Intertidal zone and herbivore density affect grazing intensity on *Ascophyllum nodosum*

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The rocky coastline of Maine is commonly inhabited by the knotted wrack (*Ascophyllum nodosum*), which serves as a foundation species in the intertidal ecosystem. A frequently occurring periwinkle snail (*Littorina obtusata*) lives within and consumes *Ascophyllum*, forming grazing scars on the algae surface that may harm the structural integrity and infection defense ability of the plant. To examine the impact of grazing activity on *Ascophyllum* success and how this impact varies across increasing tidal height (a proxy for changing stress), we manipulated grazer density at two different tidal heights and measured resultant grazing intensity. Our results show that *L. obtusata* generate wounds on the algae surface and that the intensity of this grazing increases with increasing grazer density, suggesting that snails may elicit top down control on these seaweeds and that this effect likely varies as a function of intertidal elevation.

1. Introduction

Many ecological systems are characterized by the presence of a foundation species. These species create biotic and abiotic conditions that serve to support ecosystem function and productivity from the bottom up. In sections of the Northern Atlantic coast, the fucoid wrack *Ascophyllum nodosum* has been observed to occupy this role of foundation species through providing dense macroalgal canopies in the rocky intertidal zones, which positively influence the benthic community (Bertness 1999, Jenkins 1999, Olsen 2010). In the Gulf of Maine, *Ascophyllum* grows in thick beds on sheltered shores and provides ecosystem services as a primary producer and through amelioration of physical stressors, most notably in buffering temperature changes and water loss (Bertness 1999). In addition, macroalgae directly benefit associated organisms by facilitating invertebrate recruitment and growth and providing a refuge from predation (Bertness 1999). These effects have been shown to be most positive in the high intertidal zone, where physical stresses are increased (Bertness 1999). However, the relationship is not mutual, and grazers may negatively affect host foundation species in a top-down manner (Rosemond et. al. 1993, Sala et. al. 2008) that contributes to the holistic control of the ecological system (See Fath 2004).

Viejo and Aberg (2003) observed by field survey techniques that an assemblage of grazers on Northeast Atlantic shores, including littorinids *obtusata* and *littorea* and various isopods and amphipods, create grazing wounds through herbivory that vary as a function of grazer density. Mechanical wounding, such as that caused by herbivorous grazers, leads to increased fragility and loss of biomass, especially in wave-vulnerable canopies (Toth 2006, Viejo 2003), and increases susceptibility to epiphyte recruitment, which effects macroalgal productivity (Scrosati and Longtin 2010).

Littorina obtusata graze significantly on *Ascophyllum* fronds (Watson and Norton 1987), inhabiting the macroalgal canopy in the intertidal zone when tide is low. Thus, canopy in the high intertidal zone is exposed to snails for a greater portion of each day, along with the increased desiccation, nutrient, thermal, and osmotic stresses that increase with intertidal elevation (Davison 1996).

This study aimed to determine the density dependent effects of a specialized *Ascophyllum* grazer, the periwinkle snail *Littorina obtusata*, along the rocky shore stress gradient by manipulation of intertidal elevation and grazer density and subsequent observation of grazing intensity as a function of wounded area. We hypothesized that 1) grazing intensity follows a density-dependent pattern, increasing with increased grazer population and that 2) increased intertidal elevation corresponds with increased grazing intensity due to increased combined stressors (dessication, grazing, disturbance) in higher zones.

Since noted decreases in *Ascophyllum* canopy in parts of the North Atlantic have occurred in the last several decades (Davies 2007), this study is motivated to observe a potential driver of foundation species loss.

2. Materials and Methods

2.1 Site

The study was conducted on the rocky shore of Frenchman's Bay adjacent to the Mt. Desert Island Biological Laboratory in Salisbury Cove, Maine. The shore ranges from oyster beds at the lowest intertidal zones to large sheets of rock and small boulders in the higher zone, and is exposed to moderate wave action from the east. Tides commonly range more than 13ft. in this area and the shore has a gradient of approximately 10°. *A. nodosum* is found in high density attached to rock throughout the intertidal forming >15cm thick beds, but is less common at extreme low and high intertidal elevations. The periwinkle species *L. obtusata* and *littorea* are present in all intertidal zones. *Obtusata* inhabit the macroalgal beds and graze exclusively on fronds, whereas the more generalized grazer *L. littorea* roam the shore substrate and have a greater effect in grazing *Ascophyllum* recruits, though feeding trials have also shown frond grazing (Viejo and Aberg 2003). However, the specialization of the *obtusata* grazing implies a far more significant contribution to grazing wounds. The site was representative of the predominantly rocky shorelines of the area.

2.2 Initial Survey

In order to ascertain patterns of *L. obtusata* distribution and grazing in *Ascophyllum* canopies across the intertidal gradient, background data was collected from algal beds in August 2007. During low tide, eight 50cm^2 quadrats were randomly situated in each low and high intertidal zones to record *L. obtusata* density. Grazing intensity was also scored, as a function of total cm of grazing scars displayed on 5 arbitrarily selected fronds per plot. Grazing scars were distinguished as asymmetrical light patches against the dark green *Ascophyllum* tissue and were often concentrated on air bladders.

2.3 Field Experiment

From July to September 2009, we conducted a manipulative field experiment to examine the relative importance of *obtusata* density and intertidal elevation in driving natural patterns of snail grazing intensity on *Ascophyllum*. At the Salisbury Cove study site, 64 rocks hosting *Ascophyllum* individuals of similar size (height, girth) and natural grazing intensity were collected from the intermediate intertidal. After clearing each transplant of snails, we assigned each to one of four snail density treatments (0,20,40,80) and to one of two intertidal zones (high and low). This resulted in 8 treatments, each with 8 replicates, to which appropriate snail densities were added from locally collected snail populations.

After 28 days, we recorded snail density for 3-4 randomly selected replicates from each of the 8 sets. We also scored grazing intensity on these replicates by measuring grazing scars on 5-10 fronds and then calculating intensity, both as a grazed percentage of total frond length (cm scars/total cm frond) and as scarring extent for 4 fronds (cm scars/4 fronds). At this intermediate time step, all replicates were cleared and repopulated with respective snail densities to maintain the snail density treatments.

Final data were collected in September after 84 days, and entailed recording snail density and scoring grazing intensity for each remaining sample.

2.4 Sampling and Statistical Analysis

We analyzed the relationship between snail density and grazing intensity observed in our field survey using linear regression analysis relating snail density to grazing scars. Snail densities and grazing scars in low and high intertidal zones were compared using t-tests (assuming unequal variances, α=0.05)

Upon return to the field site for final data collection in September, we found that only n=17 out of an initial n=32 replicates remained in the high intertidal. This was not predicted by results of the intermediate time step and was a result of physical removal of *Ascophyllum* individuals from host rocks due to wave action. As a result, final data from the high intertidal zone was used judiciously and seldom to extract results due to lack of replicate power. T-tests (α=0.05) were used to test for differences between snail density and tidal height treatments. A 1-way Analysis of Variance (ANOVA) was used to determine the effect of initial (treatment) snail density on grazing scars at the low intertidal zone. To assess differences in means, we performed a Tukey's post-hoc comparison test.

3. Results

3.1 Survey Data

Snail density correlated very strongly with prevalence of grazing scars for low and high intertidal data points combined (Fig. 1). Linear regression for the low intertidal zone was more representative (R^2 = 0.705) than for the high zone (R^2 = 0.394) but both showed a positive slope signifying increased grazing scars with increasing snail density. Average snail density in high zone plots was 8 times greater than in low zone plots ($P<0.0001$) (Figure 1). This corresponded with about 6.5 times higher grazing scar presence in high zone vs. low zone plots ($P<0.0001$).

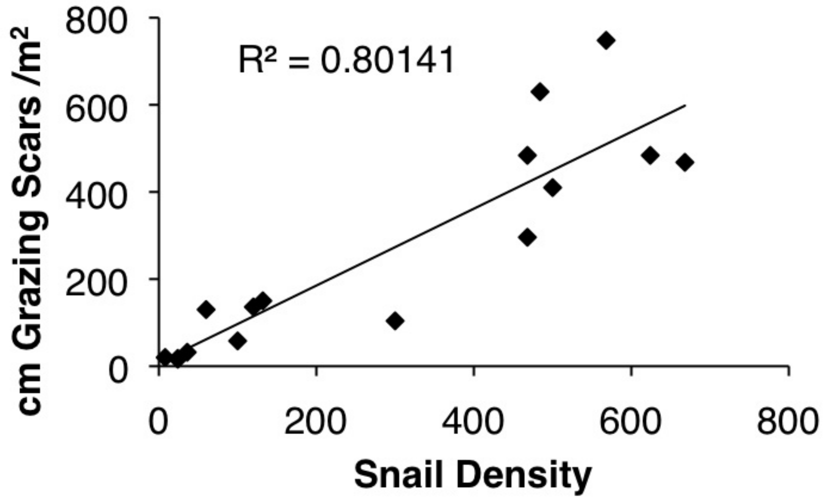

Figure 1: Grazing wounds increased with increasing snail densities in survey plots. Data points at high snail density represent, mostly, the high intertidal zone, where snail densities were significantly greater.

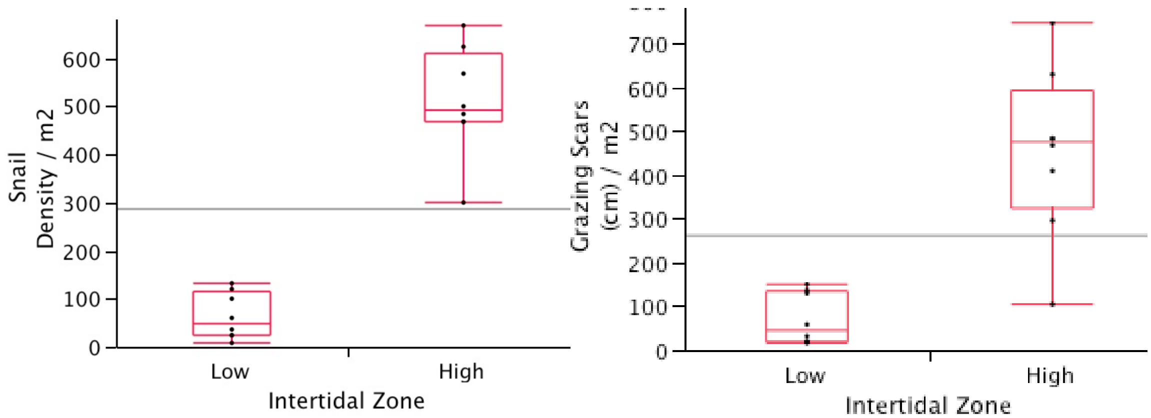

Figure 2: Survey data quantifying grazing wounds and snail density in low and high intertidal zone, determined by analysis of 50cm^2 quadrats.

3.2 Experimental Data

Results at 84 days supported survey observations that, overall, higher snail density correlates with increased grazing intensity. Data comparing intertidal zones was disrupted significantly by September wave action, which separated numerous replicates of *Ascophyllum* from host rocks at the high intertidal level, effectively decreasing final sample size (Table 1). This disturbance seemed to be focused in the early fall, as all 8 replicates for each snail density treatment were accounted for in early August but were decimated by late September. The low zone was largely intact and unaffected. Regardless of the low sample size, trends suggested increased grazing wounds with an increase in intertidal elevation (P=0.0457).

As a result of these complications, data exclusively from the low zone was used to compare snail density and resultant grazing intensity because of the higher statistical power of this replicate set. The data showed an increase in wounding of *Ascophyllum* fronds with an increase in snail density (ANOVA P=0.0062).

Table 1: Survival of replicates over time varied with intertidal zone. Though almost all replicates survived the summer, wave action in early fall stripped *Ascophyllum* holdfasts from host rock, reducing sample size for high intertidal zone and final biomass for surviving replicates. The low zone did not experience similar mortality. This implied higher disturbance in the high intertidal, where patterns of grazing intensity were also increased relative to the low zone.

Intertidal Zone	Snail Density	Remaining Replicates at 28 days (August)	Remaining Replicates at 84 days (September)	Final % Frond Wounded
Low	0	8	8	10.6 ± 1.6
Low	20	8	8	15.1 ± 1.6
Low	40	7	7	15.3 ± 1.2
Low	80	8	8	21.4 ± 2.9
High	0	8	2	8 ± 0.12
High	20	8	5	18.5 ± 1.9
High	40	8	4	27.5 ± 4.2
High	80	8	6	24.3 ± 3.3

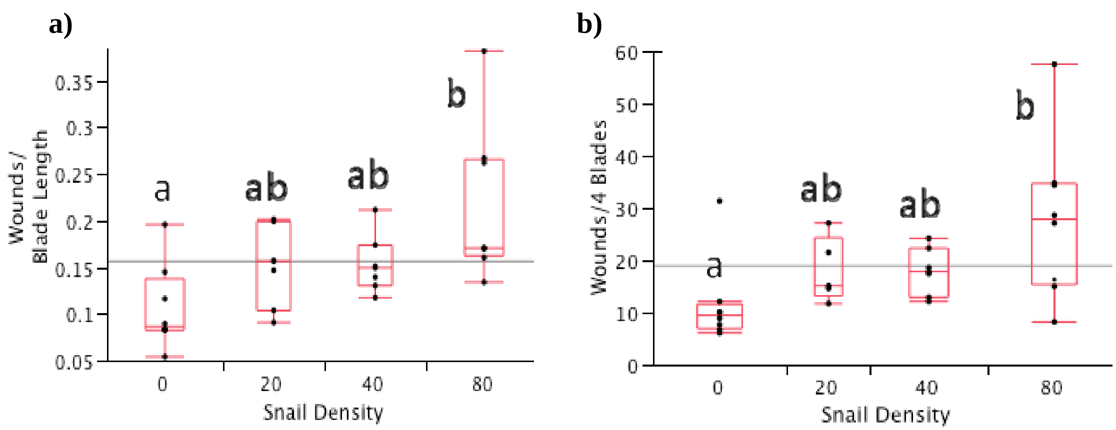

Figure 3: Grazing intensity increased with increasing snail density. Intensity was calculated as **a)** cm of wounds per total cm frond length and **b)** cm of wounds per 4 fronds. Both results showed generally increasing wounds from 0- to 80-snail densities and a significant difference between these high and low treatments.

4. Discussion

Our results show that intensity of grazing on *Ascophyllum* increases with increasing snail density and intertidal elevation. As the density of a specialized grazer increases from lower to higher intertidal elevations (as observed), grazing extent and effect are amplified in moderately sheltered rocky shores of New England. Macroalgae in the high intertidal have more physical wounds due to grazing as well as increased thermal, osmotic, and wave-related stresses that increase probability of strand breakage and susceptibility to fungal invasion. This implies a negative, top down effect of the grazer *obtusata* on macroalgal canopy that may serve, in conjunction with stressors like dessication and disturbance, to delineate the upper range of *Ascophyllum* on the shore.

The major unintended result of the manipulative experiment was a significant mortality of high intertidal zone replicates. When I returned to collect data in early fall, I observed intense wave action and higher winds compared to the summer months. A significant proportion of the high intertidal macroalgae had been stripped from their holdfasts, leaving bare rock. Though this had interesting implications for high intertidal disturbance, it greatly decreased the statistical power of the high intertidal zone data set. Thus, while the remaining high zone data followed similar trends, we used only data from the low intertidal zone for grazing intensity graphical analysis (Fig. 3).

Our study showed that grazing scars are dependent on snail density. Observed grazer density (and extrapolated grazing intensity) differed across the intertidal gradient.

This trend is likely due to an interplay between the physical stress gradients and biotic interactions characteristic of rocky intertidal habitats. The harsh physical stresses that create the upper limit for *Ascophyllum* also limit the elevation of grazer habitat, while the prolonged exposure of the macroalgae during low tides provides both a safe haven and reliable food source for the snails. Along with a likely preference for the higher intertidal zone due to food availability, biotic stresses in the low intertidal zone likely push *obtusata* up higher in the intertidal. Although *obtusata* densities have been linked more highly to bottom-up macroalgal food availability (Ingolfson 2009), predation by green crabs on the incoming tide may influence the grazer's density at the low intertidal zone.

The relationship between *obtusata* density and intertidal zone led us to the next question: does grazing intensity on *Ascophyllum* differ between low and high intertidal zones? Our results indicate a slightly higher average grazing intensity in the high intertidal (Table 1). However, due to the aforementioned mortality of high intertidal zone samples, this data could not be reliably obtained. Regardless, this mortality does have interesting implications about the potential interactions of high grazing intensity and disturbance. Increased physical wounds at higher intertidal elevation (Fig. 2) may decrease macroalgal resilience to increased wave action, creating a combined negative effect of grazers and disturbance on macroalgal success. These questions regarding interactive effects of wave action and grazing on *Ascophyllum* mortality require further study.

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