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Territorial Behavior of the Ozark Zigzag Salamander, *Plethodon angusticlavius*, Altered by Mode of Predator Cue and Sex of Intruder

Sarah E. Heimbach

Missouri State University, Heimbach521@live.missouristate.edu

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**TERRITORIAL BEHAVIOR OF THE OZARK ZIGZAG SALAMANDER,
PLETHODON ANGUSTICLAVIUS, ALTERED BY MODE OF PREDATOR CUE AND
SEX OF INTRUDER**

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

Sarah E. Heimbach

May 2019

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ABSTRACT

Territorial defense in many species must be balanced with trade-offs in activities such as reproduction and predator avoidance. Adjusting behavior based on current assessments of predation risk and the cost of maintaining or gaining a territory is one way that individuals can balance trade-offs to maximize fitness. I conducted two experiments to determine how Ozark zigzag salamanders, *Plethodon angusticlavius*, adjust their territorial behavior-based predation risk. First, I tested whether male and female territorial intruders changed their competitive behavior according to whether predation risk is assessed via unimodal (chemical) or multimodal (chemical + physical) cues. Females and males responded differently to unimodal and multimodal cues with females generally responding similarly to all predator cues, and males responding to multimodal cues in an additive manner. Second, I determined whether predation risk affected competitive behavior differentially based on whether the intruder salamander was in a territory marked by a same-sex or different-sex residents. Overall, the territorial behavior of both male and female intruders was moderated by the presence of a predator, but the effect differed based on the sex of both the intruder and the resident salamander. The results of these two experiments suggest that *P. angusticlavius* salamanders adjust their territorial behavioral in the presence of predation risk based on the source of the information (unimodal vs multimodal cues) and the sex of nearby individuals (potential mates or competitors).

KEYWORDS: multimodal cue, unimodal cue, predation, territorial, Ozark zigzag salamander, *Plethodon angusticlavius*

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Approved:

Alicia Mathis, Ph.D., Thesis Committee Chair

Brian Greene, Ph.D., Committee Member

Janice 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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INTRODUCTION

Because time and energy is limited, investment in one activity usually comes at a cost to others. For example, time spent in territorial defense leaves less time for activities such as antipredator vigilance (Jakobsson et al. 1995; Jones & Paszkowski 1997). In addition to increased predation risk due to lower vigilance, territorial advertisement signals (markings and aggressive displays) can be detected by predators via “eavesdropping”, which also increases the risk of predation (Jakobsson et al. 1995; Gwynne & O’Neill 1980). Natural selection should favor individuals that assess the level of predation risk and vary their antipredator levels accordingly, devoting more time to territorial defense when predation risk is low and more time to antipredator defense when predation risk is high. Such “threat-sensitive” trade-offs (sensu Helfman 1989) have been observed for a variety of species including dugongs, *Dugong dugon* (Wirsing et al. 2007), black-tailed deer, *Odocoileus hemionus sitkensis* (Chamaille-Jammes et al. 2014), guanacos, *Lama guanicoe* (Marino & Baldi 2008), wall lizards, *Podarcis muralis* (Amo et al. 2004), slimy sculpins, *Cottus cognatus* (Chivers et al. 2001), and larval newts, *Notophthalmus viridescens* (Mathis & Vincent 2000).

Many animals are adept at detecting predators via chemical cues, which are effective in low visibility habitat, when predators are cryptic, and persist over time allowing for earlier detection than visual cues (Mathis et al. 2003). Chemical cues do not, however, give specific predator behavior and location information. Visual cues are not accessible when visibility is low, but when accessible they give accurate behavior and location of the predator.

Most studies have focused on antipredator/territorial defense trade-offs by quantifying behavior in the presence vs. absence of predatory cues, such as cichlid blockheads, *Steatocranus*

casuarius, altering territorial behavior in the presence versus the absence of a predator (LaManna & Eason 2007). However, threat-sensitive responses can be more nuanced. For example, Helfman (1989) reported that territorial damselfish, *Stegastes planif*, varied their responses to predator models based on the size and position of the model predator. For some response variables, responses were step-wise with respect to risk levels, and, in others, there appeared to be threshold or all-or-nothing responses.

Assessment of predation risk can be more accurate when prey use multiple sources of information, such as visual plus chemical “multimodal” cues (Ward & Mehner 2010). Multimodal cues can generally be characterized as either redundant, with all cues providing similar information, or nonredundant, with different sensory modalities accessing different information (Partan & Marler 2005). Redundant cues can increase the probability of predator detection by insuring that information can still be available even if one source becomes limited, such as guppies, *Poecilia reticulata*, relying on visual cues only when chemical cues were unavailable or ambiguous (Brown & Magnavacca 2003). Nonredundant cues can allow the receiver to better tailor their responses based on the additional information, such as anole lizards, *Anolis sagrei*, that modulate their responses to visual cues from avian predators when auditory cues are also available (Elmasri et al. 2012).

Costs and benefits of predation/aggression trade-offs may not be the same for all prey individuals. For example, subordinate, but not dominant, great tits, *Parus major*, reduced aggressive behavior under risk of predation (Lange & Leimar 2001), apparently because subordinates have less to gain by being aggressive under risky conditions. Similarly, territory intruders, but not residents, of Ozark zigzag salamanders, *Plethodon angusticlavius*, responded significantly to predator exposure immediately prior to territorial contests (Parsons 2010).

During reproductive seasons, sex can also influence responses to predators, with males sometimes taking greater risks than females (e.g., Cooper & Wilson 2007). Sex can also influence antipredator/aggression trade-offs if individuals behave differently in the presence of a potential mate than when potential mates are absent (e.g., audience effects in alarm calling: Evans & Marler 1992).

This study examines trade-offs between antipredator behavior and territorial behavior in the Ozark zigzag salamander, *P. angusticlavius*. These lungless salamanders are completely terrestrial, occupying the leaf litter and underground burrows on forest floors in the Ozark Plateau region of the central United States (Conant & Collins 1998). Territorial *Plethodon* forage in the leaf litter during wet periods, but retreat to moisture refuges under rocks and logs when it becomes hot and dry (e.g. Jaeger 1971); suitable retreats are defended by territorial residents (Jaeger & Forester 1993). Both males and females of Ozark zigzag salamanders exhibit territorial defense, (Mathis et al. 2000) and advertise territorial ownership with pheromonal markers deposited on the substrate, or on fecal pellets (Mathis 2000). These pheromonal markers can provide salamanders with a host of information including sex and size (Mathis 1990), parasite load (Maksimowich & Mathis 2001; Dalton & Mathis 2014), diet/territory quality (Walls et al. 1989) and whether tail autotomy has occurred (Wise et al. 2004). These conditions can influence the outcome of territorial disputes in Plethodontid salamanders (Maksimowich & Mathis 2001; Mathis & Britzke 1999; Mathis 1990). Chemical cues are assessed via vomerolfaction during nose-tapping behaviors where the nasolabial cirri come into contact with the substrate and transport the chemicals through grooves to the salamander's vomeronasal organ (Jaeger 1984). Once salamanders have established their territories, defense is via visual displays and bites (Jaeger & Forester 1993). Common

antipredator responses of *Plethodon* and other terrestrial salamanders include avoidance of the marked area (Cupp 1994), reduced foraging (Watson et al. 2004), escape (Mathis & Lancaster 1998), and freezing (Crane et al. 2012). Intruders, but not residents, typically reduce territorial behaviors in the presence of predatory stimuli (Watson 2001; Parsons 2010).

I examined the influence of predation risk on territorial behavior of male and female Ozark zigzag salamanders in two experiments. The first experiment examined whether salamander sex and the presence of unimodal (chemical only, tactile only) versus multimodal cues (chemical and tactile combined) influenced agonistic and chemosensory behavior of intruders. The second experiment tested whether male and female intruders responded differently to cues from same- and opposite-sex residents in the presence and absence of chemical (unimodal) predatory stimuli. These results should shed light on potential costs and benefits of territoriality for male and female salamanders.

METHODS

Collection and Maintenance

This study was approved by the Institutional Animal Care and Use Committee under protocol number 17-012. Salamanders and ring-necked snakes (predators; *Diadophis punctatus*) were caught in the spring and fall of 2015-2016 at Bull Shoals Field Station, Taney Co., MO, USA. Salamanders were housed in petri dishes (14.6 × 2 cm) with moistened filter paper in a temperature-controlled environmental chamber at 15 ± 2 °C with a 12L:12D photoperiod. Filter paper was changed weekly, and salamanders were fed five flightless *Drosophila hydei* fruit flies twice weekly. Salamanders were sexed via candling (Gillette & Peterson 2001) and snout-vent lengths (SVL) were measured. Only salamanders with an SVL of at least 32 mm (likely adults, Wilkinson et al. 1993) were used in trials. Ring-necked snakes (n = 8, 20-30 cm total length) were housed in 3-L glass aquaria with paper towel substrates, water dishes, and PVC hides at room temperature (20-25 °C) with a 12L:12D photoperiod. Paper towels were misted daily with dechlorinated tap water to maintain moisture and changed weekly.

Experiment 1: Response of Territorial Intruders to Unimodal vs Multimodal Predatory Cues

Salamanders (n = 62; captured in September and November of 2015) were assigned to same-sex pairs with a maximum SVL difference of 3 mm to limit body-size differences, which can influence aggressive behavior (Mathis & Britzke 1999). In each pair, salamanders were randomly assigned to be either a resident or an intruder and retained the same residency status

throughout the experiment. Residents and intruders were maintained separately until the start of the behavioral trial.

The experiment followed a repeated-measures design, with each intruder exposed to a water blank (control) and each of three predator treatments in a randomized order immediately prior to each contest: chemical snake cue (unimodal-chemical), physical stimulated attack (unimodal-tactile), and a combination of the chemical cue and physical attack (multimodal-chemical + tactile). Resident salamanders received no treatment, and never encountered the same intruder salamander more than once to avoid possible influences of familiarity (dear enemy effects) on aggressive behavior (Jaeger 1981).

The snake chemical cue was collected after the snakes had been fasted for 4 d, at which point they were moved into individual 400-mL glass beakers. After 48 h, the snake was removed and the beaker was rinsed with 100 mL of dechlorinated tap water, which was then divided into 5-mL aliquots and frozen at $-16 \pm 2^\circ\text{C}$. The water blank was collected in the same way with no snake in the beaker. The resident salamander was moved into the testing arena ($24 \times 24 \times 2\text{cm}$) 5 d prior to testing to establish residency by depositing chemical territorial markers (Nunes & Jaeger 1989). To ensure that there were no chemical cues from prey in the testing arena, both resident and intruder salamanders were fed 10 fruit flies in their home dishes one day before being moved into the trial chambers and were not fed again until testing was complete.

Intruders were exposed to a threat treatment immediately before the start of aggressive trials. The intruder was placed under an opaque dish ($8.5 \times 1\text{ cm}$) in a new petri dish for 3 min before exposure to the randomly-assigned treatment. For the chemical cue treatments, 5 mL of chemical stimulus (blank or snake) was applied evenly to the filter paper. After 3 min, the opaque dish was removed and the salamander was exposed to the chemical stimulus for 5 min.

For the physical attack treatment, the cover dish was removed and the salamander was grasped immediately anterior to the pelvic girdle with forceps for the entire 5 min to mimic the attack of *D. punctatus* (Mathis & Lancaster 1998). For the combined chemical and physical treatment, the chemical and physical stimuli were applied as above, but the salamander's nasolabial cirri made contact with the substrate containing the chemical cue at least three times during the 5-min exposure period. While the intruder was being exposed to the stimuli, the resident was placed under an opaque dish on one side in the testing arena; because the resident did not experience any of the stress treatments, we categorize it as unstressed.

After stimulus exposure, the intruder was rinsed to remove any snake and/or alarm (Watson et al. 2004) secretions, and placed under an opaque dish on the other side of the testing chamber from the resident for 5 min of acclimation. To aid in the identification of the intruder and resident salamander, each was marked with a different colored dot of florescent powder on the top of the head. After acclimation, the opaque dishes were removed, and the intruder's behaviors were recorded for 15 min. At the end of the trial, all salamanders were rinsed and returned to their home dishes and resumed their normal feeding schedules.

The following agonistic and chemosensory behaviors were recorded based on those defined by Jaeger in 1984. (1) Bites, an overt aggressive act in which the salamander makes contact with another and grasps it with its mouth. (2) Agonistic displays (duration): All trunk raised (ATR), an agonistic display where the salamander's trunk is lifted off of the substrate; FLAT, a submissive display where the salamander's entire ventral surface is in contact with the substrate; EDGE, a presumed escape or exploratory behavior in which the salamander is in contact with the wall of the testing arena with at least one foot and the tip of the snout. (3) Passive agonistic behaviors (frequency): Look toward (LT), the salamander moves its head so

that the line of sight is directed toward the other; Look away (LA), either salamander moves its head so that the line of sight established in LT above is broken; Move toward (MT), the salamander approaches the other in a direction that will eventually result in contact; Move away (MA), the salamander moves to actively increase the distance between it and another.

(4) Chemosensory behaviors (frequency): Nose taps (NT), a chemosensory behavior where the salamander presses its nasolabial cirri to the substrate; Nose-tap fecal pellet (NTP), a chemosensory behavior where the salamander presses its nasolabial cirri to a fecal pellet

Because the data were not normally distributed, all data were align-rank transformed using the procedure in ARTool (Higgins & Tantoush 1994). Transformed data were then analyzed using the General Linear Models function in Minitab16. Factors included in the model were ID, sex, treatment, order, and all possible interactions of sex, order, and treatment.

Experiment 2: Does Predation Risk Moderate the Territorial Behavior by Same-Sex and Opposite-Sex Intruders?

In this experiment, the focal intruder salamander was tested on arena substrates that had been marked by a resident, but the resident was not physically present. Salamanders ($n = 59$; collected in November 2016) were assigned to pairs of intruder (focal) and resident (cue donor). Pairs of salamanders were within 3 mm SVL.

Sex treatments were (Intruder-Resident): Male-male, male-female, female-female, and female-male. Each salamander was randomly assigned as a cue donor or focal salamander. This experiment followed a repeated measures design; each salamander was randomly assigned to be either a focal salamander or a cue donor, and retained the same status throughout the experiment, and no focal salamander was exposed to the same cue donor more than once.

Each focal salamander was exposed to each of the following chemical-cue treatments in a randomly selected order: conspecific cue only, snake cue only, or a 50:50 combination of snake and conspecific cue. Snake cue was collected in the same manner as the previous experiment for 5 adult *D. punctatus* (26-30 cm total length) caught in September 2017. Salamander cue was collected by moving the cue donor (resident) to a clean petri dish (14.6×2 cm) with moistened filter paper for 5 d to ensure deposition of chemical markers (Mathis et al. 2000). Both cue donors and focal salamanders were fed five fruit flies the morning of trial to control for hunger. Before each trial, the cue donor was removed from its home dish and 5 mL of dechlorinated water was swirled over the filter paper for 30 s. The salamander-cue solution was pipetted into a vial which was kept at room temperature (20-25 °C) until testing. Immediately before testing, 4 ml of cue was evenly poured on a clean paper towel in the testing arena ($24 \times 24 \times 2$ cm). The focal salamander was then moved from its home dish to the center of the testing arena and placed under an opaque dish (8.5×1 cm) for 5 min to acclimate. After 5 min, the cover dish was removed and the following behaviors were recorded for 15 min: ATR, FLAT, EDGE, and Nose taps (defined above). After trials were concluded, all salamanders were returned to their home dishes.

Because the data were not normally distributed, all data were align-rank transformed using the procedure in ARTool (Higgins & Tantoush 1994). Transformed data were then analyzed with the General Linear Models procedure in Minitab16. The data were divided into two groups for statistical analyses: salamanders exposed to same-sex conspecific cue (male-male and female-female), and those that were exposed to opposite-sex conspecific cue (male-female and female-male). Factors included in the model were ID, sex, treatment, order, and all possible interactions of sex, order, and treatment.

RESULTS

Response of Territorial Intruders to Unimodal vs Multimodal Predatory Cues

Testing order had a main effect only on the number of nose taps ($F_{3,65} = 4.42$, $p < 0.01$), with both sexes decreasing their number of nose taps as the trials progressed. Females tended to be more affected by order than males (Sex*order interactions: p 's = 0.04-0.06), with data for the first trial being somewhat greater in magnitude than in the subsequent trials. There were no other significant interactions between order and treatment (p 's = 0.18-0.92). Data for order effects and ID effects are presented in Appendix A.

Time spent in the aggressive ATR posture was lower in all three predator treatments in comparison to the blank control for both males and females ($F_{3,65} = 5.52$, $p < 0.01$, Figure 1). ATR was not significantly influenced by sex ($F_{1,65} = 0.83$, $p = 0.37$) or by a sex*treatment interaction ($F_{3,65} = 1.86$, $p = 0.14$).

For the submissive FLAT posture, there was a significant interaction between sex and treatment ($F_{3,65} = 3.26$, $p = 0.02$, Figure 2). Females spent more time in FLAT for all three predator treatments compared to the blank control, whereas males spent more time in FLAT for only the combined physical and chemical predator treatment. No factors had a significant effect on time spent in FLAT (sex: $F_{1,65} = 0.99$, $p = 0.32$; treatment: $F_{3,65} = 2.21$, $p = 0.10$) independent of the interaction effect.

For EDGE behavior, the treatment*sex interaction was also significant ($F_{3,65} = 2.75$, $p = 0.05$, Figure 3). Females spent similar amounts of time in EDGE for all treatments, whereas males decreased time in EDGE for the combined predator treatment compared to the other

treatments. No other factors had a significant effect on time in EDGE (sex: $F_{1,65} = 0.01$, $p = 0.93$; treatment: $F_{3,65} = 0.19$, $p = 0.90$) independent of the interaction effect.

Nose taps were lower in the unimodal-tactile and multimodal treatments in comparison to the water blank and the unimodal-chemical treatments for both males and females ($F_{3,65} = 4.46$, $p < 0.01$, Figure 4). No other factors had a significant effect on the number of nose taps (sex: $F_{1,65} = 1.07$, $p = 0.31$; sex*treatment interaction: $F_{3,65} = 0.55$, $p = 0.65$).

Biting ($n = 3$), NTP ($n = 10$), and NTI ($n = 8$) behaviors were too infrequent for statistical testing and are thus excluded from the results.

Effect of Predation Risk on Territorial Behavior by Same-Sex and Opposite-Sex Intruders

Order effects were present in some responses for both opposite-sex pairs, and same-sex pairs, and the same trend was present as in the previous experiment (data for the first trial generally differed from the succeeding trials). There were also significant effects of individual (ID), indicating that some individuals consistently showed higher levels of a given behavior than others. Data for order effects and ID effects are presented in Appendix B. The behavior of one male was substantially different from the other males (e.g., about 300 more seconds in ATR than the next highest male); the data for this male was removed from the analyses.

For ATR, different patterns of responses to predation risk were present for intruders in the presence of pheromones from same-sex vs. opposite-sex residents. In the opposite-sex trials (Figure 5), time spent in ATR by intruders was not significantly affected by treatment, with both males and females showing a trend of lower levels of ATR in the predator-only treatment than when opposite-sex pheromones were present ($F_{2,40} = 2.30$, $p = 0.11$). Males tended to show more ATR than females, but this difference was not significant ($F_{1,40} = 3.09$, $p = 0.09$). The

sex*treatment interaction was not significant ($F_{2,40} = 0.36$, $p = 0.70$). For salamanders in the same-sex trials, time spent in ATR was significantly affected by the sex*treatment interaction ($F_{2,42} = 5.58$, $p < 0.01$). Females spent more time in ATR when exposed to the same-sex conspecific cue in any combination, than the predator cue alone (Figure 6). Males, in contrast, spent more time in ATR when exposed to the same-sex conspecific cue with no predator, and the predator alone, in comparison to the combined treatment (Figure 6). No other factors had a significant effect on time spent in ATR for salamanders in same-sex pairs (sex: $F_{1,42} = 3.75$, $p = 0.07$; treatment: $F_{2,42} = 1.26$, $p = 0.30$).

For FLAT, patterns of responses to predation risk also differed according to whether intruders were exposed to pheromones from same-sex vs. opposite-sex residents. In the opposite-sex trials (Figure 7), time in FLAT was significantly affected by the sex*treatment interaction ($F_{2,42} = 3.20$, $p = 0.05$), with males showing low levels of FLAT in all treatments, and females showing increased levels of FLAT in the predator treatments. The main effect of Treatment was also significant ($F_{2,42} = 11.33$, $p < 0.01$), with this difference driven by the very high levels of flat in the predator treatments by females. Although females tended to show higher levels of FLAT overall, the main effect of sex was not significant ($F_{1,42} = 2.57$, $p = 0.121$). In the same-sex trials (Figure 8), neither interaction effect ($F_{2,42} = 2.38$, $p = 0.10$) nor the effect of sex ($F_{1,42} = 0.07$, $p = 0.79$) were significant, but both males and females spent more time in FLAT in the presence of the predator cues ($F_{2,42} = 5.37$, $p = 0.01$).

EDGE was a frequent behavior and not strongly effected by treatment or sex for both opposite-sex and same-sex trials. In opposite-sex trials (Figure 9), time spent in EDGE behavior was not significantly affected by sex ($F_{1,42} = 0.00$, $p = 0.999$), the sex*treatment interaction ($F_{2,42} = 1.32$, $p = 0.28$), or treatment ($F_{2,42} = 1.86$, $p = 0.17$); a trend was present showing less

EDGE behavior in the presence of predator cues. For salamanders in same-sex pairs (Figure 10), there were no significant effects of sex ($F_{1,42} = 0.15$, $p = 0.70$), treatment ($F_{2,42} = 1.04$, $p = 0.36$), or the sex*treatment interaction ($F_{2,42} = 0.25$, $p = 0.78$) on EDGE behavior.

For NT (chemosensory behavior), the only significant effect was Treatment for both opposite-sex and same-sex trials, but the nature of the effect was somewhat different in the two trials. For opposite-sex trials (Figure 11), number of NT was reduced in the predator only treatments for males, and greatly reduced for both treatments involving a predator for females ($F_{2,42} = 4.55$, $p = 0.02$), with no main effect of sex ($F_{1,42} = 0.34$, $p = 0.56$) and no sex*treatment interaction ($F_{2,42} = 1.77$, $p = 0.18$). For same-sex trials, both males and females only reduced NTs in the predator-only treatment ($F_{2,42} = 5.77$, $p < 0.01$, Figure 12), and there was no significant effect of sex ($F_{1,42} = 1.93$, $p = 0.18$) or the sex*treatment interaction ($F_{2,42} = 0.76$, $p = 0.47$).

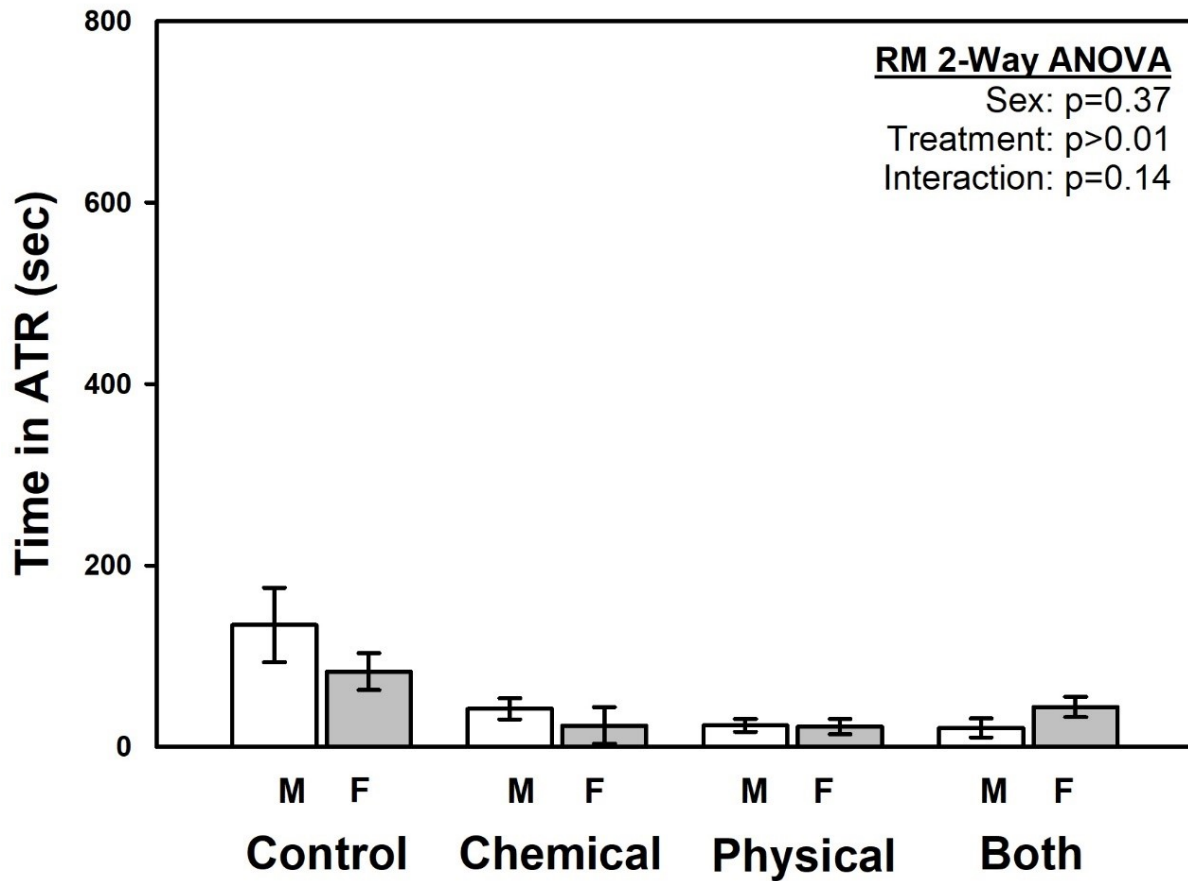


Figure 1. Mean (\pm SE) time spent in All Trunk Raised (ATR) aggressive posture after immediate prior exposure to a blank control or one of three predator treatments. White bars are males ($n=8$) and gray bars are females ($n=21$). All statistics were calculated with data transformed using the aligned rank transformation.

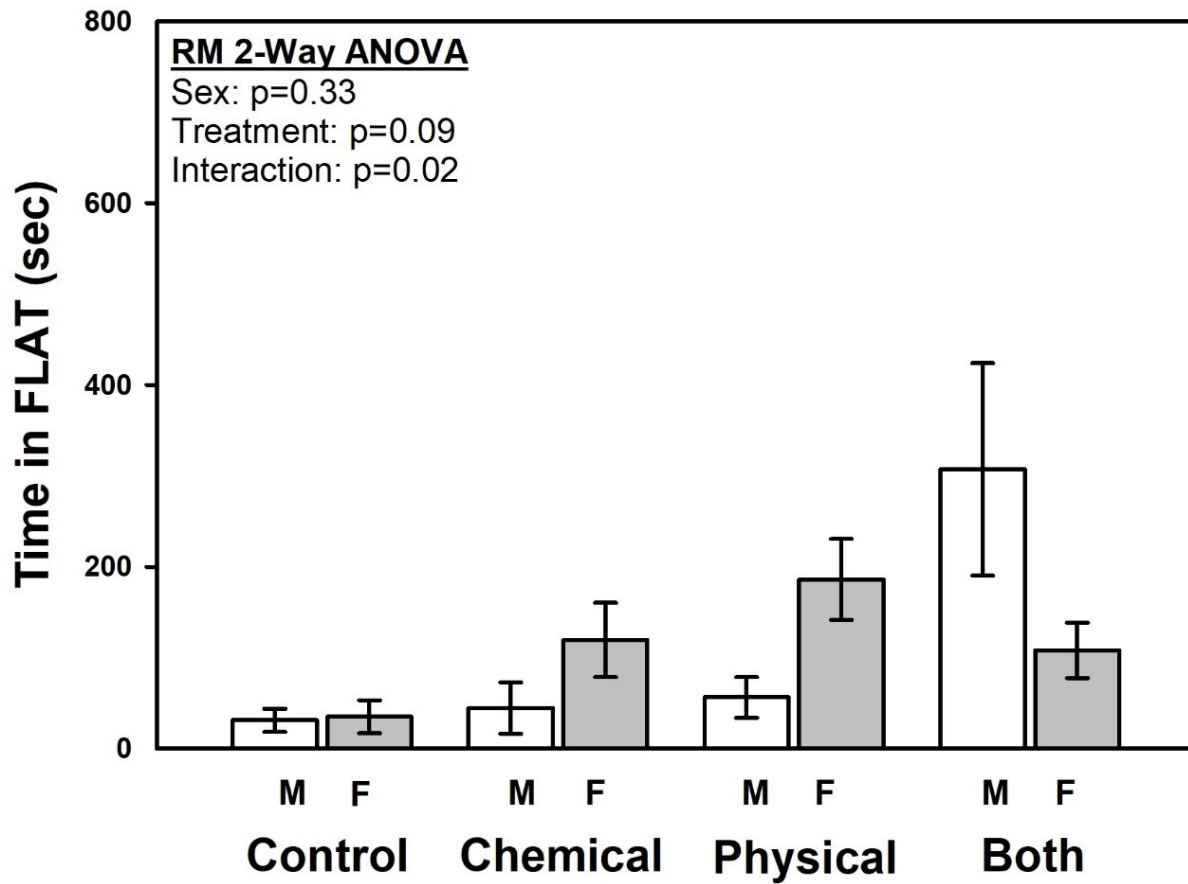


Figure 2. Mean (\pm SE) time spent in Flat (FLAT) submissive posture after immediate prior exposure to a blank control or one of three predator treatments. White bars are males (n=8) and gray bars are females (n=21). All statistics were calculated with data transformed using the aligned rank transformation.

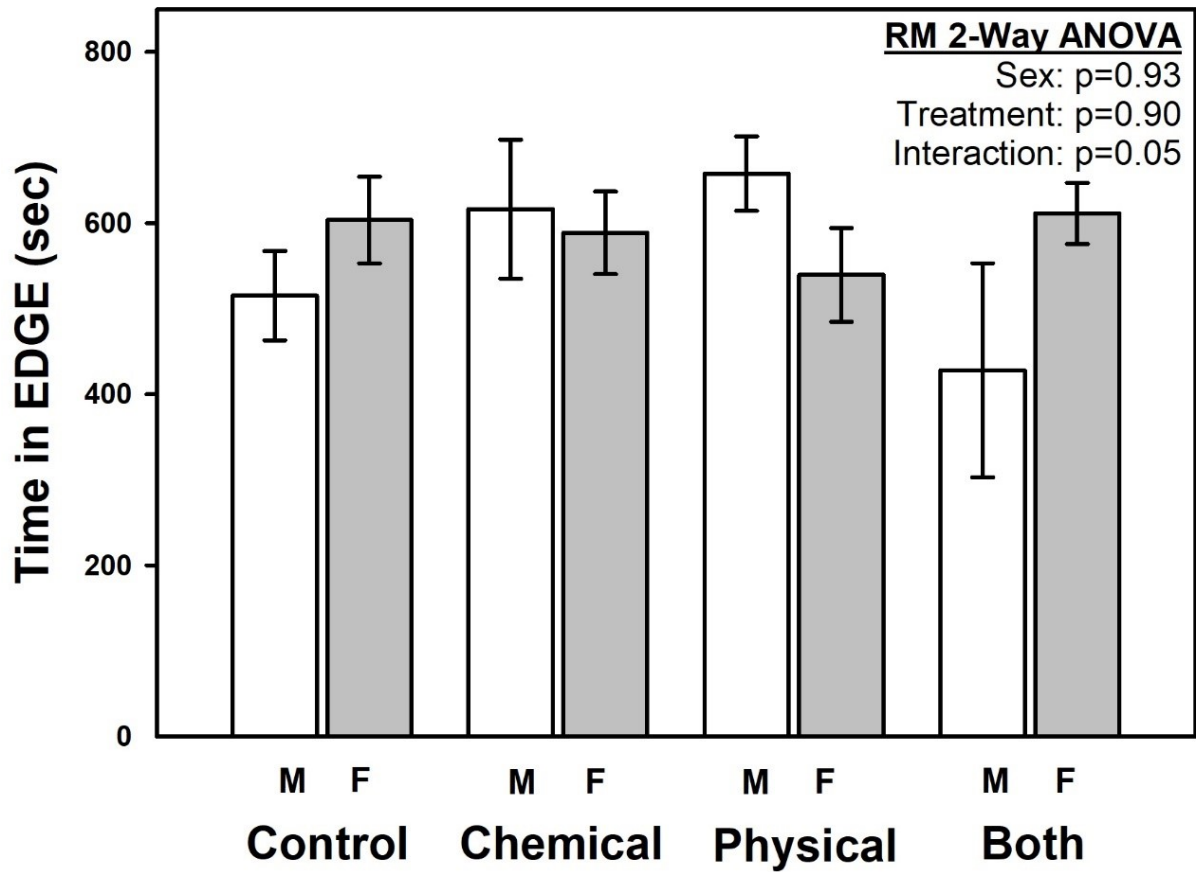


Figure 3. Mean (\pm SE) time spent around the edge of the arena (EDGE) after immediate prior exposure to a blank control or one of three predator treatments. White bars are males (n=8) and gray bars are females (n=21). All statistics were calculated with data transformed using the aligned rank transformation.

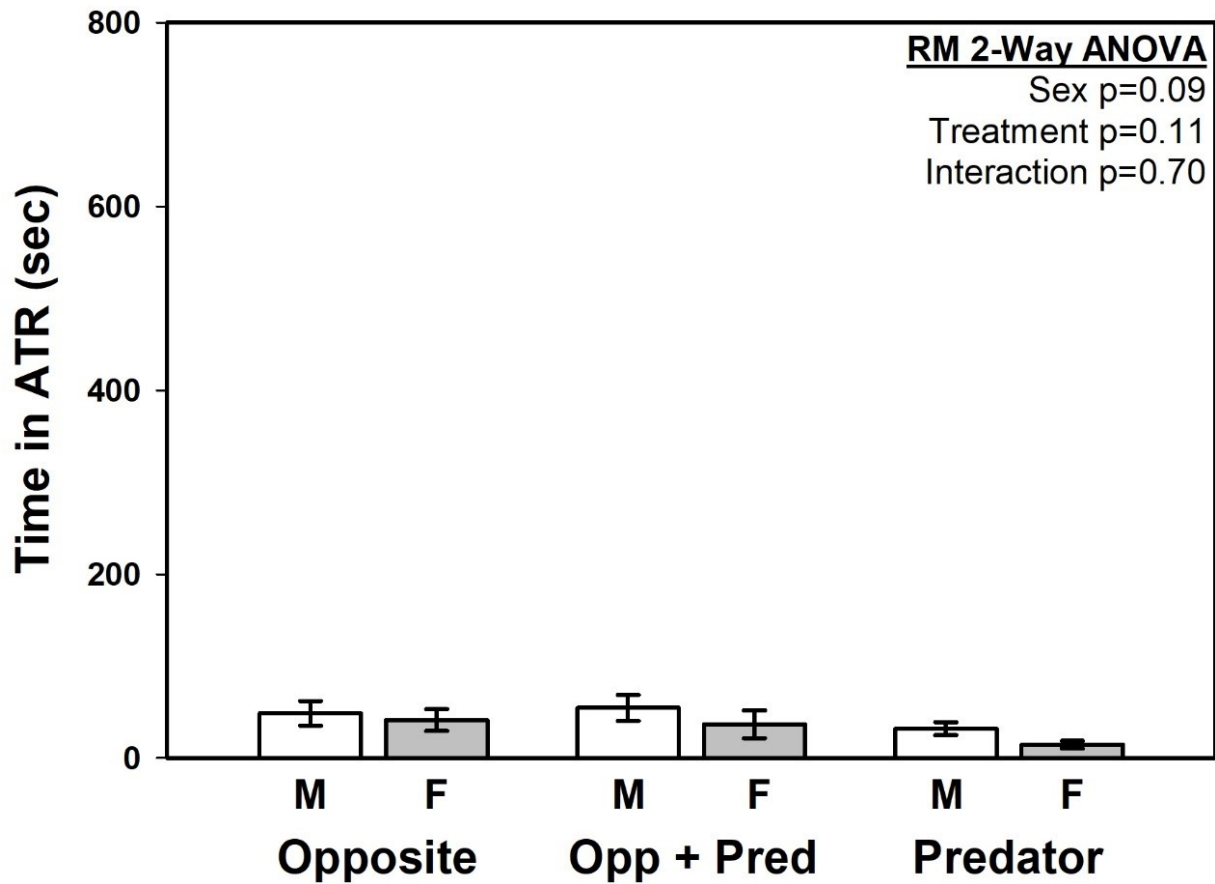


Figure 5. Mean (\pm SE) time spent in All Trunk Raised (ATR) aggressive posture after immediate prior exposure to pheromones from an opposite-sex intruder (Opposite), opposite-sex intruder pheromones combined with a predator cue (Opp + Pred), or predator cue alone (Predator). White bars are males ($n=11$) and gray bars are females ($n=17$). All statistics were with data transformed using the aligned rank transformation.

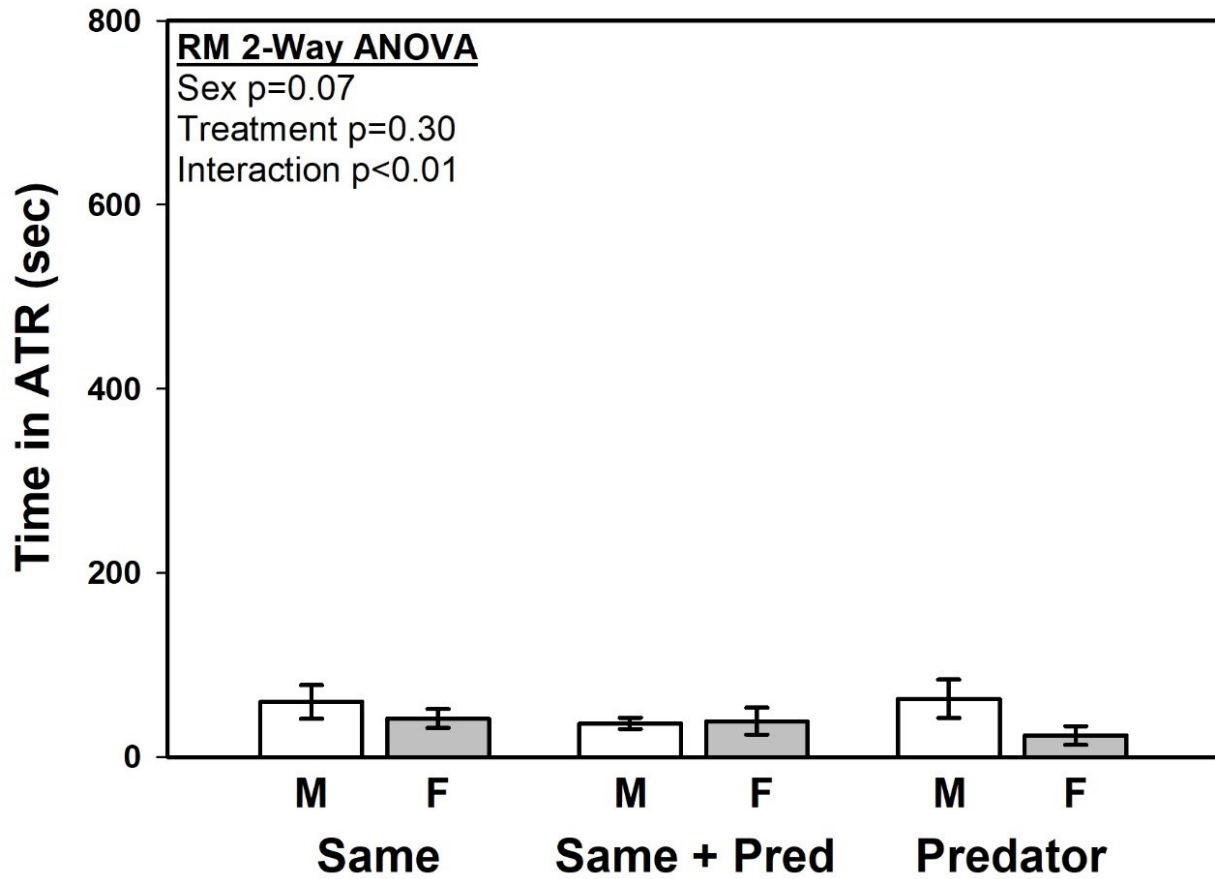


Figure 6. Mean (\pm SE) time spent in All Trunk Raised (ATR) aggressive posture after immediate prior exposure to pheromones from a same-sex intruder (Same), same-sex intruder pheromones combined with a predator cue (Same + Pred), or predator cue alone (Predator). White bars are males ($n=11$) and gray bars are females ($n=17$). All statistics were with data transformed using the aligned rank transformation.

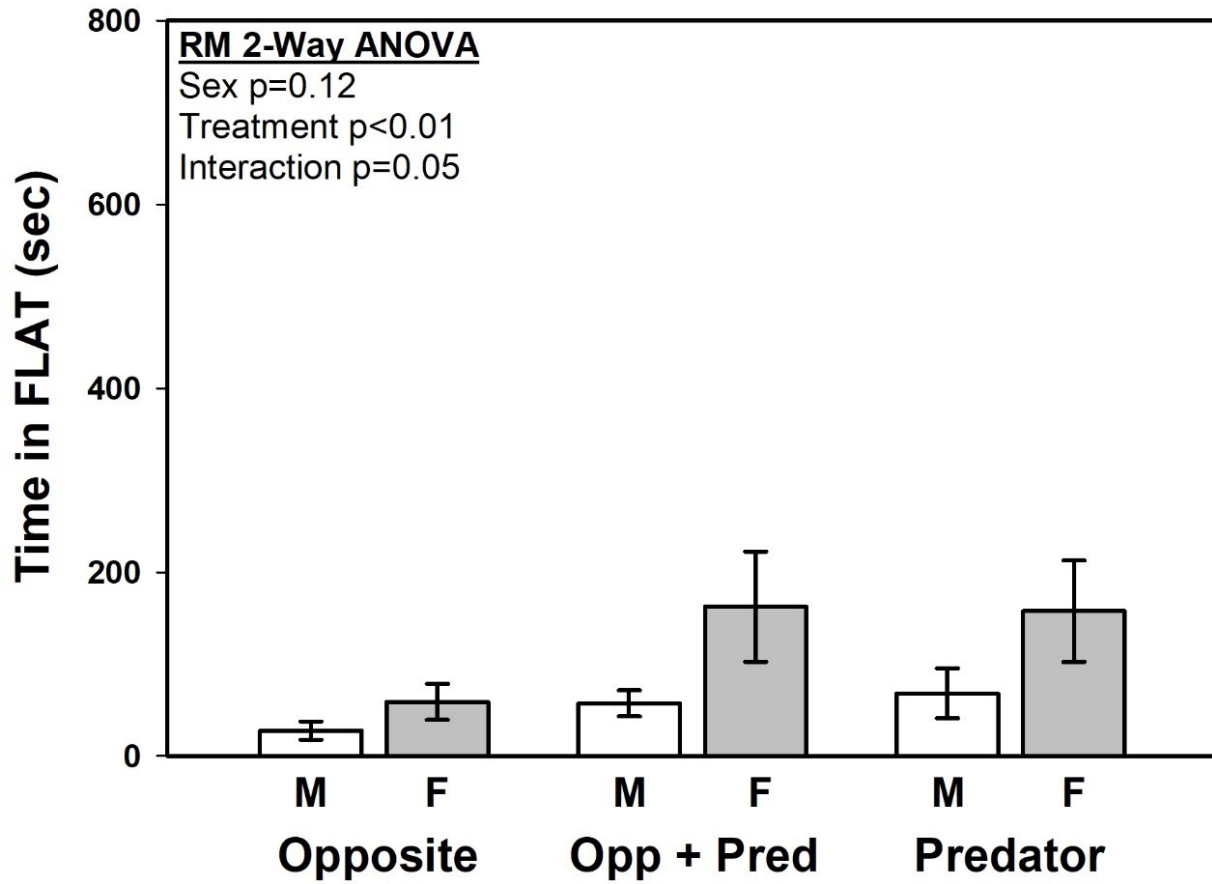


Figure 7. Mean (\pm SE) time spent in the Flat (FLAT) submissive posture after immediate prior exposure to pheromones from an opposite-sex intruder (Opposite), opposite-sex intruder pheromones combined with a predator cue (Opp + Pred), or predator cue alone (Predator). White bars are males ($n=11$) and gray bars are females ($n=17$). All statistics were with data transformed using the aligned rank transformation.

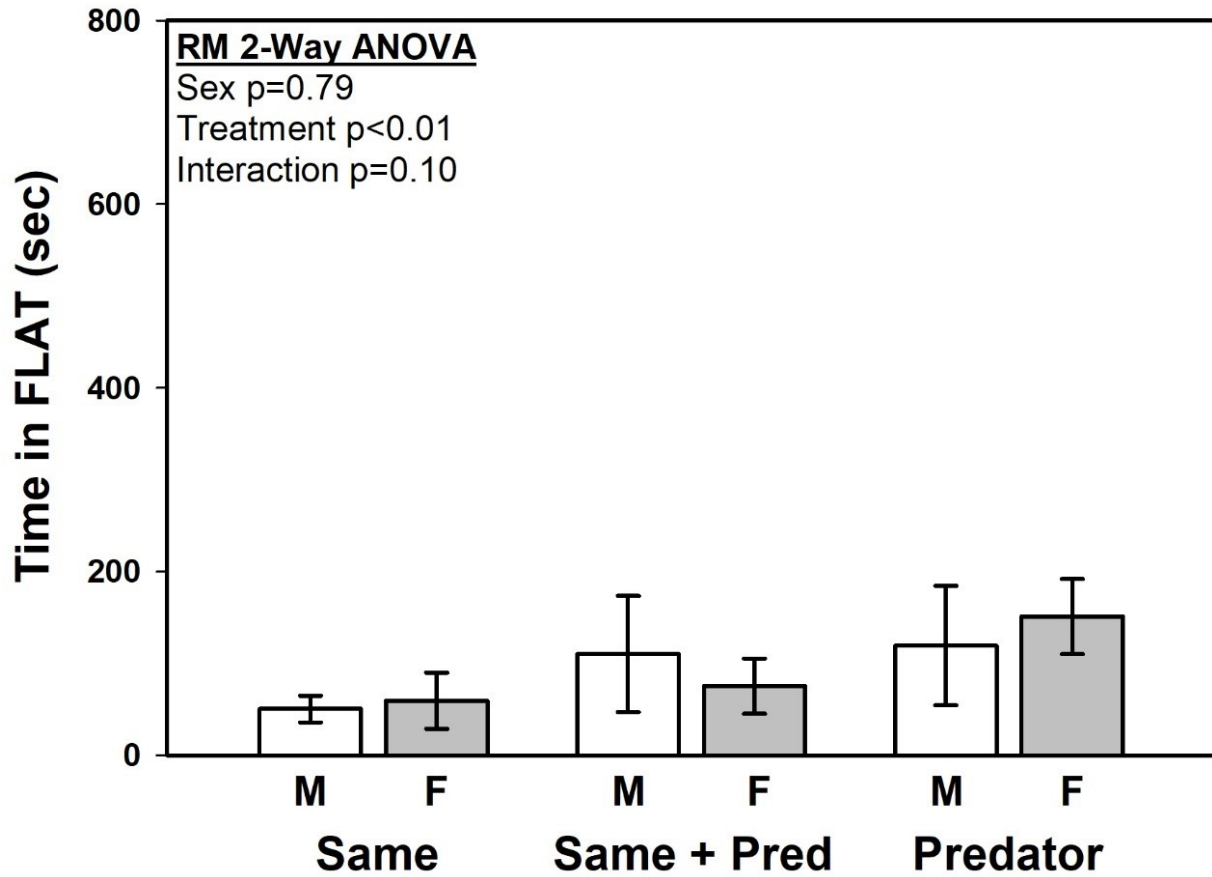


Figure 8. Mean (\pm SE) time spent in the Flat (FLAT) submissive posture after immediate prior exposure to pheromones from a same-sex intruder (Same), same-sex intruder pheromones combined with a predator cue (Same + Pred), or predator cue alone (Predator). White bars are males ($n=11$) and gray bars are females ($n=17$). All statistics were with data transformed using the aligned rank transformation.

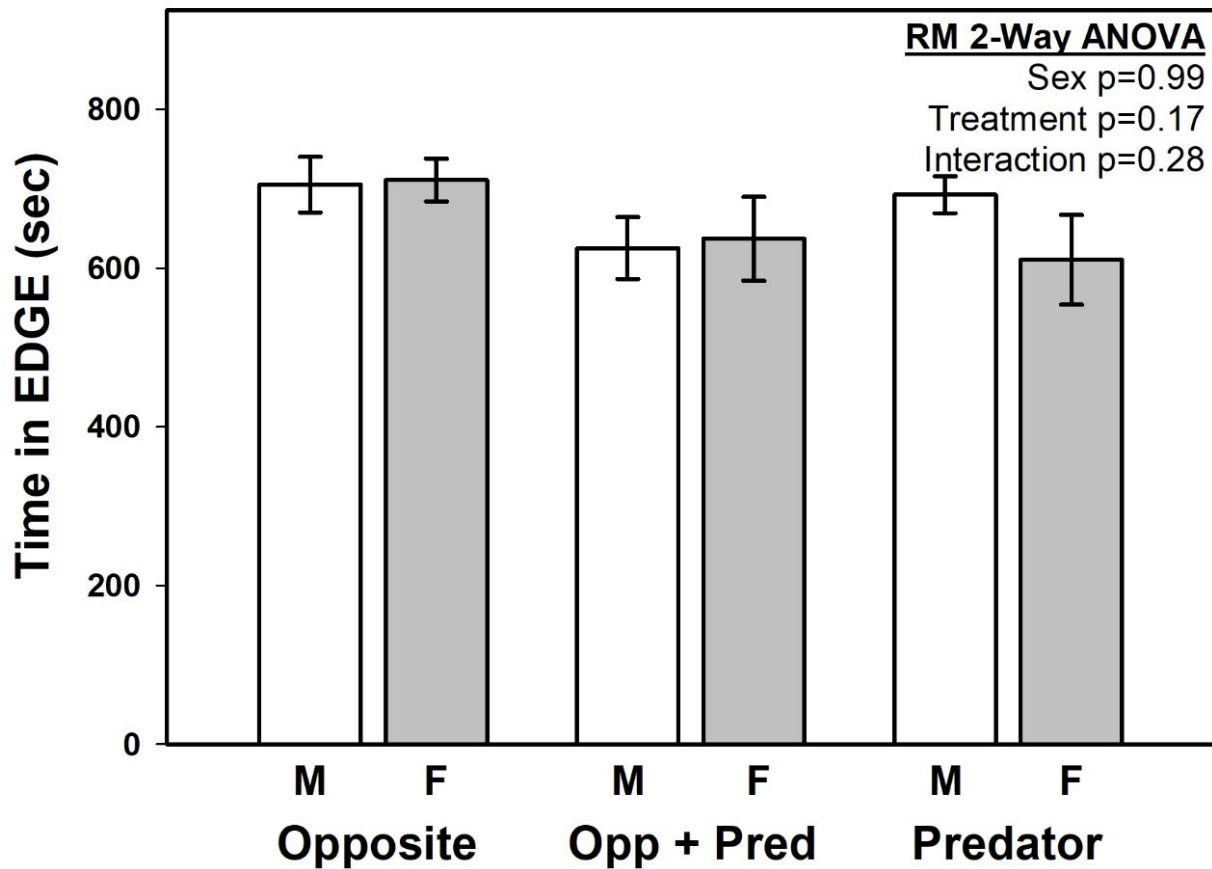


Figure 9. Mean (\pm SE) time spent around the edge of the arena (EDGE) after immediate prior exposure to pheromones from an opposite-sex intruder (Opposite), opposite-sex intruder pheromones combined with a predator cue (Opp + Pred), or predator cue alone (Predator). White bars are males ($n=11$) and gray bars are females ($n=17$). All statistics were with data transformed using the aligned rank transformation.

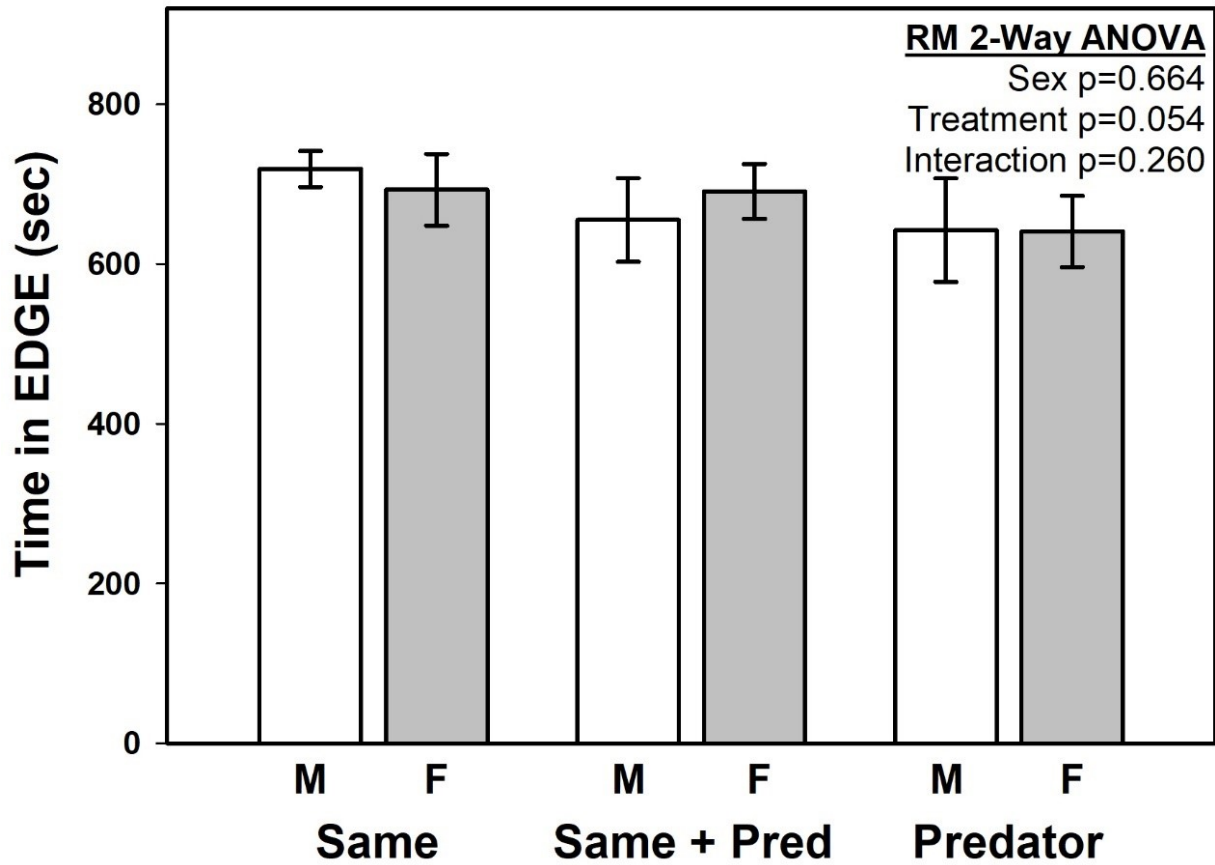


Figure 10. Mean (\pm SE) time spent around the edge of the arena (EDGE) after immediate prior exposure to pheromones from a same-sex intruder (Same), same-sex intruder pheromones combined with a predator cue (Same + Pred), or predator cue alone (Predator). White bars are males ($n=11$) and gray bars are females ($n=17$). All statistics were with data transformed using the aligned rank transformation.

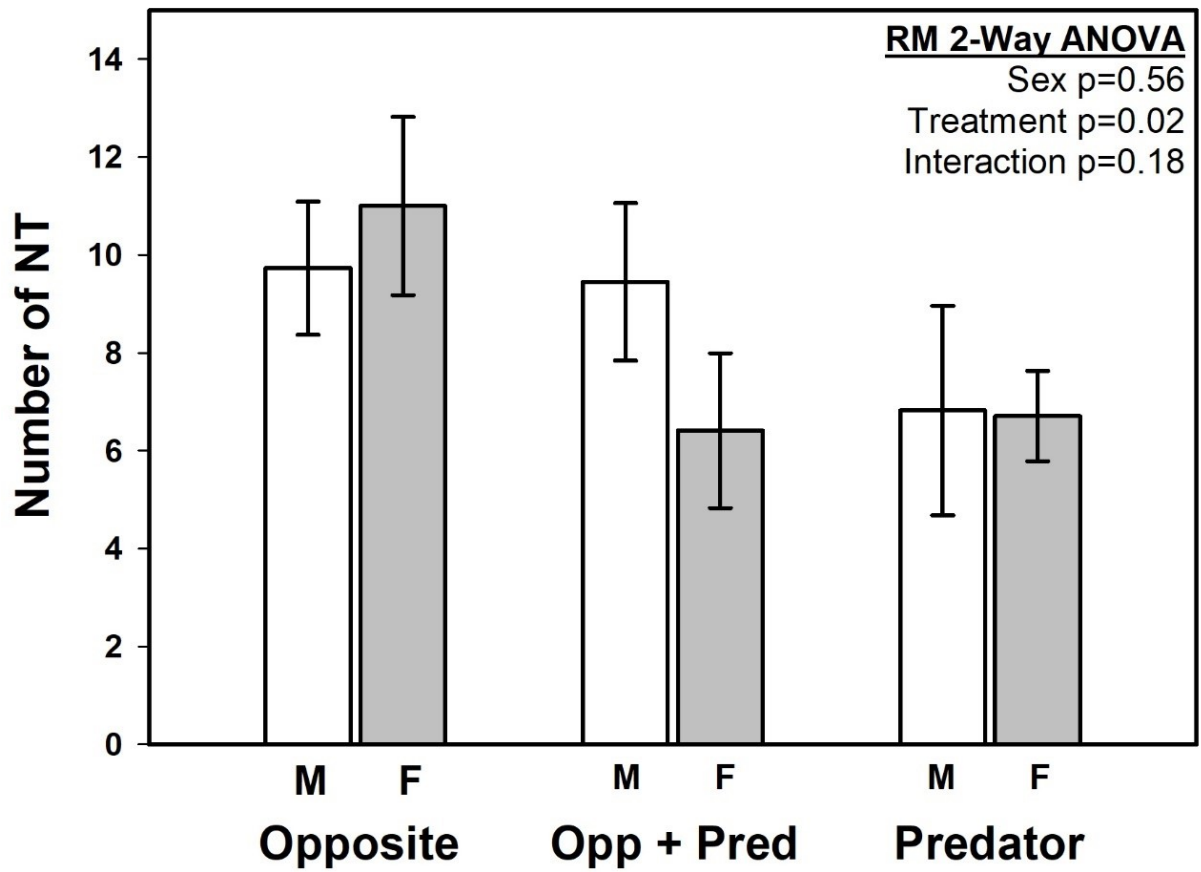


Figure 11. Mean (\pm SE) number of chemosensory nose taps (NT) after immediate prior exposure to pheromones from an opposite-sex intruder (Opposite), opposite-sex intruder pheromones combined with a predator cue (Opp + Pred), or predator cue alone (Predator). White bars are males (n=11) and gray bars are females (n=17). All statistics were with data transformed using the aligned rank transformation.

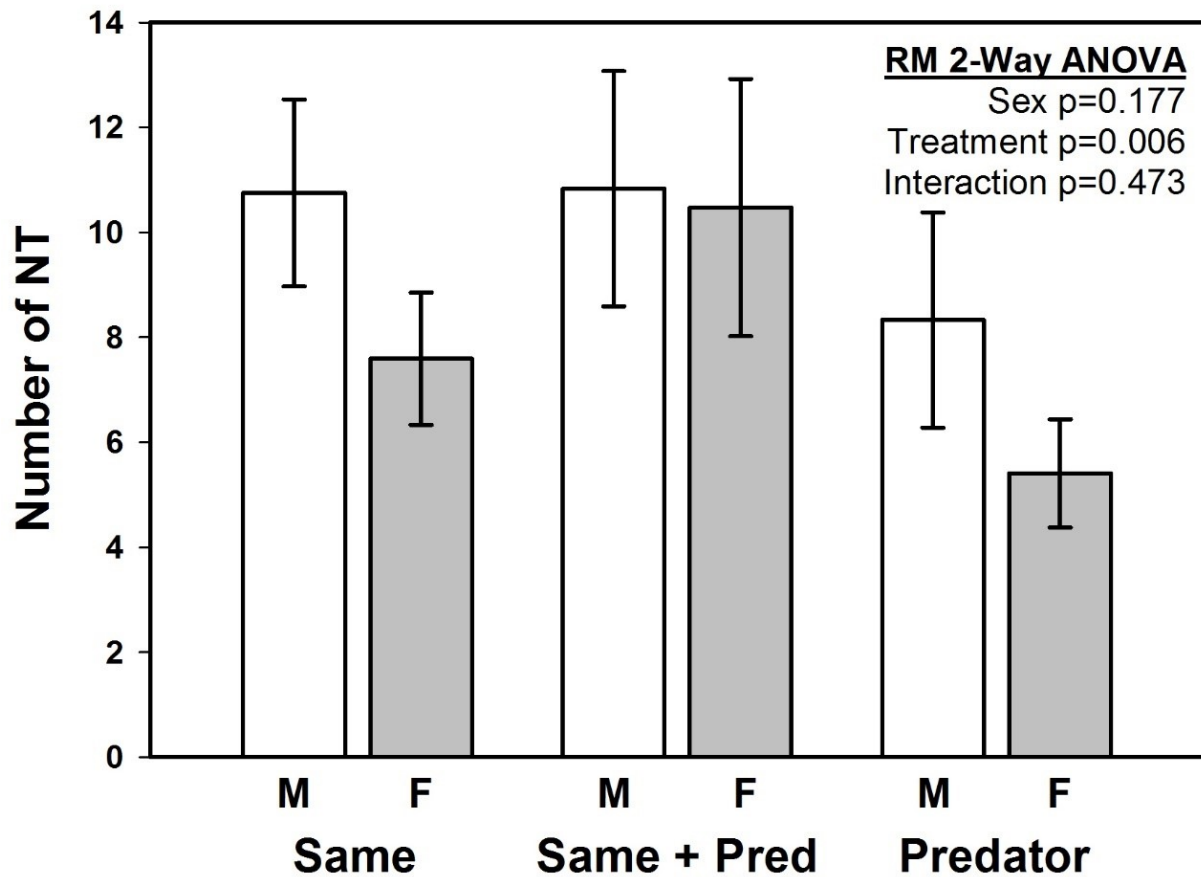


Figure 12. Mean (\pm SE) number of chemosensory nose taps (NT) after immediate prior exposure to pheromones from a same-sex intruder (Same), same-sex intruder pheromones combined with a predator cue (Same + Pred), or predator cue alone (Predator). White bars are males (n=11) and gray bars are females (n=17). All statistics were with data transformed using the aligned rank transformation.

DISCUSSION

Unimodal versus Multimodal Predator Cues

Overall, for territory intruders, exposure to predation risk led to changes in territorial behavior for both unimodal and multimodal cue types in comparison to the control. Predation risk affected agonistic posturing (ATR and FLAT), Edge behavior, and chemosensory sampling (Nose Taps). However, the patterns of responses were often different for males and females.

For female intruders, exposure to all types of predatory stimuli resulted in similar changes in frequencies of agonistic visual displays. Response to the predatory stimuli—a decrease in high-visibility ATR and an increase in low-visibility FLAT—is consistent with a function to reduce conspicuousness to potential predators. Similarly, male guppies, *Poecilia reticulata*, also reduced the frequency of high-visibility displays when predation risk was high (Endler 1987), and brown anole lizards, *Anolis sagrei*, shifted from high- to low-visibility head bob displays in the presence of predators. The lack of a difference between unimodal and multimodal cues suggests that the information concerning the level of threat indicated by chemical and tactile cues was redundant (Partan & Marler 2005), even though the information content differs. Chemical cues left by a predator can provide a range of information about the predator, including size (Mirza & Chivers 2002) and diet (Mathis & Smith 1993), whereas tactile cues from an unsuccessful physical attack provides information on the recent location, feeding motivation (i.e., actively foraging) and, possibly, identity, of the predator. In either case, it would not be known whether the predator has remained in the immediate area. Both modalities may present a similarly high level of risk for the salamander. Because all of the females we tested were gravid, they may have suffered physical constraints that may not be present in other

times of the year. A gravid female may not be able to flee as quickly or fit into a crevice or burrow that is small enough to shelter them from a predator such as small snakes. Gravid females sometimes respond differently to elevated predation risk than nongravid females or males, including decreased flight behavior and increased freezing (e.g., lizards: Bauwens & Thoen 1981; snakes: Gregory & Gregory 2006).

In contrast, time spent in EDGE behavior by female intruders was not influenced by predator treatment. EDGE is typically interpreted as a salamander seeking either to escape or to locate crevices for hiding (= escape in Horne 1988), so the lack of increased EDGE behavior in response to higher predation risk was somewhat surprising. At least in part, we attribute the lack of a treatment effect, even in comparison to the blank control condition, to the relatively high levels of EDGE behavior (about two-thirds of each trial) that was seen, which left little opportunity for increases in this behavior when predation risk was heightened. Similarly, high levels of EDGE behavior during territorial competition in the absence of high predation risk have been reported for intruders (but not for residents) in this species (Dalton & Mathis 2014).

Like females, male intruders showed an equivalent decrease in time spent in ATR in response both to unimodal and multimodal predator-related cues in comparison to the control. However, males responded differently from females with respect to EDGE and FLAT behavior, with the multimodal cue eliciting an apparently additive response compared to either of the unimodal cues. This additive response suggests that the high level of information content provided by multimodal cues is required to initiate antipredator behavior by males. Similar additive responses have been observed in other prey species, including hermit crabs *Pagurus bernhardus* (Dalesman & Inchley 2008), mosquitofish *Gambusia infinis* (Ward & Mehner 2010), Atlantic salmon *Salmo salar* (Blanchet et al. 2007) and wall lizards *Podarcis muralis* (Amo et al.

2004). For male salamanders, the costs of missed mating or territorial opportunities (e.g., Foam et al. 2005; Ferrari et al. 2008) appears to result in a higher threshold for triggering antipredator behavior than for females.

Chemosensory behavior was similarly affected by treatment (main effect) for both sexes, with the multimodal cue appearing to be nonredundant for both sexes. The chemical cue from the predator alone was insufficient to elicit changes in nose-tapping for both males and females. However, both the physical cue alone and the multimodal (chemical + physical) cue elicited similar decreases in nose-tapping behavior. The observed decreases in chemosensory behavior suggest that further chemosensory sampling following a physical attack would not provide information that would enhance antipredator behavior or that additional information would not change the response. Decreased activity is a common antipredator behavior in amphibians with visually-oriented predators and may offer the best chance of escaping detection immediately after a perceived predator attack (Brodie et al. 1974; Brodie 1977; Hayes 1989). Chemosensory nose-tapping also requires the snout to be briefly in contact with the substrate, which may interfere with effectiveness of vigilance behavior.

Effect of Predation Risk on Territorial Behavior by Same-Sex and Opposite-Sex Intruders

For both aggressive (ATR) and submissive (FLAT) displays, the response of intruders to same- and opposite-sex residents was moderated by the presence of predator cues (i.e., an interaction between resident-sex and predation treatments). The nature of the effect was different depending on whether the resident was of the same or opposite sex. However, overall,

the behavior of females generally was moderated more strongly by the presence of a predator than males.

For *Plethodon* salamanders, territories appear to be multifunctional, including both defense of food resources and also serving as areas where pairs can mate either in zones of overlap (Mathis 1990) or in areas that are co-defended by socially-monogamous pairs (Gillette et al. 2000; Lang & Jaeger 2000). Differential effects of predation risk based on the sex of the nearby individuals may lead to tolerance to neighbors that present a reproductive opportunity either at that time or in the future (Mathis 1990).

For ATR, the interaction between resident sex and predation risk occurred only when the resident was of the same sex. For females, the pattern was decreased ATR only in the predator-only treatment. Although levels of ATR were relatively low, female intruders were willing to engage in low-levels of contest escalation with other females, but not males, even in the presence of predators. Similarly, female residents of *P. cinereus* were more aggressive toward females than toward male intruders, with the authors attributing this difference to males being potential mating partners (Lang & Jaeger 2000). In contrast, for males the lowest levels of ATR were in the same-sex resident + predator treatment, suggesting an additive effect of these two factors. When only one threat (same-sex competitor, predator) was present, males showed relatively high average levels of ATR (approximately 30-60% higher than for females), suggesting that male intruders are highly motivated to secure a territory. Lang and Jaeger (2000) also reported higher levels of aggression by male residents to male intruders in comparison to female intruders.

For FLAT, the interaction between resident sex and predation risk was only present in opposite-sex treatments. In this case the pattern was the same for both males and females, with higher levels of FLAT with any stimulus that included predator kairomones (predator only, and

opposite-sex resident + plus predator). However, the effect of predation risk was more extreme for females than for males. Females showed very high levels of FLAT when predation risk was high, whereas males showed only slight increases in FLAT behavior.

This apparent sex difference in predator-moderated behavior may be due to the reproductive status of the females during the experiment. All females tested were gravid (eggs visible through the body wall) throughout the entire testing period. Two hypotheses involving female survival while carrying eggs or embryos have been proposed: the Physical Burden hypothesis (Shine 1988), and the Physiological Cost hypothesis (Brodie 1989). The Physical Burden hypothesis predicts negative correlations among relative clutch mass, locomotion, and survival during pregnancy (Shine 1988). The Physiological Cost hypothesis predicts that the broad physiological changes that occur during pregnancy should negatively affect locomotion regardless of the level of investment (Brodie 1989). Regardless of the cause, reduced locomotive ability for gravid females has been reported for many species, including fish (Plaut 2002), lizards (Miles et. al. 2000), and birds (Veasey et. al. 2001). A female laden with eggs not only faces the physical constraints of a heavy burden, but also faces an increased risk of starvation after laying her eggs due to energetic costs (Madsen & Shine 1993). Low levels of aggression and high levels of FLAT decrease visibility in the face of predatory threat and are relatively low-cost in terms of energetics in contrast to flight. In addition, in comparison to males, females may not be able to sprint to cover as effectively. A similar response was recorded in gravid female collared lizards, *Crotaphytus collaris*, that compensated for their reduced sprint speed by remaining closer to refugia and waiting longer before attempting to seek cover (Husak 2006).

As a main effect, the presence of predator cues significantly increased both the amount of time spent in the FLAT display and the frequency of nose-tapping. These effects were

significant for both same-sex and opposite-sex pairings, indicating a robust effect of predator cues. In addition to being a submissive posture in aggressive contests (Jaeger 1984), increasing time in FLAT behavior is consistent with decreasing visibility in the context of predation risk and has been reported in another study of antipredator behavior for this species (Crane et al. 2012). Reductions in nose-tapping are likely a consequence of overall reductions in activity, which is a common response to predation risk (Brodie et al. 1974; Brodie 1977; Hayes 1989). Parsons (2010) also reported that intruders decreased the number of nose-taps in the presence of cues from snake predators.

Independent of predation risk, there tended to be lower levels of aggressive displays (ATR) by intruders when the resident was male, but this difference was relatively weak and not statistically significant ($0.05 > P < 0.10$). The weakness of this effect may be due in part to the generally low levels of ATR by salamanders in this study, which has been shown for intruders in territorial contests in this (e.g. Mathis et al. 2000) and other (e.g. Cutts et al. 1999; Sacchi et al. 2009; Fuxjager et al. 2010) species. Dalton and Mathis (2014) also reported differential responses by intruders when the resident was male, although in that experiment the effect was manifested as increased EDGE behavior in the presence of chemical cues from male residents in comparison to females. Taken together, these studies (Dalton & Mathis 2014; current study) provide some evidence to support the hypothesis that males are considered to be more of a threat than females.

Overview

In both experiments, predation risk generally affected territorial behavior by decreasing levels of aggressive behavior and increased submissive behaviors of intruders. However, sex of the intruder influenced the responses to predation risk in both experiments and sex of the resident influenced the response of intruders in the second experiment. These differences support the hypothesis that salamanders can assess local factors and adjust their territorial behavior according to perceived costs and benefits. Females, which were all gravid in this study, generally were more strongly affected by predation risk, while males were more likely to engage in riskier behavior overall and particularly in the presence of pheromones from an opposite-sex salamander.

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APPENDICES

Appendix A. Unimodal vs Multimodal Data

Behavior	Factor(s)	<i>df</i>	<i>F</i>	<i>p</i>
ATR	ID	29	1.35	0.157
	Sex	1	0.83	0.367
	Treatment	3	5.52	0.002
	Order	3	0.14	0.936
	Sex*Treatment	3	1.86	0.144
	Sex*Order	3	2.67	0.055
	Treatment*Order	9	0.70	0.703
FLAT	ID	29	1.41	0.129
	Sex	1	0.99	0.325
	Treatment	3	2.21	0.095
	Order	3	1.60	0.199
	Sex*Treatment	3	3.62	0.018
	Sex*Order	3	2.58	0.061
	Treatment*Order	9	1.46	0.181
EDGE	ID	29	1.49	0.092
	Sex	1	0.01	0.929
	Treatment	3	0.19	0.902
	Order	3	1.86	0.146
	Sex*Treatment	3	2.72	0.051
	Sex*Order	3	2.87	0.043
	Treatment*Order	9	1.20	0.310
NT	ID	29	2.63	0.001
	Sex	1	1.07	0.309
	Treatment	3	4.46	0.007
	Order	3	4.42	0.007
	Sex*Treatment	3	0.55	0.650
	Sex*Order	3	0.33	0.806
	Treatment*Order	9	0.41	0.925

Appendix B. Predation Risk on Territorial Behavior Data

Behavior	Paired w/	Factor(s)	<i>df</i>	<i>F</i>	<i>p</i>
ATR	Same	ID	27	2.96	0.001
		Sex	1	3.57	0.071
		Treatment	2	1.26	0.295
		Order	2	1.41	0.256
		Sex*Treatment	2	5.58	0.007
		Sex*Order	2	8.40	0.001
		Treatment*Order	4	3.04	0.028
		Sex*Treatment*Order	4	3.61	0.013
	Opposite	ID	26	1.62	0.083
		Sex	1	3.09	0.092
		Treatment	2	2.30	0.113
		Order	2	2.32	0.111
		Sex*Treatment	2	0.36	0.697
		Sex*Order	2	0.37	0.690
		Treatment*Order	4	2.04	0.107
		Sex*Treatment*Order	4	1.37	0.261
FLAT	Same	ID	27	2.66	0.002
		Sex	1	0.07	0.795
		Treatment	2	5.37	0.008
		Order	2	4.27	0.021
		Sex*Treatment	2	2.38	0.104
		Sex*Order	2	1.80	0.177
		Treatment*Order	4	0.56	0.693
		Sex*Treatment*Order	4	0.85	0.504
	Opposite	ID	26	7.13	<0.001
		Sex	1	2.57	0.121
		Treatment	2	11.33	<0.001
		Order	2	5.74	0.006
		Sex*Treatment	2	3.20	0.052
		Sex*Order	2	1.57	0.220
		Treatment*Order	4	0.47	0.758
		Sex*Treatment*Order	4	0.79	0.536
EDGE	Same	ID	27	2.73	0.002
		Sex	1	0.15	0.703
		Treatment	2	1.04	0.363
		Order	2	0.23	0.798
		Sex*Treatment	2	0.25	0.779

		Sex*Order	2	0.66	0.524
		Treatment*Order	4	0.96	0.437
		Sex*Treatment*Order	4	2.24	0.081
	Opposite	ID	26	1.75	0.055
		Sex	1	0.00	0.999
		Treatment	2	1.86	0.168
		Order	2	0.23	0.799
		Sex*Treatment	2	1.32	0.279
		Sex*Order	2	2.76	0.075
		Treatment*Order	4	0.32	0.864
		Sex*Treatment*Order	4	1.38	0.257
NT	Same	ID	27	4.08	<0.001
		Sex	1	1.93	0.177
		Treatment	2	5.77	0.006
		Order	2	3.60	0.036
		Sex*Treatment	2	0.76	0.473
		Sex*Order	2	4.95	0.012
		Treatment*Order	4	2.26	0.079
		Sex*Treatment*Order	4	0.75	0.562
	Opposite	ID	26	2.61	0.003
		Sex	1	0.34	0.564
		Treatment	2	4.55	0.017
		Order	2	1.85	0.170
		Sex*Treatment	2	1.77	0.184
		Sex*Order	2	1.38	0.264
		Treatment*Order	4	0.85	0.501
		Sex*Treatment*Order	4	0.75	0.561