Non-Genetic Littoraria Fitness: How Size, Environment, and Health Affect Survivorship of Predator Interactions

Thomas Murphy

Under the supervision of Dr. Brian Silliman,
Department of Marine Science & Conservation, Duke University

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Research Supervisor

Faculty Reader

Director of Undergraduate Studies

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Abstract

Marsh periwinkles (*Littoraria irrorata*) have many predators. When they encounter one, traits of both the periwinkle and its environment should contribute to whether or not it escapes. A better understanding of how these interactions are affected could provide greater insight into how changing habitats will affect ecosystem dynamics in Atlantic salt marshes. By counting the scars from such interactions on periwinkles hand-collected from several sites in salt marshes near Beaufort, NC, the effect of the environment (i.e. density and height of vegetation, distance from ocean access) and the periwinkle's own non-genetic characteristics (i.e. size/age) on survivorship were determined. Using Single and Multiple Linear Regression analyses, no correlation between these features and the rate of scarring was determined.

Introduction

The predator/prey struggle ranks among the most important drivers of evolution and ecological change. Prey animals that successfully escape an encounter with predators are more likely to survive and reproduce, even though they may have scars commemorating the escape. This is a consistent factor through all ecosystems, but it is especially clear in the relationships between marsh periwinkle snails (*Littoraria irrorata*) and blue crabs (*Callinectes sapidus*) in salt marsh environments. In these, generations of snails and crabs have lived and died in bands of mud between ocean and dry land, both adapting to better counter the others' adaptations. However, many aspects of those predator-prey interactions are not dependent on genetics; differences in time, health, and environment easily change an encounter's outcome. For instance, a snail may more easily escape from a crab, even if it is left visibly marked by the interaction, by being younger and smaller than others.

Snails and crabs are a particularly useful model for such interactions due to their shells. The hard exoskeleton of the blue crab is its primary tool for destroying the protective shell of the periwinkle; the claw crushes the shell, cracking or breaking it outright, but the snail may still escape. If it survives the injury, the shell can heal, but a scar will be apparent (Blundon and Vermeij, 1983; Dietl and Alexander, 2008). Because little else in the environment can cause such scarring, the presence of marks can tell a story of successful escapes.

These visible marks can be useful tools for recognizing individual animals that have survived interactions with predators. Since scarred individuals have been proven to be more likely to survive an interaction with a predator, they have been established as "fitter" in that way than their peers. While this shows which individuals are exceptional, it does not show what makes them so. In order to determine the distinguishing features, it is necessary to investigate those other key aspects of the snail that correlate with greater or lesser fitness in predator survivorship, such as size, habitat, and health.

The habitat of these snails is dominated by salt-resistant plants, primarily marsh cordgrass. *Littoraria* tend to live on or between the cordgrass stalks, which blue crabs cannot climb. Because of this, areas of denser and higher cordgrass offer more space for the snails to take refuge out of the crabs' reach. The density of the snails also has an effect on this, as the refuge space is defined by how many individuals seek shelter there; in the same vein, the concentration of crabs has an effect, as it determines how common threats to the snails are. The cordgrass, snails, and crabs do not exist in a vacuum, however; they are also influenced by distance from the shoreline and the grazing habits of larger animals. Many of the marshes around Beaufort are on barrier islands, and there can be a great deal of ecological variation depending on position relative to the tides, and how well certain organisms compete in environments of different salinity. For instance, while cordgrass typically dominates the intertidal zone, it is bounded on the water side by oyster colonies, which handle submersion better than cordgrass, and on the land side by less salt-tolerant plants, which can out-compete the cordgrass in other areas. This means that the cordgrass stalks' density and height are in part dependent on their distance from competitors. Additionally, the barrier islands of North Carolina are historically home to a population of feral horses that sometimes trample or graze on the cordgrass. This can endanger the snails by temporarily eliminating their refuge space. These external forces all have unique effects on the likelihoods of both encountering a predator and surviving that encounter.

While there is no specific size for a snail at which it becomes more threatened, blue crabs have been shown to be more effective in hunting larger periwinkles in an experimental environment (Schindler et al, 1994). Using scarring rates, researchers have also shown that crab predation on periwinkles decreases as one moves further from the edge of the sea into denser cordgrass, and increases once past the cordgrass (Dietl and Alexander, 2008). The well-being of an individual snail may also have an effect; a previously-injured or parasitized periwinkle will have a different likelihood of survivorship than a completely healthy one. For instance, a University of Maryland experiment showed that repaired parts of a periwinkle shell are no weaker than any other part of the same shell (Blundon and Vermeij, 1983). In fact, crabs tend to consume more unscarred individuals than scarred ones when both are present in similar numbers, indicating that the repaired shells are in fact stronger (Greenfield, Lewis, and Hinke, 2002). Additionally, while the periwinkle has not been studied in this way, the New Zealand mud snail (*Zeacumantus subcarinatus*) has been shown to produce more reparative material for the shell when parasitized by certain trematodes, strengthening the shell and healing the wound faster (MacLeod, Poulin, and Lagrue, 2017). These studies all show that there are relationships between the separate factors of the periwinkle's life, but no study has yet compared all of them to one another until this one. By addressing this gap, it will be possible to develop a more complete understanding of the interconnected systems of salt marsh life, connecting seemingly disparate elements like snail parasites and cordgrass density.

For this study, approximately three hundred marsh periwinkles were collected from six distinct sites in the cordgrass-dominated salt marshes surrounding the Duke University Marine Lab in Beaufort, North Carolina. By inspecting them closely, a relationship among size, habitat, and health of the snails and their likelihood of surviving a predator interaction was investigated, in order to determine the relationship between individual non-genetic features and ability to evade predation. All other factors being roughly equal, younger and smaller periwinkles living in areas of lower and thinner cordgrass should show more scars than others, and those with multiple scars should not be uncommon. In terms of proximity to the shoreline, there should be an optimal distance at which the most snails show no scarring, and on either side of which there are more signs of successful predation; this should match the density of the cordgrass, which is thinnest when it has more competition at the upper and lower limits of its range. All of this would be consistent with the alternate hypothesis that small snails are the most likely to escape a crab's claws, snails living in harder-to-navigate areas would see the fewest predators, and previous interactions do not make snails any less fit to survive future encounters. This stands in opposition to the null hypothesis, which states that no such correlations between individual or environmental characteristics and the likelihood of surviving a predator encounter exist.

Methods

For the purposes of this study, the main organism of study was *Littoraria irrorata* (the marsh periwinkle). Approximately 300 *Littoraria* were collected by hand from the *Spartina* (cordgrass) marshes in the vicinity of the Duke University Marine Lab in Beaufort, North Carolina in November of 2021. Within the marshes, six distinct collections were made in areas along a creek with cordgrass of varying heights. The environmental characteristics of each site were recorded. These variables included snail, crab, and cordgrass population density based on a 0.5m^2 quadrat, as well as average cordgrass height based on the average of twelve randomly-selected stalks, percentage of the area visibly affected by large herbivore grazing, and distance from the creek's mouth in meters. The creek mouth was chosen as a representative point for the shoreline as a whole, in order to ensure uniformity in the collected data.

These subjects were then inspected for evidence of prior predator interaction, typically characterized by a scratch, partial fracture, or scar in the shell. As they were inspected, the number of marks was recorded, and the length of the shell from spiral tip to bottom of the aperture was measured in millimeters using a caliper. The subjects were also checked for fouling organisms such as limpets or barnacles; if any were found, they were counted and the number recorded. Finally, the shells were numbered using a permanent marker, returned to their collections, and sent to an expert in internal parasites, who was to inspect them for trematode colonization. This process would have involved breaking the shells, removing the soft tissues, and inspecting them under a dissection microscope. If any parasites were recognized, their presence was to be noted for the specific individual based on its number. The snail remains were then disposed of.

The numbers collected in this process were placed into a spreadsheet and subjected to individual and multiple linear regression tests using the program R.

Results

286 *Littoraria* were collected between the six locations. During this process, certain features of each plot were identified and compared. The collected data were recorded in Table 1.

Plot	**Number of Snails in 0.5m^2 Quadrat**	**Total Number of Snails Collected**	**Mud Crab Burrows in 0.5m^2 Quadrat**	**Average Cordgrass Height (cm)**	**Number of Cordgrass stems per 0.5m^2**	**Percent of Area that was Grazed**	**Distance from Creek Mouth (m)**
1	8	30	1	19.417	421	25%	0
2	105	51	0	29.667	110	0%	82
3	79	52	0	18.583	91	0%	152
4	74	49	0	49.417	115	0%	249
5	79	53	0	38.833	174	0%	381
6	55	51	0	15.417	216	40%	512

Table 1: Plot Analysis (Features of Plots 1-6)

This table, while not very useful for analysis, gives an overview of the variables being studied and how useful they may be. It is immediately apparent that Plot 1 was home to the fewest *Littoraria*. While Plot 1 also showed signs of having the most mud crabs, a statistic of one instead of none is not very useful for predictions. Additionally, while Plot 1 had the greatest density of cordgrass stalks by a wide margin, the average height was not exceptional in any way. With the exception of the average cordgrass height, Plots 2-5 had much more in common with one another than any of them had with Plot 1. The data on mud crab density and grazing area, however, were not sufficiently robust to use for inclusion in later statistical analysis, as each was only represented by one or two sites, both of them minimally.

The main focuses of the post-collection study involved the size and scars on the individuals, however.

Plot	**Count**	**Mean Size (mm)**	**Median Size (mm)**	**Mean Scar Count**
1	30	7.086	7.535	0.77
2	51	7.286	7.330	0.82
3	52	7.646	7.800	0.19
4	49	7.966	7.920	0.10
5	53	7.847	8.000	0.17
6	51	7.628	7.750	0.25

Table 2: Size, Scar data (Average size and scar count based on Plot)

This makes it clear that, although there are many scars present among each Plot population, there remain a number in each area that exhibit no scars whatsoever. This is consistent with the expected circumstances; not every snail has evaded a crab, only some of them have. Unscarred individuals are just those that have not yet been injured in an encounter. Unfortunately, this provides little insight into whether one scar or more than one scars is more common for scarred individuals. For more information on this, the data were reanalyzed, this time treating scarring as a binary variable: either scarred or not. In this case, the number of scars was immaterial, so long as it was not zero.

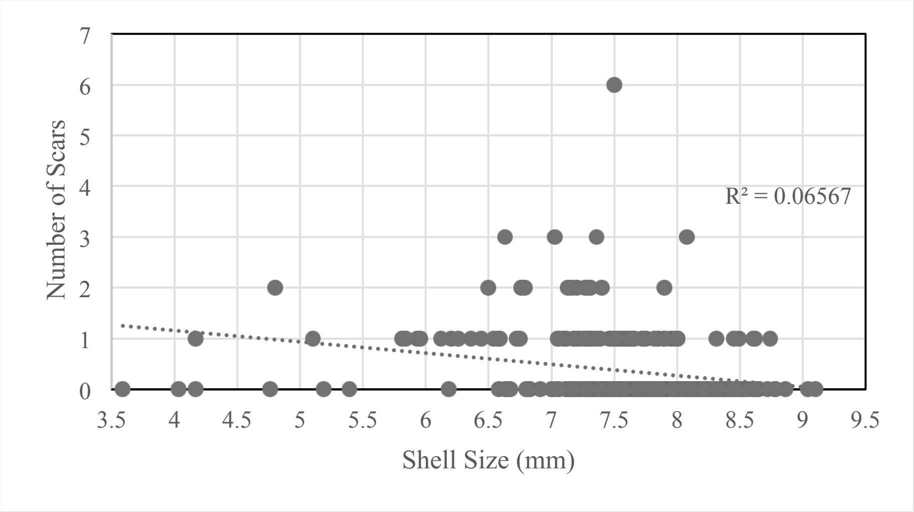
Plot	**Number of Snails Collected**	**Number of Scarred Snails**	**Percentage of Snails that were Scarred**	**Mean Scar Count**
1	30	11	36.67%	0.77
2	51	29	56.86%	0.82
3	52	9	17.31%	0.19
4	49	5	10.20%	0.10
5	53	9	16.98%	0.17
6	51	13	25.49%	0.25

Table 3: Binary Scar Statistics (Percentage of snails with scars of the total each Plot) Compared to Mean Scar Count

These data in Table 3 indicate that only a minority of individuals in Plots 3-6 had more than one scar, as the percentage of snails that were scarred was almost identical to the ratio of total scars to snails. However, Plots 1 and 2 had significantly more scars than scarred individuals, with a number of them exhibiting two or three. In fact, Plot 1 even had one individual with six scars.

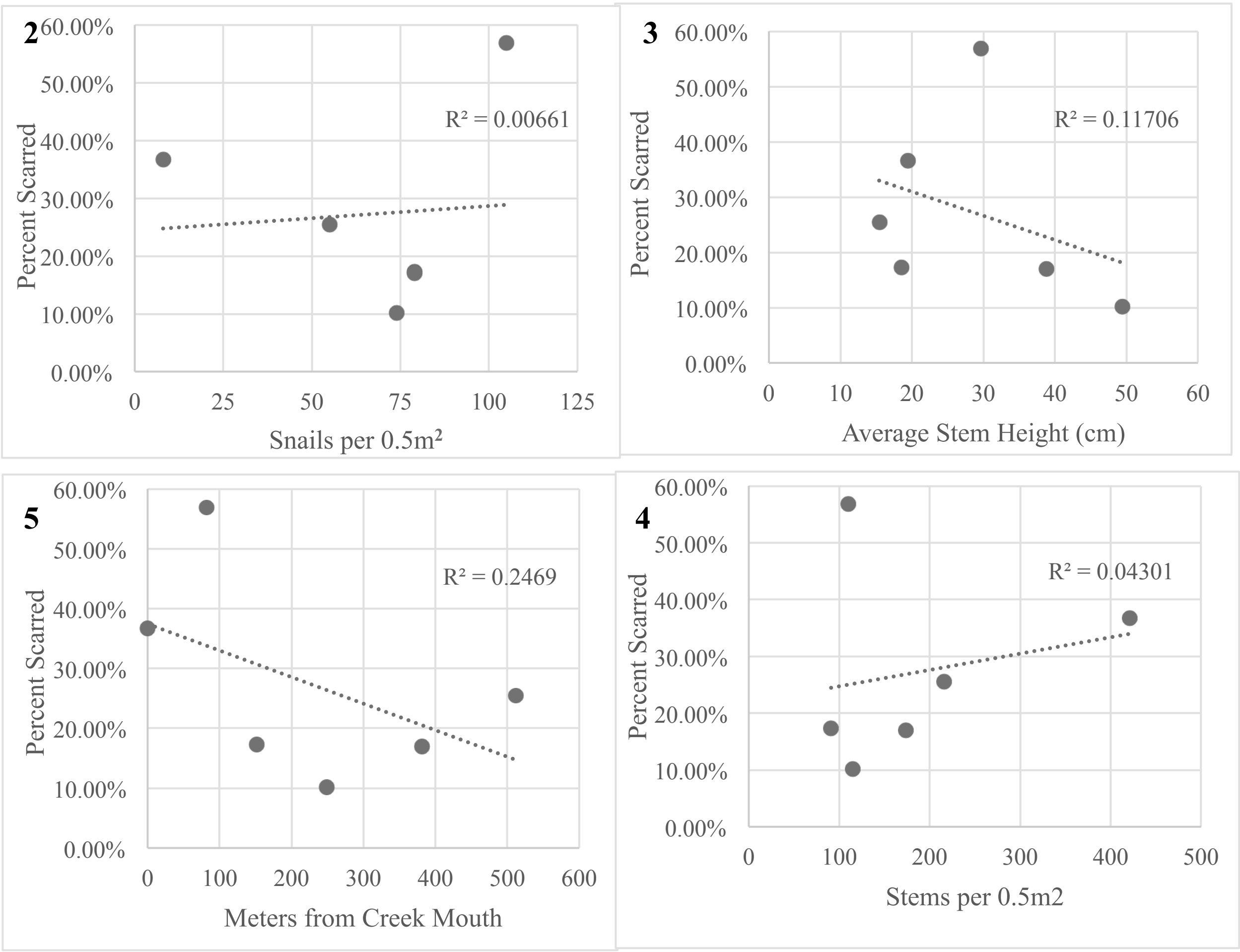
This all can be applied to the question of whether scarring makes an individual more or less likely to survive another predator encounter, but there are many other questions. In order to better understand the roles of individual- and habitat-specific factors in survivorship, the direct relationship between scarring and those other variables must be measured.

Figure 1: Number of Scars as a Function of Shell Size



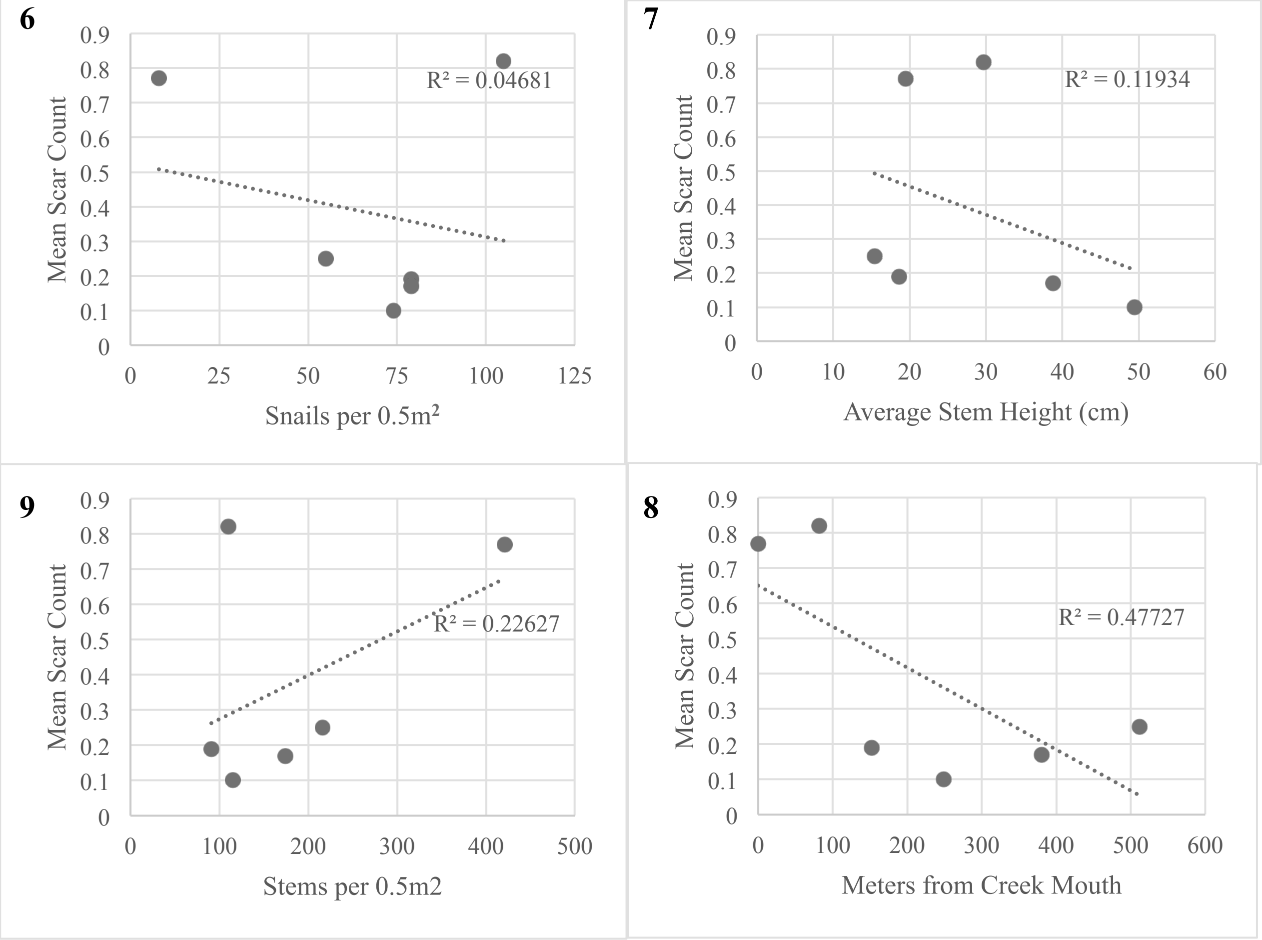
While the most scars can be found on larger snails, there is no consistent trend that can be used to predict the number of scars based on shell size. Although the number of individuals with scars increased with size, so did the number of unscarred individuals. However, these are individual-level statistics; investigation into larger-scale trends may provide greater insight. Using data from the sites at which each sample population was collected, some environmental connections to scarring may be identified.

Figures 2-5, Clockwise from Top Left: Percentage of Snails that were scarred as a function of Snails per $0.5m^2$ Quadrat, Average Cordgrass Stem Height (cm), Cordgrass Stems per $0.5m^2$ Quadrat, and Distance from Creek Mouth (m)



Figures 2-5 show that snail density (p-value: 0.8783), cordgrass density (p-value: 0.6934), cordgrass height (p-value: 0.5068), and distance from the creek mouth (p-value: 0.316) are all ineffective predictors of the percentage of collected snails that had scars. In none of these cases did the R^2 value approach significance, nor did the p-values approach the 0.05 that would have been required to indicate a correlation. The graphs for average number of scars based on plot showed similar results.

Figures 6-9, Clockwise from Top Left: Average Number of Scars per Snail as a function of Snails per 0.5m^2 Quadrat, Average Cordgrass Stem Height (cm), Distance from Creek Mouth (m), and Cordgrass Stems per 0.5m^2 Quadrat



Once again, snail density (p-value: 0.6805), cordgrass density (p-value: 0.3403), cordgrass height (p-value: 0.5024), and distance from the creek mouth (p-value: 0.1286) did not reach significance in either the R^2 statistic or the p-value. The only one of these variables that could be interpreted as anything approaching meaningful would be the correlation between distance from the creek mouth and average number of scars; while it is definitely not a linear relationship, snails collected within 100m of the creek mouth averaged significantly more scars than those further away. However, there were not enough site collection points to use this conclusion.

While the intention had been to include data on parasites, the samples disappeared en route to the parasitology expert. Because the shipping company listed the shipment as “Delivered,” this was attributed to package thieves. Without those samples, determining relationships between parasitism and predation was not possible.

All of these tests compared only a single variable at a time to scarring statistics. In order to determine whether all the variables together showed any correlation, a multiple linear regression model was used. When tested using this model, the four variables of snail density, cordgrass density, cordgrass height, and distance from the creek mouth did not meet the requirements to reject the null hypothesis, with a p-value of 0.5398 for the percent of snails that had any scars and a p-value of 0.4318 for the average number of scars. Similarly, no basis for the presence of a non-linear multi-variable relationship was identified. This indicates that neither alone nor in conjunction with one another do these variables show any correlation whatsoever with a snail’s likelihood of having one or more scars.

Discussion

Based on these data, no link can be established between trends in scarring on a marsh periwinkle shell, an indicator of predator encounters, and the size of the shell, the height or density of the cordgrass in which the periwinkle lives, the density of periwinkle living, or distance from the mouth of the creek along which they are found. This applies both to the total number of scars and the percentage of collected periwinkles that had any scars. By comparing these scarring statistics to the features of the individual subjects and the sites from which they were collected, no definitive connections could be drawn.

The direct comparison of shell scars to shell size, shown in Figure 1, had the greatest predictive power of these relationships, as it involved the individual characteristics of each periwinkle rather than depending on a more generic, site-based approach for the independent variable. As such, the lack of correlation between those two would have the greatest predictive power when applied to the population at large. There is, however, a major flaw with taking this conclusion as the gospel truth: because snails were hand-collected, the smallest individuals would have been passed over during the collection process. This leaves a significant gap in the sample population. It is possible that snails too small to have been noticed by the collectors would also be small enough to better evade crabs; at the same time, the marsh is home to crabs of all sizes, so it is unclear how these effects would balance. Without data on the smallest snails, however, this is all speculation. All that can be said with confidence is that, among this sample population, there appears to be no correlation between size and scar count.

The variables that describe cordgrass status, periwinkle density, and distance from the creek mouth do not share the same pitfall as the variable of size, but they do have their own. With only six areas of collection, the scope of the statistical analysis was limited, and the variance in some of the measured variables was not sufficiently large to analyze at all. The data collected during this process were useful for drawing broad conclusions about how little these variables affected the survivorship of periwinkles encountering crabs, but for more definitive results, research would need to be conducted involving many more collection sites.

This lack of correlation disagrees with most of the literature and on the subject, as well as the hypothesis of this paper. While it has been found that scarred snails are generally preyed upon less successfully than their unscarred counterparts (Greenfield, Lewis, and Hinke, 2002), suggesting that periwinkles with signs of surviving multiple encounters should be found, most scarred individuals had only one scar. Even among those that had multiple, it was unclear whether they had been the result of the same encounter or different ones. Additionally, there was no apparent connection between size and scarring, at least among the sizes measured in this process, as Schindler et al. found in 1994. The only possible connection that could be made was between the site's proximity to the creek mouth and predation; the plot closest to the creek mouth had the smallest collection, as the collectors could not find enough individuals to match the numbers of the other sites. However, this was also the only plot that included a mud crab burrow in the area, establishing the presence of a predator in the site. Additionally, scarring neither increased nor decreased as collection moved further from the creek mouth, in contrast with the findings of Dietl and Alexander from 2008. As such, more research would be necessary before this could be applied on a larger scale. A useful focus might be the density of mud crab burrows compared to the proximity to the creek mouth or tide line.

These results indicate that the research is far from settled on the predator-prey dynamics that involve marsh periwinkles. These differences from the literature suggest that there is some factor at play that has not yet been accounted for, whether that was caused by limited sampling or some feature unique to this population. These individuals were collected near Beaufort, North Carolina in November of 2021; different regions and time of year might demonstrate different trends in crab and snail behavior as the snails experience differences in weather, climate, predator ranges, and other key aspects of their unique ecosystems. The best way to address this discrepancy would be to first conduct a larger-scale version of this experiment, one that includes enough variation in collection sites to better account for grazing and crab density while also allowing sufficient diversity in the cordgrass, snail density, and creek mouth distance variables analyzed here. The next step would be to replicate the same procedure at different times of year and in different cordgrass marshes. In doing so, researchers may then be able to reconcile these findings with those already published. In doing so, researchers may come to better understand the unique features of different wetland environments, and develop actionable steps to preserve their diversity.

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