
Trait-mediated Indirect Interactions: Predator evasion, alarm signaling, and threat assessment in a marine gastropod and their influence on community composition

Katharine Canning

Abstract

Historically, the study of the effects of trophic interactions on community compositions has dealt with density-mediated indirect interactions (DMII). Trait-mediated indirect interactions (TMII) are a new way of considering food webs, and may have an equal or greater community-wide effect than DMII. In my experiments, I looked at how predator identity and alarm signaling can elicit varying degrees of escape response in a marine gastropod, *Tegula funebris*, and how these varying levels of response correspond to variable operation of TMII in a system. From observing the behavior of *T. funebris* when exposed to possible predators, I found four predators that elicited escape responses: two predatory sea stars and two predatory gastropods. By recording the orientation and traveling time of *T. funebris*, when placed in the same container with one of the predators or a crushed conspecific, I was able to determine an escape response gradient. I found that of the four predators, *Leptasterias hexactis* elicited the strongest response and *Acanthina spirata* elicited the weakest. There was also a statistically significant difference between the degree of response elicited by predatory sea stars and that elicited by predatory gastropods. In a second experiment I studied the feeding behavior of *T. funebris* in the presence and absence of *L. hexactis*, *Acanthina spirata*, and a crushed conspecific. I collected data on the percent cover of diatoms remaining on glass slides from a total of eight experimental treatments. Measurements of snail and predator sizes were also taken and recorded. I found that predator identity had a significant effect on diatom cover when *T. funebris* was present. However, *L. hexactis* was the only predator that significantly affected diatom cover

in the lab. Snails in the presence of sea stars grazed on fewer diatoms than when sea stars were absent, indicating that the presence of sea stars altered the feeding behavior of *T. funebris*. The difference between the two types of predators can possibly be attributed to differences in hunting modes and habitat domains. The lack of significant results for crushed conspecifics indicates that *T. funebris* are quite discerning in assessing predation risk.

1. Introduction

Trophic cascades are widely recognized as determinates of community compositions (Schmitz et al., 2004). Until recently, our understanding of trophic cascades was limited to density-mediated indirect interactions (DMII), which are the result of predation on herbivores. Removal of herbivores from the population by the predator leads to a positive indirect effect on the plant community (Trussell et al., 2002). The alternatives to DMII are trait-mediated indirect interactions (TMII), which are the result of a change in prey behavior or in morphology in the presence or absence of its predator (Schmitz et al., 2004).

Changes in morphology and behavior of organisms in response to predator threats have been well documented in many animals. These include protozoans, crustaceans, gastropods, insects, fishes, amphibians, and mammals (Rochette et al., 1997). Behavioral changes are often more recognizable than morphological ones, and include refuge seeking and changes in grazing rates and patterns for herbivores (Trussell et al., 2003; Jacobsen and Stabell, 2004). Without actually decreasing in abundance themselves, herbivores that are able to employ these defensive behaviors have positive effects on plants, as fewer plants are consumed. This is the essence of TMII.

To examine the effects of just a single predator, however, would be to ignore the complexity of interactions that occur between prey and predators in a given environment. Many studies have shown that prey organisms are often very adept at distinguishing one predator from another and at assessing the overall threat that the predator may pose (Legault and Himmelman, 1993; Rochette et al., 1997; Bernot and Turner, 2001). Other studies have shown that conspecific alarm signaling is an important component of predator evasion as well (Rochette et al., 1997; Jacobsen and Stabell, 1999; Jacobsen and Stabell, 2004). How these prey abilities might affect TMII, however, has been studied very little.

A prey species' ability to discern threats may confer a selective advantage. The absence of this ability means that escape behaviors will use up energy that might otherwise be used to stay in the area and find reproductive opportunities or continue feeding (Rochette et al., 1997). Lima and Bednekoff (1999) call this the Predation Risk Allocation Hypothesis. If one assumes that prey weigh the costs and benefits of their behaviors, then the strength of a defensive response is correlated with the threat of predation (Legault and Himmelman, 1993; Rochette and Himmelman, 1996). In that case, what are the varying degrees of behavioral responses to different predators, and do these levels of response also differ in their effects on community composition?

In my research, I sought to determine whether *Tegula funebris* do indeed show predator sensitivity and response to conspecific alarm signaling, with the hope of connecting these to TMII in a simple food chain. Using preliminary observations of the behavior of *T. funebris* with various possible predators, I was able to determine two predatory sea stars and two predatory gastropods to which the snails responded with escape behaviors. Next, by recording the orientation of snails exposed to each predator, along with other relevant data, I hoped to find a difference in the degree to which *T. funebris* responded to the different predators. Finally, by documenting the percent diatom cover on slides in both the presence and absence of a predator that elicited a strong response, a predator that elicited a weak response, and alarm signals from crushed conspecifics when *T. funebris* was present, I hoped to discover whether predator

identity or conspecific alarm signaling alone had a positive indirect effect on the abundance of diatoms. If this effect were found to exist in the absence of actual predation of *T. funebris* by the predators, then I would have evidence to support the existence of TMII in this system.

2. Materials and Methods

All organisms were collected from the rocky intertidal of Horseshoe Cove on the Bodega Marine Reserve.

2.1 PRELIMINARY OBSERVATIONS

The purpose of this part of the experiment was to determine the predators that induced an escape response in *Tegula funebris*. Various possible predators of *T. funebris* were collected and brought into the lab, including *Pachygrapsus crassipes*, *Pisaster ochraceus*, *Leptasterias hexactis*, *Nucella emarginata*, *Acanthina spirata*, *Pagurus samuelis*, and *Asterina miniata*. Snails along with each of the predators were placed in large fingerbowls containing saltwater, and their behavior was observed and recorded. Predators were noted as having induced an escape response if the snail turned and crawled away from it. Observations were also made on whether contact was necessary to induce a response and on whether a climbing response was elicited. Five trials, using five different snails, were performed with each predator.

2.2 DETERMINING STRENGTH OF PREDATOR THREAT

From my preliminary observations I found that four of the seven predators tested elicited an escape response in *T. funebris*. These four predators (*P. ochraceus*, *L. hexactis*, *N. emarginata*, and *A. spirata*) were used in the next step of the experiment. All specimens were collected approximately 48 hours before their use. None of the animals was kept in the lab longer than a week.

I obtained 10 large rectangular plastic containers in which to conduct my experiments. I drew a circle with reference angles at one end of each of the containers. All trials were performed on a water table with running water. Approximately 24 hours prior to each trial, snails were placed in the containers in 1800 ml of salt water, in order to allow them to acclimate. The next day, a treatment was chosen at random for each container. For four of the treatments, one of the four predators was added to the container, and for the fifth, crushed conspecifics were added. All of these were placed at the far end of the rectangle, at the edge of the circle. At the same time, the snails were placed in the center of the circles facing the predator. The angle at which the snails began to move away from the predator was recorded, as well as the time it took the snail to travel 3 cm. If the snail did not move for several minutes, or the predator did not move toward the snail, then forced contact between snail and predator was induced by placing the two side-by-side. After 15 and 30 minutes I recorded the location (in, out, or in/out of the water) and position (horizontal or vertical) of the snail, as well as whether contact between the snail and predator occurred.

I also conducted the same experiment on a control group, which underwent all the same steps except that no predator or chemical cue was added to the containers. Between each set of replicates, the containers were rinsed thoroughly with salt water and allowed to dry. In total, I ran ten replicates of each treatment. At the end of the experiment, the snails and predators were measured and recorded for later statistical analysis. Rayleigh's test for circular uniformity was run on the circular data, and Watson-Williams Multisample test was run to compare the mean angles of each treatment. ANOVAs were run on all noncircular data.

2.3 DETERMINING THE OPERATION OF TRAIT-MEDIATED INDIRECT INTERACTIONS

From the above experiment, I determined that *L. hexactis* elicited the strongest escape response, while *A. spirata* elicited the weakest, and crushed conspecifics elicited an intermediate response. Therefore,

these three treatments were used in the following experiment. Prior to beginning this experiment, microscope slides were set out on a running water table two weeks in advance, so that diatoms could collect and grow on them.

In total, eight experimental treatments were applied: *T. funebris* absent with (1) no predator present, (2) *L. hexactis* present, (3) *A. spirata* present, (4) crushed conspecific present; *T. funebris* present with (5) no predator present, (6) *L. hexactis* present, (7) *A. spirata* present, and (8) crushed conspecific present. The treatment containing neither *T. funebris* nor a predator species was a control to account for loss in diatom cover not due to grazing by *T. funebris*. The treatments with only a predator or crushed conspecific present acted as a secondary control to account for any direct effects the predators might have on the percentage of diatom cover.

Fifteen replicates of each treatment were conducted and their order randomized so that three replicates of each treatment were run simultaneously. Before any snails or predators were added, I poured 125 ml of fresh seawater into each of the small fingerbowls used in the experiment, and added the diatom covered slides. Finally, the animals were added according to the treatment.

I made observations of snail location (water, air, or water/air interface), and current feeding behavior (yes or no) at two 30-minute intervals following the beginning of the experiment. After the second set of observations, the experiment was terminated and the size of the snails and predators was measured. The percentage of diatom cover of the slides was also estimated at this time. Two factor ANOVAs were performed on the data. One factor ANOVAs were performed on the presence or absence of a predator versus the location and feeding behavior of *T. funebris*.

3. Results

3.1 PRELIMINARY OBSERVATIONS

Four of the seven predators tested elicited escape responses from *Tegula funebris*. For these four, there was a general trend of contact between predator and prey, or of close proximity between the two, before a response was elicited. Contact was particularly necessary for *T. funebris* to respond to *Acanthina spirata* and *Nucella emarginata*.

3.2 DETERMINING STRENGTH OF PREDATOR THREAT

T. funebris turned furthest away from *L. hexactis* (25.24°; Rayleigh's, $p < 0.005$). Conversely, *T. funebris* turned the least far away from *A. spirata* (71.95°; $p < 0.05$). All other treatments resulted in intermediate turning responses. For *P. ochraceus*, the mean angle was 59.85° ($p < 0.001$); for *N. emarginata* the mean angle was 51.11° ($p < 0.01$); for crushed conspecifics the mean angle was 67.51° ($p < 0.01$); and for the control group the mean angle was 1.71° ($p < 0.05$) (Fig. 1). Running the Watson-Williams Multiple Comparisons test, I found that the mean angles for each of the treatments were significantly different from each other ($p < 0.0005$).

The average time it took the snails to travel 3 cm after responding to the predator was found to be statistically different among treatments (ANOVA, $p < 0.001$). There was a very noticeable difference between the traveling times of snails moving away from the two sea stars as opposed to the two predatory snails. *T. funebris* took less time to travel when responding to *L. hexactis* and *P. ochraceus*, and took a longer time to travel in response to *N. emarginata* and *A. spirata* (Fig. 2). Traveling times for the two snail treatments, the crushed conspecifics treatment, and control treatment were similar (Tukey's, all $p > 0.10$). Traveling time for the two sea star treatments was also similar (Tukey's, $p = 0.999$).

Fifteen minutes after responding to the predator, *T. funebris* was found out of the water most frequently in the treatment containing *P. ochraceus*. *T. funebris* was most frequently found in the water, either on the horizontal or vertical surface, in the treatment containing *A. spirata* (ANOVA, $p < 0.001$)

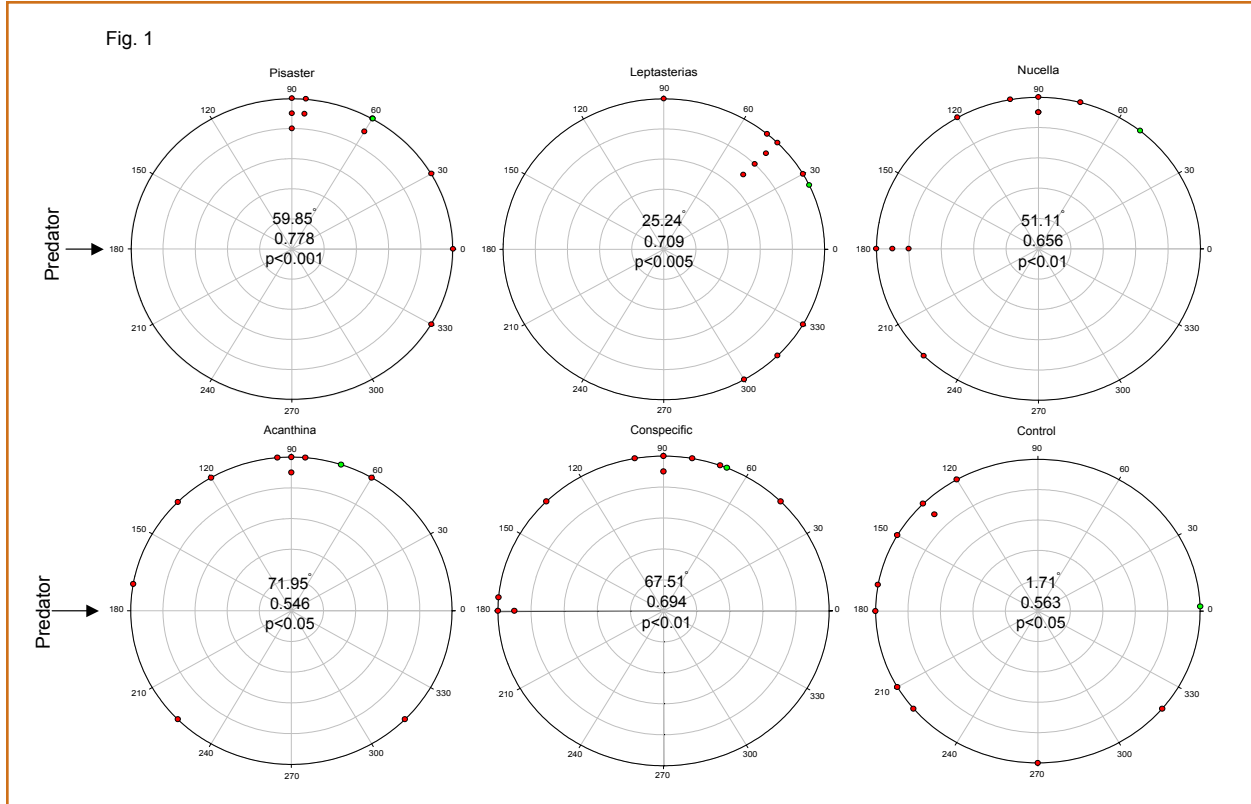


Fig. 1. Orientation of movement of *Tegula funebralis* away from (1) *Pisaster ochraceus*, (2) *Leptasterias hexactis*, (3) *Nucella emarginata*, (4) *Acanthina spirata*, (5) crushed conspecific, (6) nothing (control). Each filled dot represents the angle at which each individual *T. funebralis* began moving. The unfilled dot on each plot represents the mean angle of all data points for that treatment. This is the first number listed in the center of the plot. The second number is the value of the r-statistic, and the last number is the p-value of Rayleigh’s test for circular uniformity. The arrows on the left point in the direction in which the predator or crushed conspecific was placed.

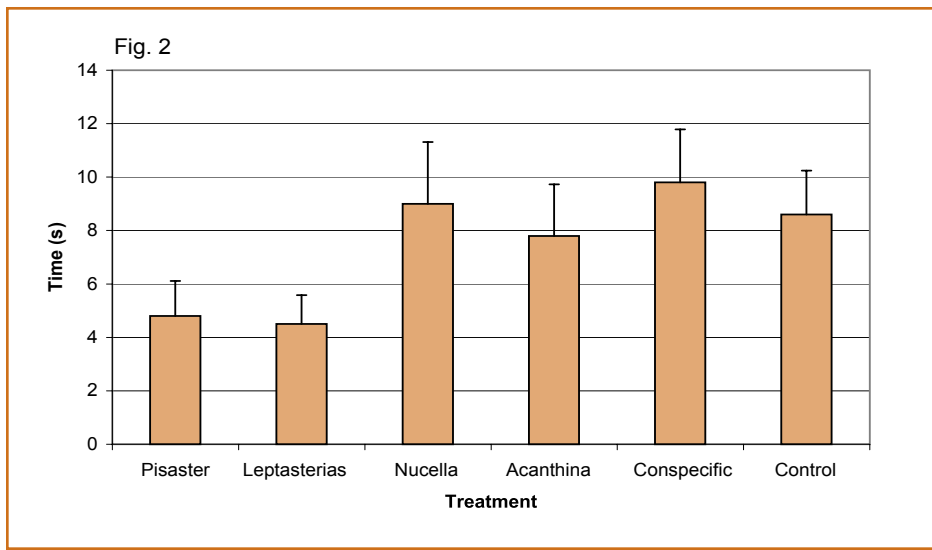


Fig. 2. Average time (+/- SD) it took for *T. funebralis* to travel 3 cm after responding to one of four predators or a crushed conspecific. Differences among groups were found to be statistically significant (ANOVA, p < 0.001).

(Fig. 3a). Thirty minutes after responding to the predator, this was still true (ANOVA, $p < 0.001$) (Fig. 3b). As with the traveling time data, there is an apparent difference in the location of the snails between the sea star treatments and the predatory snail treatments. This suggests that it took the snails longer to move out of the water when exposed to predatory snails than when exposed to predatory sea stars. This also appears to be true for the crushed conspecific treatment.

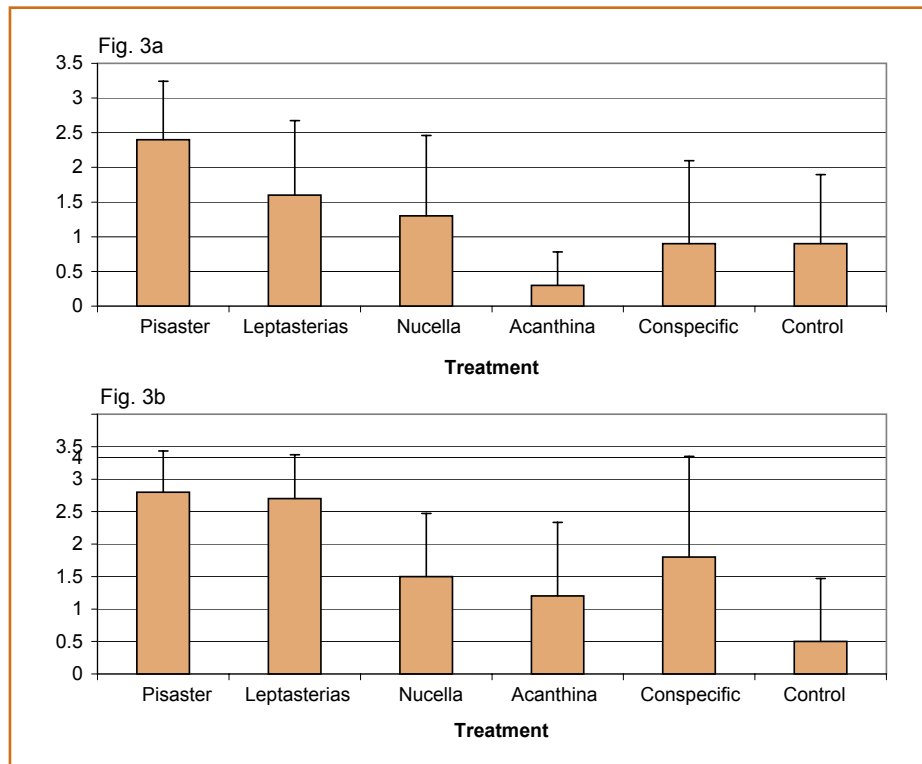


Fig. 3. Average location (+/- SD) of *T. funebris* (a) 15 minutes after being exposed to a predator or a crushed conspecific and (b) 30 minutes after being exposed. These plots were made by assigning a numerical value to categorical responses: 0 = in the water on the horizontal surface, 1 = in the water on the vertical surface, 2 = in/out of the water, 3 = out of the water. A low average indicates that the snails were found in the water more often than out of the water. Differences among groups at both time points were found to be statistically significant (ANOVA's, $p < 0.001$).

3.3 Determining the operation of trait-mediated indirect interactions

In all the treatments with *T. funebris* present, there was less diatom cover at the end of the experiment than in the treatments where *T. funebris* was absent. The only predator that appeared to have any direct effect on diatom cover was *A. spirata*; however, this effect was not significantly different from the other treatments where *T. funebris* was absent.

Predator identity, however, did have an effect on diatom cover as mediated by *T. funebris* (ANOVA, $p = 0.014$). When *L. hexactis* was present, *T. funebris* ate less than when the sea star was absent (ANOVA, $p = 0.007$). When *A. spirata* was present, *T. funebris* consumed the same amount as when it was absent (ANOVA, $p = 1.00$). When crushed conspecifics were present, *T. funebris* consumed less than when the alarm signal was absent; however, this was not quite a significant effect (ANOVA, $p = 0.069$) (Fig. 4).

Thirty minutes after the beginning of the experiment, *T. funebris* was found out of the water more often when *L. hexactis* was present than when it was absent, or when *A. spirata* or conspecifics were present (Fig. 5a). The opposite was true when conspecifics or *A. spirata* was present. In these instances, more snails were found in the water. The difference among groups was found to be significantly different (ANOVA, $p = 0.006$). After sixty minutes, these same trends were observed once again (ANOVA, $p = 0.001$) (Fig. 5b).

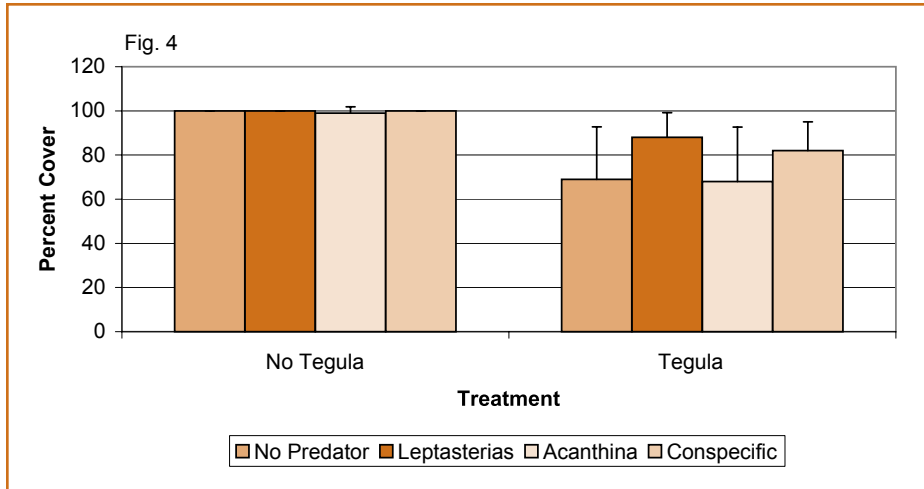


Fig. 4. Average percent diatom cover (+/- SD) at the end of the experiment for each of the eight treatments. *T. funebris* grazed fewer diatoms when *L. hexactis* was present than when it was absent. The same was true for crushed conspecifics; however, this was not true for the predatory gastropod *A. spirata*. A statistically significant interaction between predator identity and diatom cover was found (ANOVA, $p=0.014$).

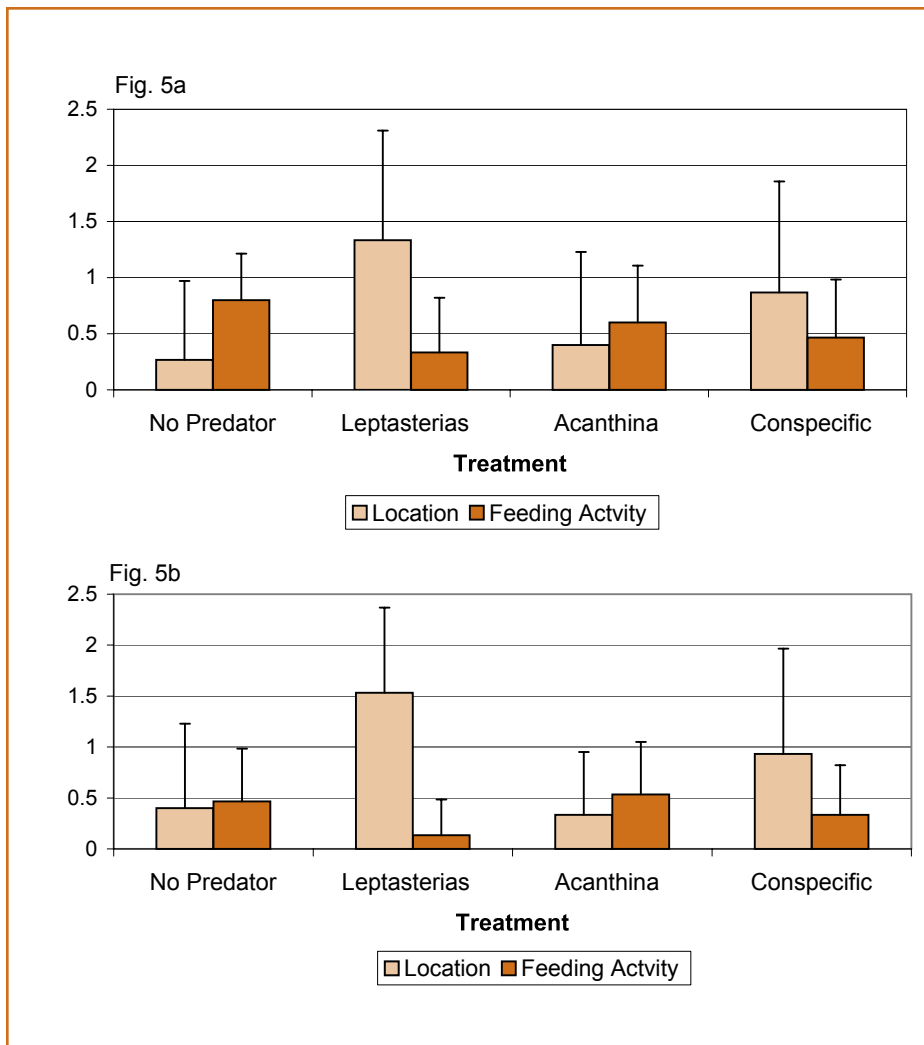


Fig. 5. Location and feeding activity of *T. funebris* (a) 30 minutes and (b) 60 minutes after the start of the experiment. These plots were made by assigning a numerical value to categorical responses. For location: 0=in the water, 1 = at the air/water interface, and 2 = in the air. For feeding activity: 0 = not feeding and 1 = feeding. A high number for location indicates that the snails were found most often out of the water. A high number for feeding activity indicates that the snails were most often found eating. Differences in location among groups were found to be statistically significant for both 30 and 60 minutes after the beginning of the experiment (ANOVA, $p=0.006$, $p=0.001$, respectively). Differences in feeding activity among groups were not found to be statistically significant for either 30 minutes or 60 minutes after the start of the experiment (ANOVA, $p=0.065$, $p=0.112$ respectively).

Although it appears that, after 30 minutes, there were fewer *T. funebris* feeding in all treatments containing a predator or crushed conspecifics, these results were not found to be statistically significant (ANOVA, $p=0.065$) (Fig. 5a). The same was true after 60 minutes (ANOVA, $p=0.112$) (Fig. 5b). I found that snail size and the size of the predator had no statistically significant effect on percent diatom cover ($p_{\text{snail}}=0.897$, $p_{\text{predator}}=1.00$), indicating that relative sizes of the animals did not affect the observed interactions.

4. Discussion

Different predators can elicit varying degrees of escape response intensity. Often this is because predator identity is closely related to hunting mode. In a review by Schmitz et al. (2004), he identifies three different classes of predators: (1) Sit-and-wait predators, (2) Sit-and-pursue predators, and (3) Active hunters. According to this model, habitat range of the predator and prey species is also important in determining the behavioral response of the prey species and whether TMIs or DMIs will dominate.

From the portion of my experiment used to determine predator sensitivity, there was an obvious difference in the intensity of *Tegula funebris*' escape response between predatory sea stars and predatory gastropods. The results from my experiment to determine trait-mediated indirect interactions (TMII) clearly show that *Leptasterias hexactis* has a positive indirect effect on diatom cover mediated through a change in behavior of *T. funebris*. *Acanthina spirata*, on the other hand, shows little or no positive indirect effect on diatom cover. This finding indicates that TMIs are operating in the former system but not in the latter. These results could be explained by the differences in predator hunting mode.

When both predator and prey have broad habitat ranges (i.e., they occupy a wide range of the habitat), then prey organisms rarely change their behavior or shift habitat sites in response to the predator (Schmitz et al., 2004). This observation likely explains the reduced escape response of *T. funebris* in the presence of the two predatory gastropod species. *T. funebris* and *A. spirata* are able to live anywhere from the high to low intertidal zones, while *N. emarginata* can be found anywhere from the mid to low zones (Sheldon, 1999). In this case, both predator and prey have broad habitat regions.

However, when a prey organism with a broad habitat domain encounters a predator with a narrow habitat domain, it frequently exhibits escape behaviors (Schmitz et al., 2004). This observation helps explain the higher escape response intensity of *T. funebris* in the presence of *L. hexactis* and *P. ochraceus*. Both of these sea stars have narrow habitat ranges, occupying only the lower middle to subtidal zones where tide pools are more frequent (Sheldon, 1999).

With this knowledge, it is then easy to infer from the hunting mode of the predator whether TMIs or DMIs will dominate. From my preliminary observations, I observed that the sea stars were the more active of the two kinds of predators. They would often move in pursuit of *T. funebris* by following its slime trail. The two gastropods did not seem to actively pursue their prey, however, often remaining in one area until contact occurred. As both *A. spirata* and *N. emarginata* feed on prey by drilling holes in their shells, it would seem less important for *T. funebris* to escape quickly, and thus the difference in dominance of TMIs in the system. An active predator in a narrow domain, such as a predatory sea star, will be more abundant and harder to escape, requiring more haste by the prey species. In this situation it is easy to imagine that TMIs will operate on the system.

Although *T. funebris* did respond to crushed conspecifics, it had little or no positive indirect effect on diatom cover. According to a study conducted by Jacobsen and Stabell (2004), conspecific alarm signaling elicited the greatest behavioral response when combined with predators feeding on conspecifics. This finding may help to explain my results. Injured conspecifics alone may not be enough to elicit strong escape responses, perhaps because injuries to conspecifics can occur in the absence of predators. Because energy acquisition is important to organisms, it might be too energetically expensive to respond with

great intensity to conspecific alarm signals as the signal may not always mean that there is an immediate threat from a predator (Rochette et al., 1997).

Acknowledgments

I would like to thank Kathryn Freeman and Marissa Bauer for their assistance in specimen and data collection. I would also like to thank Steven Morgan, Amber Mace, and Ben Miner for their help in experimental design. This project was made possible by the comedic efforts of KKMFN_© (you know who you are). Lastly, I would like to thank the Bodega Marine Laboratory for allowing me this amazing and challenging opportunity.

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