

# FROM FOES TO BEAUS: PREDATOR PRESENCE MEDIATES TERRITORIAL CONFLICT IN *STEGASTES LEUCOSTICTUS*

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**Abstract:** Intraspecific territoriality and predator-prey dynamics are essential processes for any ecosystem, and their interaction often structures prey behavior. For example, prey species experience non-consumptive effects of predation, altering their territorial behavior when sheltering. To understand how territory intrusion behavior changes with intruder size and predation risk, we conducted behavioral manipulations on beaugregory damselfish collected in reefs surrounding Little Cayman. We found that competitive behavior experienced by an intruder displays a quadratic relationship with intruder size, as extremes in size may elicit lower territorial behavior. We also found that the presence of a predator decreases total competitive behavior between damselfish, thereby dampening the quadratic relationship with size. With rapid coral degradation jeopardizing fish habitat within tropical marine systems, our study provides insight into how damselfish navigate complex intraspecific territoriality over limited habitat to cope with the threat of predation.

**Keywords:** damselfish, non-consumptive effects, predator-avoidance, territoriality

## INTRODUCTION

Territoriality, or the aggressive defense of an area against intruders (Brown 1964; Clark 1970), is a common and essential behavior across a diversity of marine and terrestrial species. Territoriality can provide animals exclusive access to resources, advantages in attracting mates, protection for their young, or a constant source of shelter from predators (Schoener 1987; Grant 1997; Davies and Houston 1984). These territorial species often come into conflict as intruders attempt to claim desirable territories from residents. However, for both intruders and residents, territorial aggression is expensive, requiring energy, time, and the risk of suffering injury from physical conflict (Cowlshaw 2014). Furthermore, territorial behaviors risk attracting predators, which may occur when an individual exhibits aggressive behavior and is unable to take shelter (Abby-Lee et al. 2015). Animals can therefore be predicted to attack or defend territories only when the benefits of holding said territory exclusively outweigh the costs of territorial behaviors (Itzkowitz 1979; Grant 1997).

Among the main factors driving the development of territoriality is predation, which plays a foundational role in structuring community dynamics in every ecosystem. Predators not only directly regulate the population of prey organisms by reducing their numbers, called consumptive effects, but also indirectly influence the behavioral and morphological characteristics of prey organisms through their presence, called non-consumptive effects (Davis 2017). Prey species may evolve morphological traits such as camouflage, pheromones, and physical defenses or develop anti-predator behaviors such as schooling, alarms, and sheltering (Peacor and Werner 2008; Davis 2017). Predator avoidance behaviors are necessary for reef fish and many other taxa to decrease the chance of being consumed (Lima and Dill 1990). However, these avoidance behaviors not only expend energy, but also divert time away from activities such as foraging, mating displays, and intraspecific territory defense, which are necessary for survival and reproductive success (Ydenberg and Dill 1986; Lima 1998; Frid and Dill 2002; Stamoulis et al. 2020). Many reef fish therefore use shelter — by hiding within tight spaces such as coral branches, conch shells, or sand burrows, they may escape predation with relatively little energy expenditure (Fontoura et al. 2019). Shelters often, however, have limited capacity, and the emphasis on sheltering behaviors is one of several factors that lead to the development of territorial behavior in many reef fishes (Ebersole 1977).

Damselfishes (family Pomacentridae) are small fish commonly found in tropical marine environments (Allen 1991). Some damselfish species select and aggressively defend territories (Abarca and Ornburn 2019), which act as shelter from predation (Gauff et al. 2018; Helfman 1988) and as nesting sites

(Itzkowitz and Makie 1986). Damselfish with high-quality territory experience greater reproductive success (Itzkowitz and Makie 1986). Thus, damselfish spend much of their time defending a territory (Abarca and Ornburn 2019; Medeiros et al. 2010) or attempting to intrude upon higher quality territory (Jan et al. 2003). Damselfish size could determine territory intrusion success, given the role that size hierarchies can play in damselfish social dynamics. Larger damselfish have been observed to have greater success in territorial disputes (Forrester 1991). Additionally, although being the exclusive inhabitant of a desirable territory may maximize the fitness of an individual fish (Itzkowitz and Makie 1986), territorial defense behavior has been found to decline with predator presence (Helfman 1988), suggesting prioritization of immediate survival. Predator-avoidance could make a territory more easily intruded upon, as an intruder urgently needs to escape a predator, and the resident fish may be preoccupied with self-preservation. If an intruder successfully takes a territory, the intruder could benefit from enhanced reproductive success and shelter (Itzkowitz and Makie 1986; Gauff et al. 2018; Helfman 1988). However, intrusion behavior may come with risk of failure and harm by the resident damselfish, which could lead to a decreased capacity for further territorial behavior and increased vulnerability to predation. As such, it is important to understand how damselfish navigate intraspecific competition for desirable territory and how this behavior may change with damselfish size and with an immediate threat of predation. As fish habitat decreases in coral reefs as a result of climate change (Clarke et al. 2020; Hollowed et al. 2013; Villéger et al. 2010), understanding how damselfish may compete with conspecifics for increasingly limited territory, especially in the face of predation, could provide insight into the prospect of damselfish persistence in a changing ocean.

To understand how damselfish territory intrusion behavior changes with intruder fish size and predation threat, we simulated conspecific territory intrusion with beaugregory damselfish collected in reefs surrounding Little Cayman. Intruder damselfish with body size closely matching that of the resident fish may experience more competitive behavior, as these fish may be more physically and energetically capable of competing for desirable territory and may pose a more significant threat to resident fish. On the other hand, larger intruders may be more capable of severely hurting the resident and vice versa, so both fish may be more hesitant to compete. Further, in the presence of a predator, intruder damselfish may engage in less territorial competition with the resident, as both fish may prioritize immediate survival before competition for exclusive territory occupancy.

## METHODS

### *Specimen collection*

We collected 42 beaugregory damselfish (*Stegastes leucostictus*) of various sizes by hand netting around dead conch shells, which are frequently used as territories, between February 28 and March 2, 2024. We collected individuals from Preston Bay and South Hole, two barrier reef lagoons around the island of Little Cayman. We also collected a schoolmaster snapper (*Lutjanus apodus*) measuring approximately 25 cm in length using rod and reel methodology at St. Mary's Bay, Little Cayman to act as the predator.

### *Experimental setup*

We separated a 55-gallon tank into two compartments: the first compartment (35 gallons) served as a predator-holding area for the snapper, while the second (20 gallons) was where we observed damselfish behavior (Fig. 1). To divide the tank, we constructed a barrier with plexiglass and mesh to allow for the transmittance of both visual and chemical predator cues. Within the experimental area, we added a thin layer of sand, several rocks, and an empty conch shell to provide a desirable territory for damselfish. We set the tank temperature to 27 °C to match the ocean temperature and aerated the water using a Tetra Whisper 110-volt air pump. We taped a 3 x 3 grid of 15 x 15 cm squares on the viewing pane to delineate nine different zones. We defined the three zones closest to the barrier as the near-predator zone, while we named the middle and farthest zones as near-conch and farther-from-predator zones, respectively. We did not modify the predator-holding zone.

### *Behavior trials*

We randomly selected two individuals from each reef site to act as "residents" for the experimental trials with fish from the same reef. To control for resident length, we only picked individuals among the largest fish collected, measuring 71.5, 74, 74, and 77 mm. We placed the resident in the experimental tank at least two hours before trials to acclimatize it to the tank and conch. Only one resident was present in the experimental tank for all 9 - 10 trials of fish from that reef site.

For each trial, we added one "intruder" damselfish, varying in length from 13 - 76 mm, to the experimental area containing a resident occupying the conch as territory. We monitored the intruder's behavior and movement for five minutes. We recorded the total amount of time in seconds that the intruder spent in each zone and how many times it exhibited avoidance and approach behaviors. Avoidance occurred when the intruder moved swiftly to avoid aggressive behaviors (chases) from the resident damselfish. An approach occurred when the intruder moved aggressively toward the resident. We also took qualitative notes of behavior. After running 19 trials without a predator, we added the *L. apodus* to the predator compartment and conducted 19 more trials with new damselfish in the presence of a predator. We measured the intruding fish for total length (from snout to caudal fin tip) following each behavioral trial of both treatments to minimize sampling stress.

### *Statistical analysis*

To assess whether predator presence affected damselfish intrusion behavior, we conducted t-tests with predator presence as the independent variable and the number of avoidances, number of approaches, and time spent in a given zone as the dependent variables. We also assessed the relationship between damselfish intruder size and intrusion behavior by generating regressions with the length difference between intruder and resident as the independent variable and the number of avoidances and approaches as dependent variables. To determine the regression that conserved the most information, we assessed differences in the Akaike information criterion adjusted for small sample sizes (AICc). Finally, we tested for potential confounding variables, such as experimental trial order and resident individuality, through one-way ANOVA tests.

## RESULTS

### *Size*

In our qualitative observation, there appeared to be a tradeoff between size and competitive interaction. The resident did not leave the shell as much in response to small individuals. Additionally, we noticed that larger intruder fish briefly shared the territory directly under the conch's spire with the resident, swimming alongside one another and rapidly fluttering their fins. This behavior would often result in either the resident surrendering its territory or the intruder backing away from the territory. The only intruders to displace the resident and take over the conch shell ( $n = 4$ ) were the same size or larger than the resident. Intruders that were 15 to 40 millimeters smaller than the resident, hereafter called intermediate-sized individuals, appeared to compete with the residents the most.

Accounting for the length of the resident, we found a significant quadratic relationship between intruder length (mm) and the number of avoidances ( $F_{2,35} = 3.37$ ,  $P = 0.0457$ ,  $r^2 = 0.16$ ; Fig. 2a), and this model was well-supported compared to a least squares linear regression (AICc difference = 288.2 - 291.2). There was no relationship between the size of the intruder and the number of approaches by the intruder using either a linear (slope  $\pm$  SE =  $0.0376 \pm 0.043$ ,  $P = 0.39$ ,  $r^2 = 0.021$ ) or quadratic regression ( $F_{2,35} = 0.93$ ,  $P = 0.40$ ,  $r^2 = 0.051$ ).

### *Predation*

Predator presence considerably dampened conspecific competitive interactions. When we introduced the predator, the intruders spent significantly more time in the zone farthest from the predator ( $t = 3.01$ ,  $P = 0.0048$ ,  $df = 36.0$ ; Fig. 3), and the resident was qualitatively more active and stressed. This demonstrates that both fish observed the predator and behaved in response to the stimulus. The intruders spent roughly

the same amount of time in the middle zone of the tank, near the conch (Fig. 1), whether the predator was present or absent ( $t = 0.43$ ,  $P = 0.67$ ,  $df = 32.0$ ).

In the presence of a predator, there were significantly fewer avoidances ( $t = 3.05$ ,  $P = 0.0057$ ,  $df = 22.9$ ) and approaches ( $t = 3.99$ ,  $P = 0.0008$ ,  $df = 18.5$ ) than when the predator was absent (Fig. 4). Further, when we separated the relationship between length-difference and avoidances by whether the predator was present or absent, both treatment groups showed different quadratic regressions. Within the predation treatment, the regression ( $F_{2,16} = 3.88$ ,  $P = 0.0423$ ,  $r^2 = 0.33$ ; Fig. 2b) is a flatter curve, with less variation in avoidance. The no-predator group displayed a nonsignificant relationship ( $F_{2,16} = 3.13$ ,  $P = 0.0712$ ,  $r^2 = 0.28$ ; Fig. 2b), but there was more variation in avoidances. Again, both quadratic regressions likely conserved more information than a linear regression (predator: AICc difference = 115.1 - 118.6; no predator: AICc difference = 153.8 - 156.2).

### *Controlling for confounding effects*

The behaviors of the residents and intruders did not substantially change throughout our trials and manipulations. The order in which intruders were tested with each resident (e.g., third intruder vs. fifth intruder in a session) had no significant effect on the total number of avoidances ( $F_{10,27} = 1.04$ ,  $P = 0.44$ ) or approaches ( $F_{10,27} = 0.37$ ,  $P = 0.95$ ). Accounting for the predation treatment and size differences, we found no significant differences between the residents ( $F_{3,34} = 2.34$ ,  $P = 0.090$ ), so individuality in resident behavior was not a concern.

### DISCUSSION

We found that differences in length between intruders and residents generated significant differences in conspecific aggressive interactions. The number of avoidance behaviors by the intruder varied with size difference along a quadratic regression, indicating that the extremes in intruder size may elicit lower territoriality from the resident. The predator's presence dampened the aggression response of the intruder, reducing the overall aggression between damselfish. The imminent risk of predation may outweigh the benefits of territorial behavior in both the resident, who displayed less effective chases, and the intruder, who approached less and spent more time in zones farther away from the predator.

The observed quadratic relationship between the difference in damselfish lengths and the amount of avoidance behavior observed suggests differences in intruder threat levels. Avoidance behaviors are directly associated with the resident fish behavior, as the resident provokes intruder avoidance by chasing and biting to defend territory. Small intruders may not pose a significant threat to a resident fish, so they receive fewer competitive advances. Other damselfish species (*P. partitus* and *P. planifrons*) are also less aggressive toward smaller juveniles due to the residents' judgment of threat level and distinct adult-juvenile territorial interests (Harrington 1993). On the other hand, large intruder fish may experience less avoidance behavior with similarly-sized resident fish due to the potential energetic and physical cost of a more balanced competition. The behavior we noticed, in which similarly-sized damselfish swam alongside one another while fluttering their fins, could be a potential agonistic display to resolve conflict by comparing size or body condition without resorting to costly aggressive behavior (Mathis and Britzke 1999; Briffa 2010). Intermediate-sized intruders (15 to 40 mm smaller than the resident) appear to experience the greatest defensive behavior from residents. An intermediate intruder size may generate this behavior as it is large enough to threaten the damselfish territory but not large enough to present a significant threat from physical conflict. This means territorial defense is necessary but less costly, thereby resulting in a higher level of aggression. Although we only studied territoriality between large residents and smaller intruders, the success of an intruder could differ with smaller residents. For example, the intermediate-sized intruders in our study may more successfully intrude upon the territory of similarly sized or smaller individuals. Relative size has been shown to determine damselfish success in territorial disputes (Forrester 1991). Thus, we suspect that the size difference between intruder and resident, rather than the absolute length of either individual, may drive levels of territorial conflict between damselfish.

The decrease in competitive interactions when the predator was present suggests a suppression in intruder fish behavior induced by predatory visual and chemical cues. When an intruder recognizes a predator, it may prioritize moving into the nearest refuge as quickly as possible and avoiding the energetic cost of territorial conflict. The intruder also displayed less avoidance behavior, suggesting that the resident fish may dedicate more energy to sheltering and less to maintaining an exclusive territory. With a predator nearby, competitive behavior could draw unwanted predator attention to both the intruder and resident fish, as increased water movement from competitive interactions can attract potential predators through visual and olfactory cues (Elvidge and Brown 2012; Hughes et al. 2010). Predator presence may also mediate the relationship between intruder size and the resulting competitive behavior. Though competitive behavior generally decreased for individuals of all sizes when a predator was present, this decrease was most profound for intermediate-sized individuals who experienced the greatest competitive behavior in the absence of a predator. As a result, the non-consumptive effects of predation may be strongest for intermediate-sized individuals who would otherwise be unlikely to occupy desirable territory due to high levels of aggressive territorial behavior from larger resident fish.

Despite the decrease in territorial behavior in the presence of a predator, damselfish predator-avoidance strategies and their outcomes could depend on territory availability and, therefore, the density of conspecifics. While an intruder could share a territory with its resident fish, as we occasionally witnessed in our study, this strategy may only grant an intruder a temporary refuge, as territorial behavior could quickly resume as soon as the predator moves past (Helfman 1988). Additionally, spatial limitations of a territory may prevent sharing. In our study, when the intruder did not share the territory with the resident, the intruder often occupied the zone farthest from the predator, likely to avoid the predator's line of vision or to maximize distance from the predator. Despite the reduction in direct intraspecific competition when a predator was present, a damselfish predator-avoidance strategy could still depend on conspecific presence and territory size. Preferred refuges, such as conch shells, could be limited in how many fish can effectively shelter inside. This space limitation could exclude some fish from the shelter, necessitating an alternate predator-avoidance strategy, such as flight.

Studying competitive behavior over a high-quality shelter in a controlled environment imposes some limitations on our study. Although we maintained the same predator for all behavioral trials, further studies could assess if a similar pattern of behavioral suppression is conserved in the presence of an egg-predator or a novel predator, such as the invasive lionfish. While damselfish have well-documented size-based hierarchies (Forrester 1991), our results also suggest that damselfish may be capable of resolving differences in length to the millimeter, generating additional questions on their sensory ecology. Throughout behavioral trials, we observed changes in the vibrancy of intruding damselfish coloration, indicating another understudied behavioral strategy that may reflect both intraspecific competition and predation.

Understanding how predator-prey dynamics mediate territorial behavior is increasingly relevant with heightened habitat degradation through climate change. Increasing coral reef degradation may limit high-quality habitat and sheltering (Fontoura et al. 2019) and disrupt damselfish alarm cues, decreasing their ability to establish meaningful territories and recognize predator cues (Chivers et al. 2016). Additionally, increasing temperatures may threaten damselfish populations by increasing predator attack speeds and reducing prey-escape speeds and responsiveness (Allan et al. 2015). Further research should seek to investigate how altered predator-prey interactions may also generate non-consumptive effects on territoriality and reproductive success. Understanding how environmental stressors may influence predation's consumptive and non-consumptive effects will be crucial in predicting how habitat degradation will disrupt reef fish communities.

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#### AUTHOR CONTRIBUTIONS

All authors contributed equally.

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## FIGURES

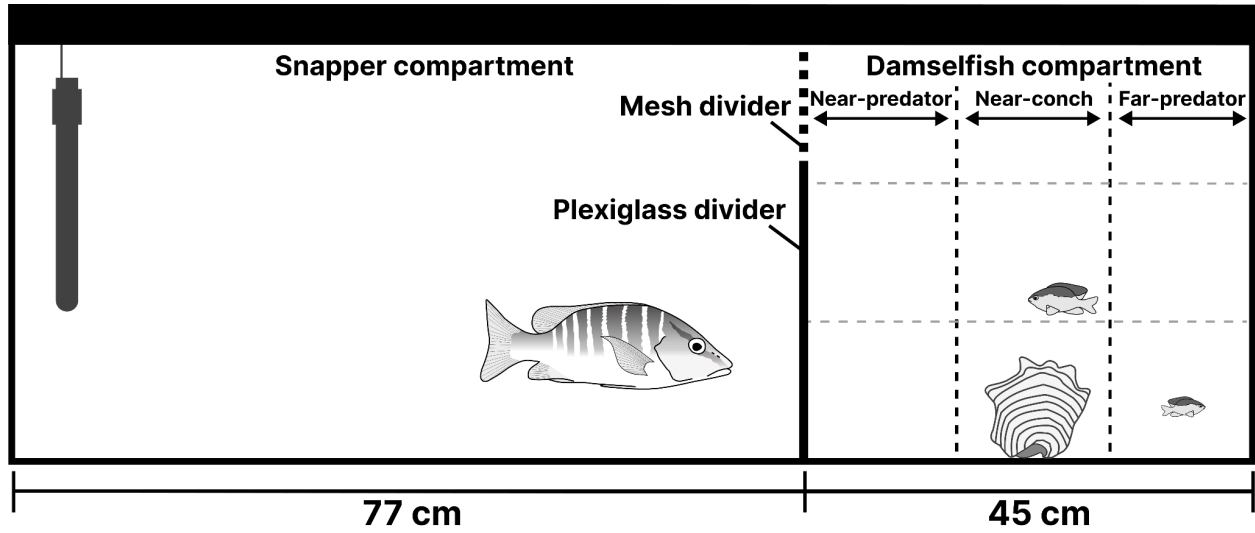


Figure 1. Tank setup including snapper compartment and damselfish compartment. Mesh and plexiglass dividers allow for the transmission of both visual and chemical cues between compartments.

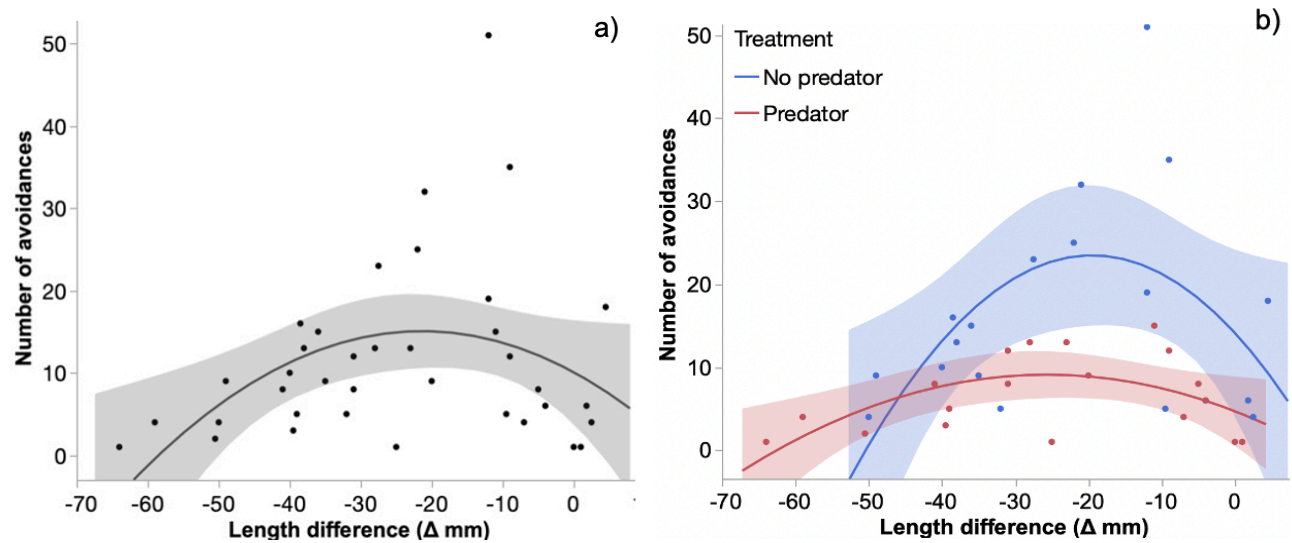


Figure 2. a) Quadratic regression of number of avoidances by length difference (intruder length - resident length in millimeters) and b) further separated by whether the predator was absent or present during the trial. Least squares regressions with 95 % confidence interval.

## Little Cayman

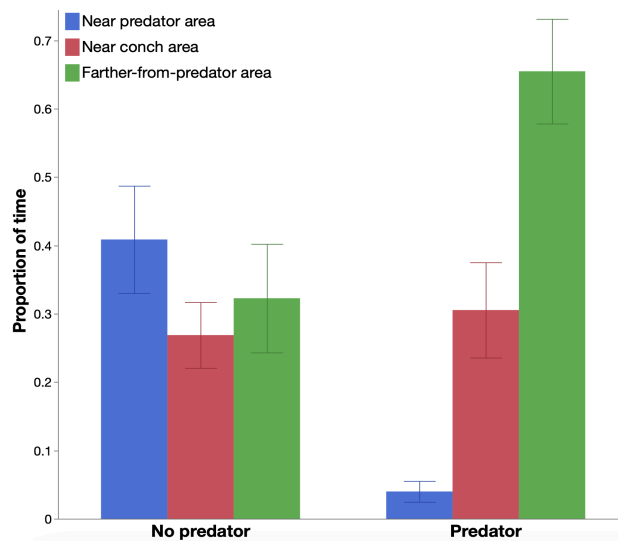


Figure 3. Mean proportion of time spent in each zone (near predator compartment, near conch, and far from predator compartment) in the absence and presence of a predator. Bars represent one standard error from the mean.

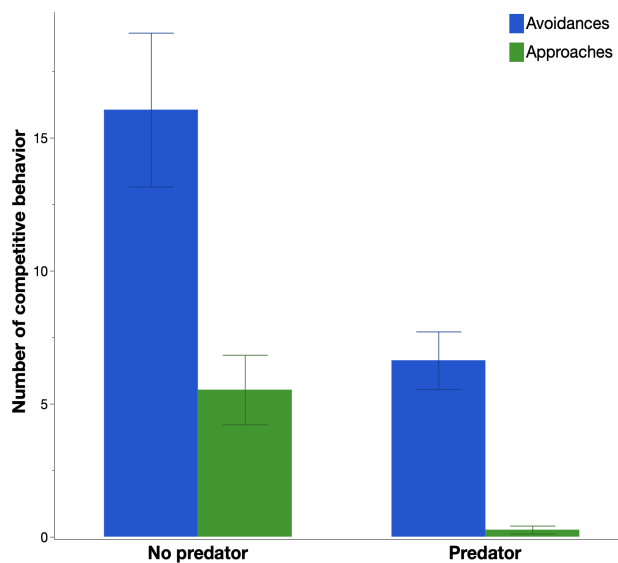


Figure 4. Mean number of avoidance and approach behaviors by the intruder in the absence and presence of a predator. Bars represent one standard error from the mean.