy is a drag: hydrodynamics, kinematics and performance in pre- and post-parturition bottlenose dolphins (*Tursiops truncatus*). — J. Exp. Biol. 214:4151–4159.

Petranka, J.W. (1998). Salamanders of the United States and Canada. — Smith. Inst. Pr., Wash., D.C., USA.

Qualls, F.J. & Shine, R. (1997). Geographic variation in 'costs of reproduction' in the scincid lizard *Lampropholis guichenoti*. — Func. Ecol. 11:757–763.

Reeder, T. W. (2013). A behavioral syndrome in the southern red-backed salamander (*Plethodon serratus*). — MS Thesis. Missouri State University, Springfield.

Rittenhouse, T.A.G., Semlitsch, R. D., & Thompson, F.R. III. (2009). Survival costs associated with wood frog breeding migrations: Effects of timber harvest and drought. — Ecology 90:1620–1630.

Ryser, J. (1989). Weight loss, reproductive output, and the cost of reproduction in the Common Frog, *Rana temporaria*. — Oecologia 78(2):264–268.

Seebacher, F., Ward, A.J.W., & Wilson, R.S. (2013). Increased aggression during pregnancy comes at a higher metabolic cost. — J. Exp. Biol., 216(5), pp.771–776.

Shine, R. (2003). Effects of pregnancy on locomotor performance: an experimental study on lizards. — Oecologia 136:450–456.

Svare, B., Miele, J., & Kingsley, C. (1986). Mice: Progesterone Stimulates Aggression in Pregnancy-Terminated Females. — Horm. Behav. 20:194–200.

Thomas, J. S., Jaeger, R. G., & Horne, E. A. (1989). Are All Females Welcome? Agonistic Behavior of Male Red-backed Salamanders. — Copeia 1989(4): 915–920.

Venesky, M.D., DeMarchi, J., Marbach, R., Pariyar, K., Hickerson, C.A.M. & Anthony, C.D. (2020). Female Salamanders Experience Higher Parasitism Compared to Males: A Cost of Female Reproduction? — J. Herp., 54(3):293–298.

Watson, R. T. (2001). The influence of predation risk on the foraging and territorial behavior of the Ozark Zigzag Salamander (*Plethodon angusticlavius*). – M.S. Thesis, Southwest Missouri State University, Springfield, MO.

Watson, R.T., Mathis, A., & Thompson, R. (2004). Influence of physical stress, distress cues, and predator kairomones on the foraging behavior of Ozark zigzag salamanders, *Plethodon angusticlavius*. — Behav. Proc., 65(2):201–209.

Whitford, W. G. & Hutchison, V. H. (1965). Effect of photoperiod on pulmonary and cutaneous respiration in the spotted salamander, *Ambystoma maculatum*. — Copeia:53–58.

Wise, S. & Jaeger, R. G. (2021). Maternal body size and condition predict measures of reproductive success and future reproductive allocation in territorial Eastern Red-Backed Salamanders. — J. Ichth. Herp. 109:55–63.

Wood, S. C. & Orr, L. P. (1969). Effects of photo- period and size on the oxygen consumption of the dusky salamander *Desmognathus fuscus*. — Oh. J. Sci. 69(2):121–125.

Zhang, J.-X., Cao, C., Gao, H., Yang, Z.-S., Sun, L., Zhang, Z.-B., & Wang, Z.-W. (2003). Effects of weasel odor on behavior and physiology of two hamster species. – Phys. Behav. 79:549–552.

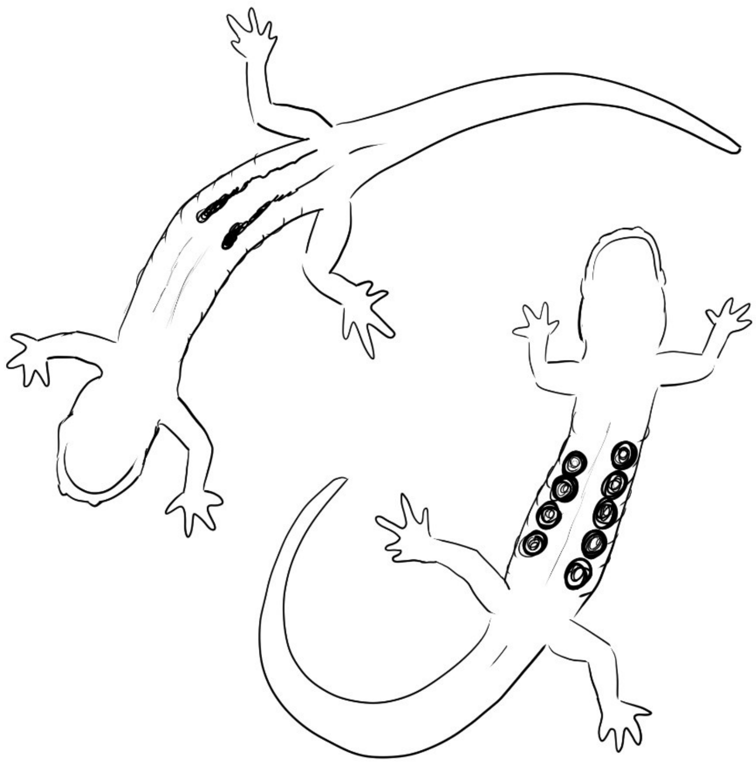

Figure 1. Researchers can determine sex of a salamander by candling. Males (left) are distinguished by the presence of black testes. Gravid females (right) are distinguished by the presence of enlarged follicles.

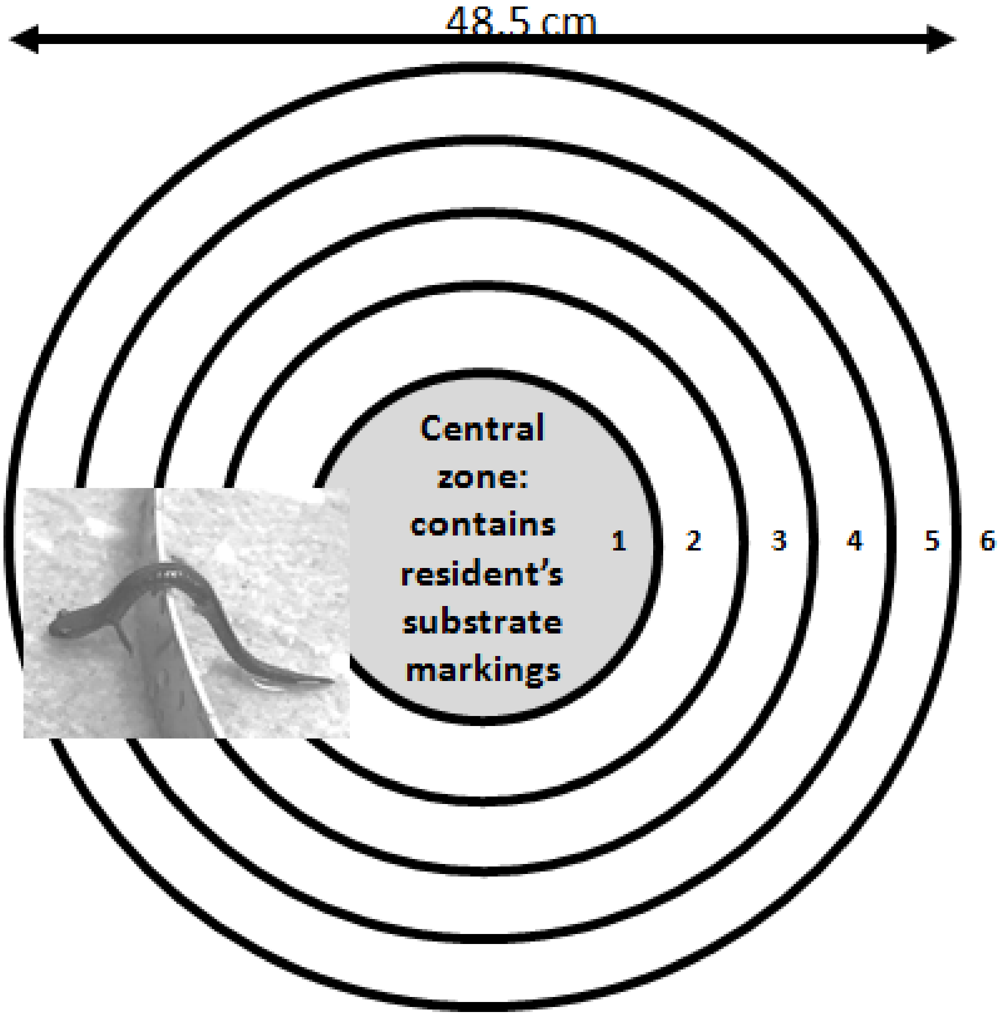

Figure 2. Diagram of the Experimental Ring Design used to examine exploratory behaviors in *P. serratus*. Zone 1 was created by placing the salamander's home dish in the center of the ERD.

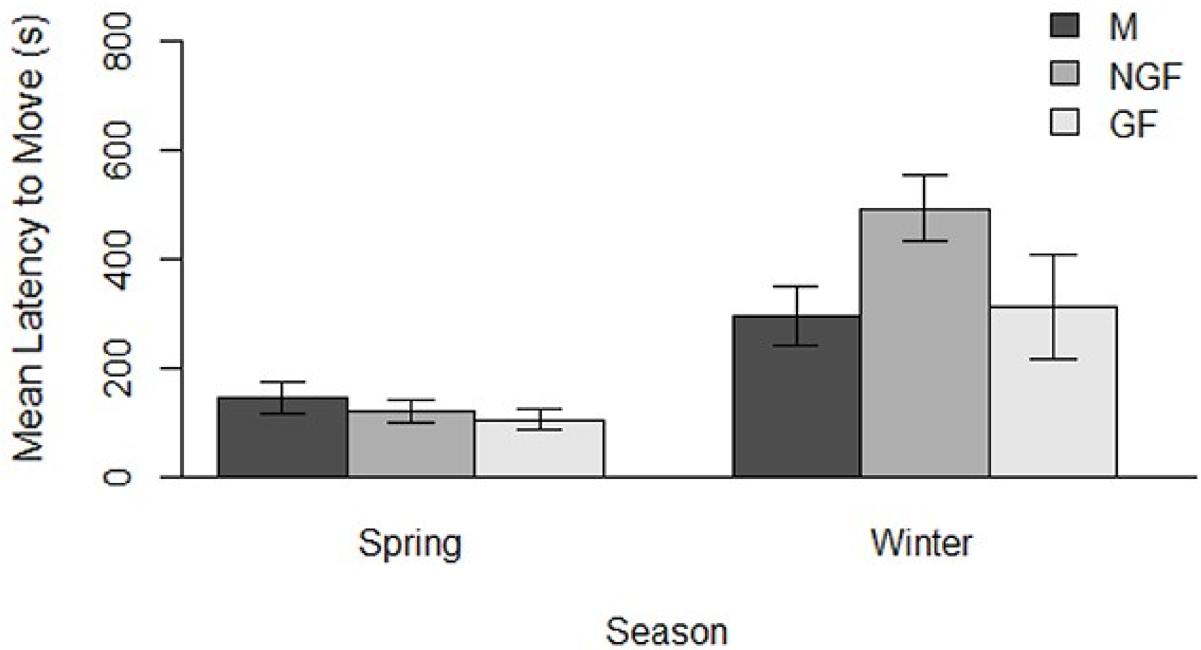

Figure 3. Mean latency to move (±SE) for males (M), nongravid females (NGF), and gravid (GF) females during the spring and the winter.

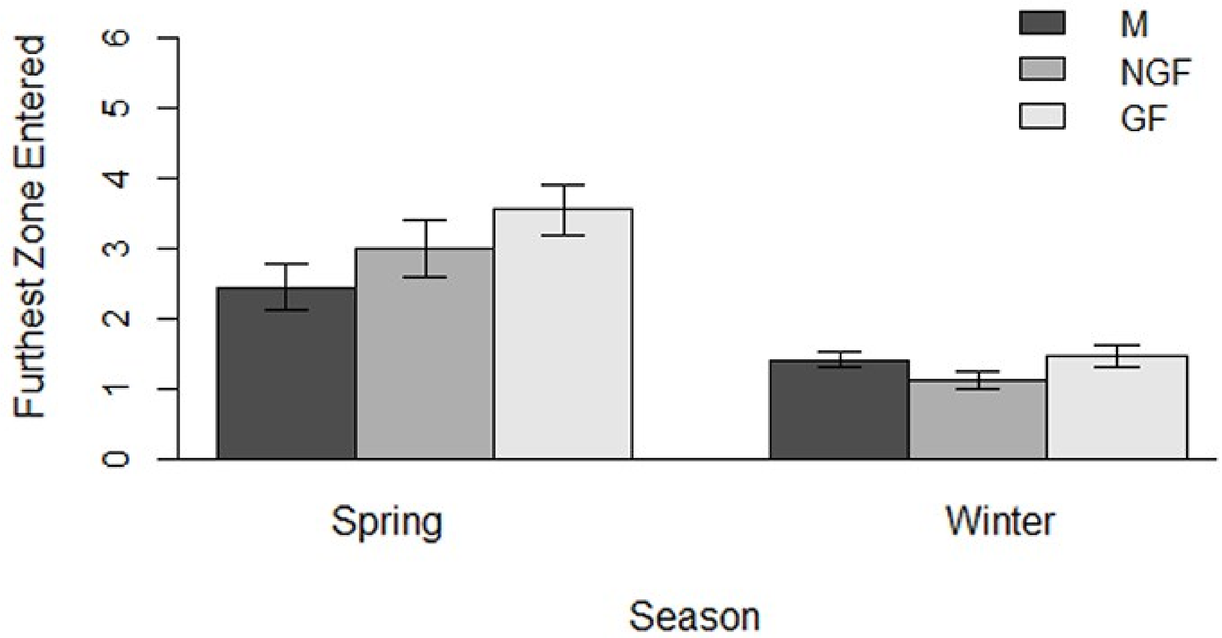

Figure 4. Mean furthest zone entered (±SE) for males (M), nongravid females (NGF), and gravid (GF) females during the spring and the winter.

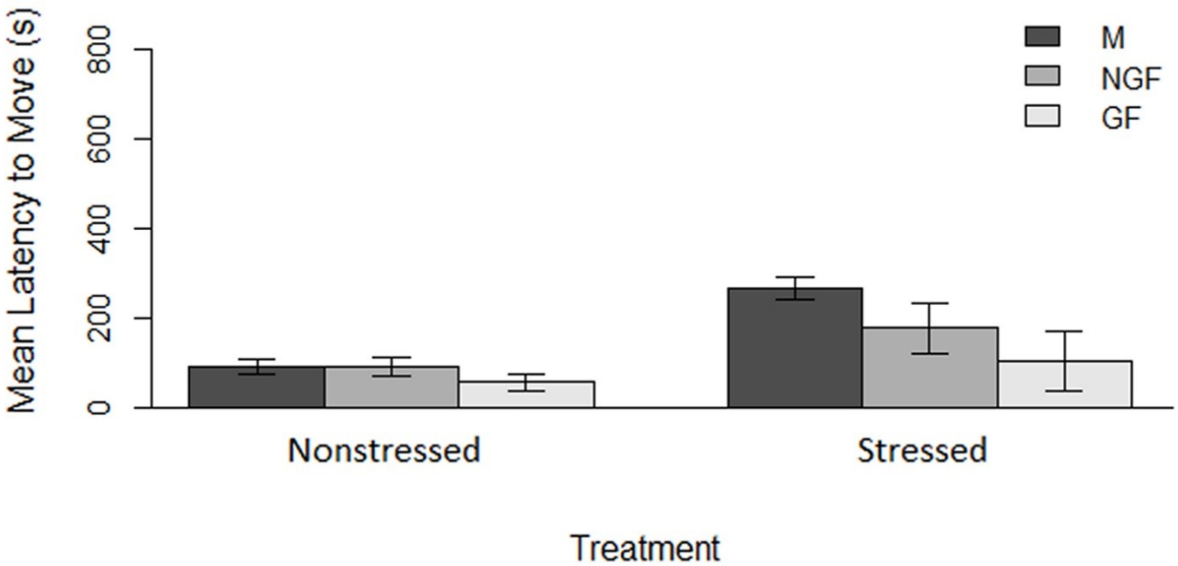

Figure 5. Mean latency to move (±SE) for stressed and non-stressed males (M), nongravid females (NGF), and gravid (GF) females.

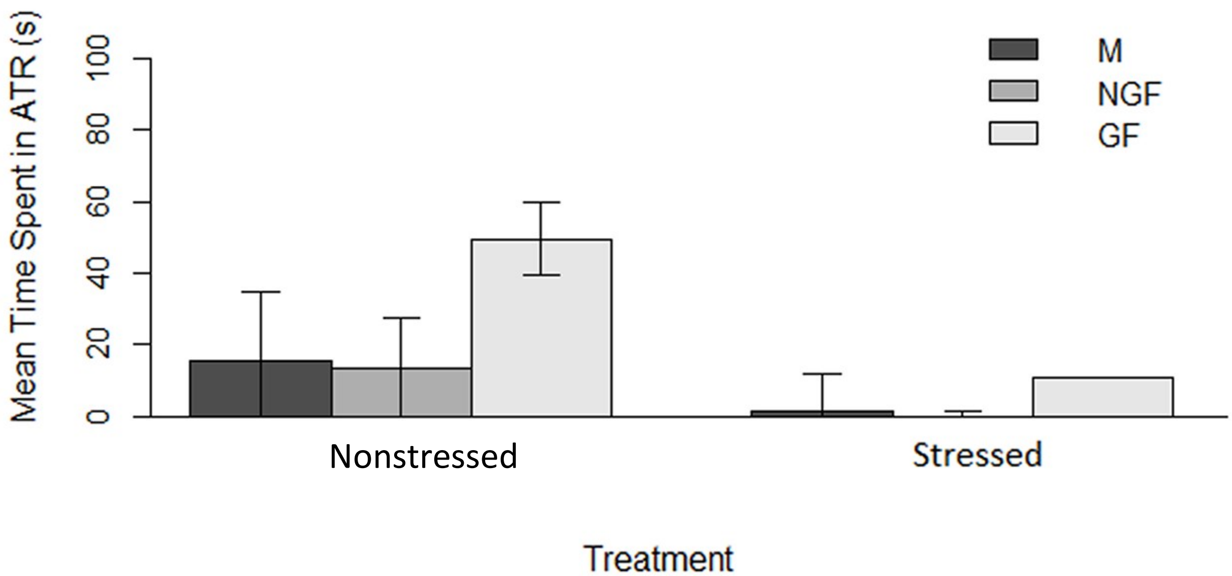

Figure 6. Mean time spent in the “all-trunk raised” display (±SE) for stressed and non-stressed males (M), nongravid females (NGF), and gravid (GF) females.

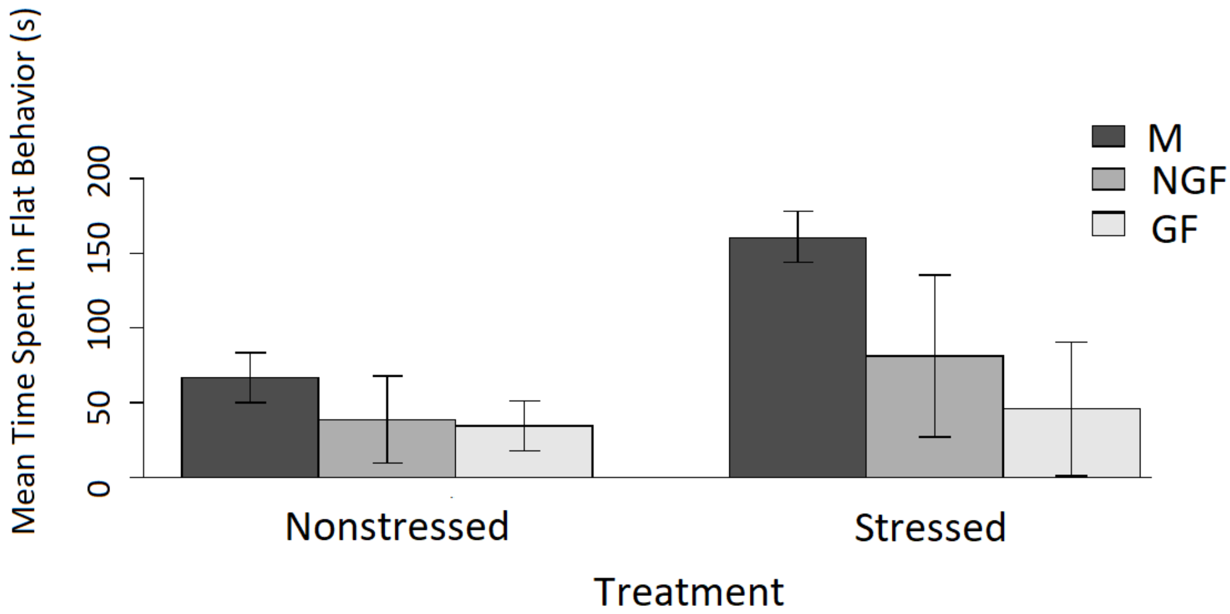

Figure 7. Mean time spent in the "flat" posture (±SE) for stressed and non-stressed males (M), nongravid females (NGF), and gravid (GF) females.

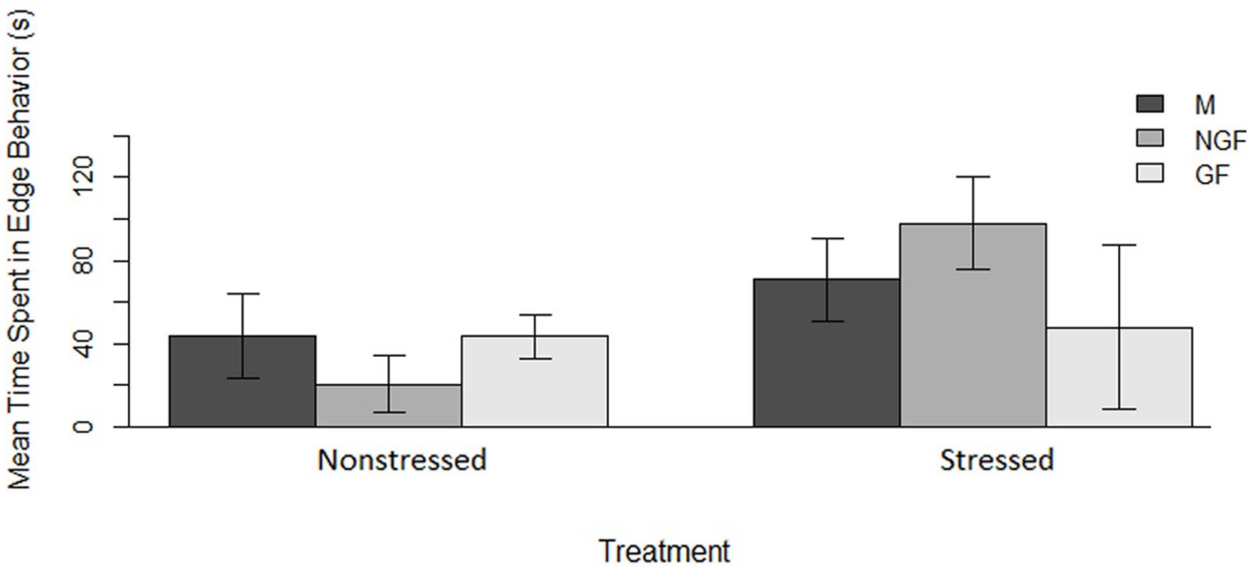

Figure 8. Mean time spent in the "edge" posture (±SE) for stressed and non-stressed males (M), nongravid females (NGF), and gravid (GF) females.

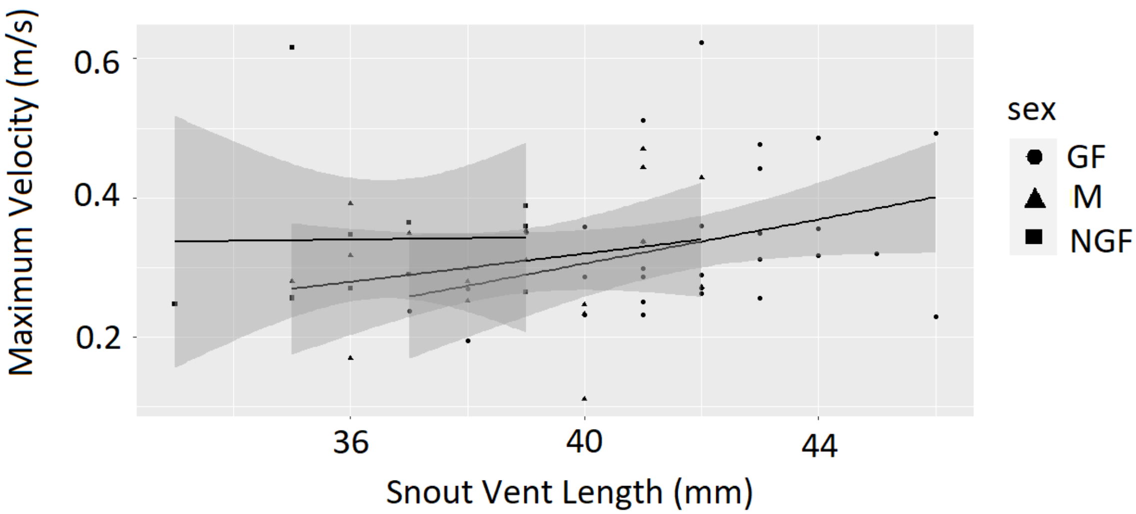

Figure 9. Maximum Escape Velocity for gravid and nongravid females and males using snout-vent length (SVL) as a covariate. Gray shapes indicate 95% confidence intervals.

APPENDIX: MISSOURI STATE UNIVERSITY IACUC APPROVAL NOTICE

From: Institutional Animal Care and Use Committee (IACUC) <iacuc_no_reply@missouristate.cayuse424.com>
Sent: Wednesday, February 15, 2017 2:42 PM
To: Mathis, S Alicia <AliciaMathis@MissouriState.edu>; Mathis, S Alicia <AliciaMathis@MissouriState.edu>; Dalton, Benjamin D <Dalton87@live.missouristate.edu>
Subject: 17-012.0 Approval Notice

Project Title: **Salamander Responses to Predators, Competitors, and Conspecifics: Behavioral and Physiological Effects**
IACUC ID: **17-012.0**
Species: **Multiple Species**

The above-referenced Application has been approved and the approval can be viewed at:

https://missouristate.cayuse424.com/acap/

If you have any questions, please do not hesitate to contact the Missouri State Office of Research Compliance at iacuc@missouristate.edu or 417-836-8405.

Application to Use Live Vertebrate Animals (Amendment)

PI: S. Alicia Mathis — Page: 4 of 5
Dept: Biology
IACUC ID: 17-012.0 — Web ID: 450

Phone: 417-836-5127 **Email:** aliciamathis@missouristate.edu		Weighing and Measuring
Name: **Benjamin D Dalton** **Dept:** 152024 - Biology **Campus Box:** Springfield MO 65897-0027 **Phone:** **Email:** Dalton87@live.missouristate.edu	Co-Investigator (Faculty or Staff) Email Contact Laboratory Coordinator	Anesthesia - Administering Anesthesia - Monitoring Aseptic Technique Forced Exercise Handling and Restraint Netting Sexing Voluntary Exercise Weighing and Measuring
Name: **Katlyn Gardner** **Dept:** 152024 - Biology **Campus Box:** 901 S National Ave Springfield MO 65897-0027 **Phone:** **Email:** gardner013@live.missouristate.edu	Student Investigator	Handling and Restraint Netting Sexing Weighing and Measuring
Name: **Sarah A White** **Dept:** 152024 - Biology **Campus Box:** 901 S National Ave Springfield MO 65897-0027 **Phone:** **Email:** sarah1696@live.missouristate.edu	Student Investigator	Handling and Restraint Sexing Weighing and Measuring
Name: **Joel Medley** **Dept:** 152024 - Biology **Campus Box:** 901 S National Ave Springfield MO 65897-0027 **Phone:** **Email:** medley66@live.missouristate.edu	Student Investigator	Handling and Restraint Netting Voluntary Exercise Weighing and Measuring
Name: **Megan Mosier** **Dept:** 152024 - Biology **Campus Box:** Springfield MO 65897-0027 **Phone:** **Email:** mosier753@live.missouristate.edu	Student Investigator	Handling and Restraint Sexing Voluntary Exercise Weighing and Measuring