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SHORT COMMUNICATION

BURROWING BEHAVIOR OF MARSH PERIWINKLES *LITTORARIA IRRORATA* IN RESPONSE TO PREDATOR CUES[§]

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INTRODUCTION

Predators pose an existential threat to prey survival, and prey species have evolved numerous strategies for mitigating predation risk (Preisser et al. 2005, Weissburg et al. 2014). Prey may alter their behavior (Lima and Bednekoff 1999, Smee and Weissburg 2006), morphology (Relyea 2002, Miner et al. 2005), or life history (Kats and Dill 1998, Brown et al. 2013) to reduce their vulnerability to consumers. Behavioral responses to consumers are ubiquitous and occur in organisms ranging from bivalves (Smee and Weissburg 2006) to mammals (Fortin et al. 2005) and can be effective in reducing mortality caused by predation (Smee and Weissburg 2006, Flynn and Smee 2010).

Marsh periwinkles *Littoraria irrorata* are commonly found in salt marshes along the coastlines of the Atlantic Ocean and Gulf of Mexico (GOM). They can decimate marsh grasses and create large bare patches when uncontrolled by predators (Silliman and Bertness 2002). Periwinkles provide an important food source for numerous species of fish and invertebrates (Zengel et al. 2016). Climbing behavior of periwinkles (Warren 1985, Robinson and Rabalais 2019) as well as other snails in both marine and freshwater systems is a well—known response to chemical exudates from predators (Jacobsen and Stabell 2004, Belgrad and Smith 2014). To avoid consumers such as blue crabs *Callinectes sapidus*, periwinkles will climb the stems of marsh plants including *Spartina alterniflora* (Warren 1985, Carroll et al. 2018, Robinson and Rabalais 2019).

Studies examining the climbing behavior of periwinkles are often conducted on scales of hours to days (Henry et al. 1993, Robinson and Rabalais 2019). We tested shorter term responses of marsh periwinkles to predation risk cues from blue crabs and measured both climbing and burrowing behaviors as well as time inactive. Burrowing is not typically investigated in this species, but we noticed periwinkles burrowing in preliminary observations. We sought to examine behavior on shorter time scales to ascertain how quickly periwinkles react to predation risk and if their short—term behavior is consistent with those documented over longer durations. Further, using periwinkle climbing behavior as a bioindicator in behavioral assays can be a useful experimental tool, and short—term experiments are preferred for this purpose.

MATERIALS AND METHODS

Marsh periwinkles were collected from Airport Marsh on Dauphin Island, AL during the summer of 2022. Blue crabs were collected from crab pots in Mobile Bay, AL. Periwinkles were housed in aerated seawater tanks (salinity 20) until use in experiments. They remained in the lab for at least 72 h before being tested and were used within 2 weeks from the date of original collection. Periwinkles were not fed after collection. Blue crabs were housed in a separate tank and fed an ad libitum diet of periwinkles, oysters, and fish scraps 3 times per week. We did not document food preferences, but all food items were consumed within 24 hours.

Behavioral assays were conducted in 2.0 L round glass aquaria (15 cm diameter x 25 cm tall). Sand was collected from beaches on Dauphin Island, thoroughly flushed with freshwater and allowed to dry, and then added to each jar to a depth of 5.0 cm. One liter of seawater (salinity 20) was added to each aquaria. Seawater was taken directly from the GOM and stored in a tank (2.0 m diameter x 0.5 m deep) for 3—7 days to allow particulate matter to settle out and ambient chemical cues to breakdown. Salinity was adjusted to the desired level of 20 by adding deionized water or salt (Instant Ocean™).

Our experiment consisted of 2 treatments: a control of seawater and a predation risk treatment containing water from a tank housing blue crabs. To make the predator water treatment, 6 blue crabs were housed in a 238 L mesocosm and fed 3—5 hours before behavior experiments were initiated. For control aquaria, 1.0 L of seawater was added, and for aquaria in the predation risk treatment, we added 0.5 L of seawater and 0.5 L of water from the blue crab tank. One periwinkle was added to each aquarium and manipulated with a stick so that its aperture opening faced the sediment, and its behavior was monitored for 10 min. Eighteen pairs of aquaria were tested such that a control and treatment were tested simultaneously.

[§]*The first author conducted this research as part of the Dauphin Island Sea Lab's Research Experience for Undergraduates in the coastal and nearshore marine science program.*

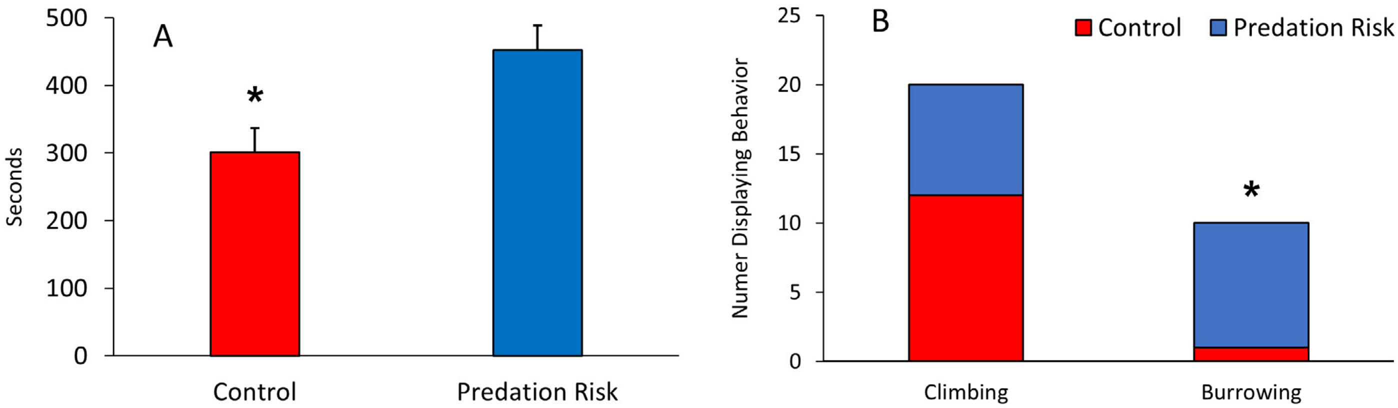

FIGURE 1. *Behavior of marsh periwinkles* (Littoraria irrorate) *in laboratory experiments. Control—clean seawater. Predation risk—seawater from an aquariam housing blue crabs,* Callinectes sapidus*. A. Mean ± se seconds marsh periwinkles were active in control and predation risk treatments. * indicates significant difference (ANOVA, p < 0.05) B. Number of marsh periwinkles displaying climbing or burrowing behavior in control and predation risk treatments. * indicates significant difference (Chi-Square test, p < 0.05) between control and predation risk.*

We measured the time(s) taken for snails to initiate movement and the total time spent active. These behaviors were compared among treatments (control, predator water) using ANOVA in JMP Pro 14.0. We also compared the frequency of climbing on the sides of the aquaria and burrowing using a binary response of yes the behavior occurred or no it did not occur. Some individuals exhibited both behaviors and were scored as yes for both. Five individuals from each treatment remained inactive for the duration of the experiment and were excluded from analysis, resulting in n = 13 for each treatment. Chi—square tests were used to compare the frequency of climbing or burrowing between treatments in JMP Pro 14.0.

Results and Discussion

Periwinkles became active within 5 minutes of placement into the aquaria, and we did not find significant differences in the time for periwinkles to initiate activity among predation risk treatments and controls ($F_{1,25} = 1.43$, $p = 0.24$). However, periwinkles in predation risk treatments were significantly more active than those in controls ($F_{1,25} = 8.76$, $p < 0.01$, Figure 1A). Unlike previous studies, we did not find significant differences among treatments regarding frequency of climbing ($\chi^2 = 3.47$, $p = 0.06$, Figure 1B), and climbing occurred more frequently in controls than in predation risk treatments. However, we found significantly more periwinkles burrowed in the predation risk treatments than in controls ($\chi^2 = 10.4$, $p < 0.01$, Figure 1B). In the controls, only a single periwinkle burrowed, while 9 or 70% burrowed in the predation risk treatments. In the predation risk treatments, of the 9 periwinkles that burrowed, 5 of them climbed the side of the jar first, before returning to the sediment and burrowed.

Climbing behavior is a well—known response to predation risk in marsh periwinkles (Warren 1985), and we were surprised that climbing was not different among treatments in our study and tended to be more common in controls than predation risk treatments. Although we measured climbing on the sides of our aquaria, in preliminary trials we also used PVC dowels (sensu Carroll et al. 2018) but our periwinkles did not climb on those either. A couple of methodological differences between this work and prior studies examining climbing behavior may account for differences observed. For example, earlier studies have been conducted over the span of hours (Warren 1985, Carroll et al. 2018) to days (Robinson and Rabalais 2019) and use multiple individuals per treatment (Carroll et al. 2018, Robinson and Rabalais 2019). Perhaps a longer experimental duration would have resulted in similar findings as periwinkles in the predation risk treatments tended to be more active, and given time may have found a preference to be out of the water. However, burrowing behavior in response to predation risk has rarely been reported for this species, and our results suggest that periwinkles may have different types of responses to predation risk. Burrowing responses may have been more noticeable in our experimental set up than in larger scale mesocosms where this behavior could be easily overlooked. We also used predator—conditioned water rather than live crabs as used by other scientists, which might also account for differences in responses (Carroll et al. 2018, Robinson and Rabalais 2019). The climbing behavior of periwinkles may vary among populations, which might also account for notable differences found in this study (Carroll et al. 2018).

Our results indicate that marsh periwinkles may burrow in response to predation risk, and future studies examining anti—predator behavior in this species should monitor this behavior as well as climbing frequency. The methods here provide a short—term behavioral assay that can be used to ascertain periwinkle responses to predation risk and provides a useful tool as a bioindicator. Such short—term experiments like this one are probably less ecologically relevant than previous work performed for longer durations (Robinson and Rabalais 2019). However, short—term bioassays such as this can be useful for investigating the specific identity of chemical exudates released by predators and provide a new behavior and time frame for such investigations.

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