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Ecological determinants of rockweed performance: interactions among herbivores,
epiphytes and tides

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Abstract

Ecological determinants of rockweed performance: interactions among herbivores, epiphytes and tides

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Ecology seeks to understand how biotic and abiotic factors influence species distribution and abundance in order to make predictions about the outcome of interactions between species and their environment. The rocky intertidal zone is characterized by strong environmental gradients over small spatial scales, making it an ideal habitat to study how changes in abiotic and biotic factors influence the distribution of species. The rockweed *Fucus distichus* is an important benthic primary producer and habitat forming species that experiences gradients of tidal immersion, herbivory, and epiphytes across its vertical range in the intertidal zone. Since these factors act together to influence *F. distichus* performance, studying their effects singly may not adequately characterize the outcomes of their combined interactions. This research examines three interaction scenarios through mesocosm and field experiments: 1) effect of herbivores (*Littorina sitkana*) on *F.*

distichus performance, mediated indirectly through inducible defenses, 2) multiple stressor effects of herbivores, epiphytes, and tidal immersion on *F. distichus* performance, and 3) direct and indirect interactions between herbivory and *F. distichus*, mediated by epiphytes. In contrast to some other furoid algae, *F. distichus* did not produce inducible defenses in response to waterborne cues from snails feeding. However, *F. distichus* grown with snail feeding cues had lower carbon to nitrogen ratios and higher subsequent consumption by snails. Thus, uptake of snail metabolic wastes by *F. distichus* may influence subsequent feeding preferences. Multiple stressor results demonstrate that herbivores and epiphytes negatively affect *F. distichus* performance, and each is additive with tidal effects. *F. distichus* grew slower when always immersed, even when protected from herbivores or epiphytes, pointing to immersion as a determinant of the lower limit of *F. distichus*. Finally, when snail herbivores fed on both epiphytes and *F. distichus*, the net effect of the snails on *F. distichus* switched from a positive indirect interaction to a negative direct interaction with increased snail density, mediated by epiphyte load. The interactive effects of immersion, herbivory, and epiphytes on *F. distichus* are complex but clarified by this research involving careful experimental manipulation of environmental factors that vary across the intertidal zone.

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DEDICATION

To the academics in my family

Mom, Dad, and Grandpère

CHAPTER 1

Responses of the rockweed, *Fucus distichus*, to waterborne cues from a littorine snail increase its susceptibility to herbivory but not defensive chemistry or morphology

Abstract

In the variable environment of the rocky intertidal zone many seaweeds exhibit phenotypic plasticity in response to environmental cues including temperature, light, water motion, and herbivore damage. However, the effect of waterborne cues from herbivores on seaweed chemistry and morphology is not well understood. The intertidal rockweed *Fucus distichus* has a range of morphologies in the field and responds chemically and morphologically to mechanical damage from herbivores. To determine if waterborne cues from herbivores induce morphological or chemical responses in *F. distichus* we grew juvenile *F. distichus* in outdoor mesocosms for 70 days from August to October in the presence of waterborne cues from: 1) the snail *Littorina sitkana* feeding on adult *F. distichus* (snail cue), or 2) adult *F. distichus* (control). We compared these treatments in terms of morphology, growth, tissue carbon and nitrogen, phlorotannin levels, and palatability to *L. sitkana*. The treatments showed no difference in morphology, growth, or phlorotannin levels, but the ratio of carbon to nitrogen was significantly reduced in the presence of waterborne cues from *L. sitkana*. Tissue from the experimental treatment was significantly preferred by *L. sitkana* in a choice feeding trial, and consumption was positively related to tissue nitrogen levels. Waterborne cues from *L. sitkana* do not induce a defense response in juvenile *F. distichus*, perhaps due to an inability to detect or respond to cues. However, *L. sitkana* can detect

small differences in *F. distichus* tissue nitrogen levels, suggesting a feedback loop in which utilization of snail metabolic wastes by *F. distichus* influences subsequent feeding preferences.

Introduction

Phenotypic plasticity is an important strategy by which seaweeds deal with the spatial and temporal variation that exists in marine environments (Blanchette et al. 2002). The morphology and chemistry of seaweed phenotypes develop in response to abiotic factors including temperature (Hay 1981), light (Haring and Carpenter 2007, Monro and Poore 2009), water motion (Ferrier and Carpenter 2009) and nutrients (Lobban and Harrison 1994, Edwards 2006). The effect of biotic factors on plasticity in morphology and chemistry of seaweeds has primarily been investigated in terms of herbivory. Variation in thallus morphology can influence herbivore feeding preferences and the vulnerability of seaweeds to herbivore attack (Steneck and Watling 1982, Norton and Hawkins 1990, Hay and Kappel 1994, Thornber et al. 2008). The production of chemicals that deter herbivory appears to be widespread among seaweeds in response to direct grazing (Norton and Hawkins 1990, Van Alstyne 1990, Amsler and Fairhead 2006, Molis et al. 2006, Toth and Pavia 2007, Rohde and Wahl 2008). There is also evidence that waterborne cues from herbivores feeding on seaweed can induce defense responses in neighboring individuals (Toth and Pavia 2000, Yun et al. 2012) but the ubiquity of this response, and the type of defense that is induced, is not well understood.

Theoretically, inducible defenses evolve when herbivory is spatially and temporally variable, there is a reliable cue for risk, and defenses are costly to produce (Karban 2011). Herbivory in marine habitats is spatially and temporally variable (Hay 1981, Lobban and Harrison 1994) and

inducible defenses seem to be present in many seaweed species (Toth and Pavia 2007). Understanding whether these defenses are induced by direct versus indirect cues has been a focus of seaweed inducible defense research in the last decade (Toth and Pavia 2000, Sotka et al. 2002, Toth and Pavia, 2007, Rhode and Wahl 2008). These studies of waterborne cues are motivated by research in terrestrial ecosystems that have shown that volatile compounds, released in response to herbivory, induce chemical defenses in neighboring plants (Karban et al. 2006, Karban 2011). Marine examples of waterborne cues inducing defense include phytoplankton that produce defensive chemistry in the presence of waterborne cues from herbivorous zooplankton (Selander et al. 2006). Additionally, some populations of the furoid alga *Ascophyllum nodosum* produce phlorotannins and show increased resistance to herbivory in response to waterborne cues from nearby feeding snails (Toth and Pavia 2000, Long and Trussell 2007). There is evidence that *Fucus spiralis* and *Fucus vesiculosus* can induce defensive chemistry in response to waterborne cues from herbivores feeding on neighboring furoid algae that are not conspecific (Yun et al. 2012).

The rockweed *Fucus distichus* exhibits highly variable morphology in the field, both among and within sites (personal observation, Fig. 1). Some of this variation is likely driven by abiotic factors such as light and wave exposure (Williams and Dethier 2005), however, the high variation in morphology observed over very small spatial scales may not be fully accounted for by these abiotic factors alone. Herbivory is known to affect both the morphology and chemistry of *F. distichus* (Van Alstyne 1988, Van Alstyne 1989, Van Alstyne 1990, Dethier et al. 2005, Dethier and Williams 2009). *F. distichus* produces phlorotannins in response to mechanical damage by herbivores, which may be an induced defense against herbivory (Van Alstyne 1988,

Van Alstyne 1990). Morphological responses to herbivory include growth (Dethier et al. 2005) and adventitious branching (Van Alstyne 1989). We hypothesized that waterborne cues from herbivores would induce morphological and chemical defenses in *F. distichus*, and that this could explain some of the morphological variation present in the field.

The goal of our study was to determine whether waterborne cues from herbivores affect the morphology, growth, tissue chemistry, and susceptibility to herbivores of *F. distichus*.

Specifically, our research addressed three questions. First, do waterborne cues from herbivores affect the growth or morphology of *F. distichus*? Second, do waterborne cues from herbivores induce phlorotannin production in *F. distichus*? Third, since we hypothesized that the effects of waterborne herbivore cues on *F. distichus* chemistry and morphology could be related to defense, we tested whether exposure to waterborne cues from herbivores reduces the palatability of *F. distichus* during subsequent herbivore attacks.

Materials and Methods

Study sites and Organisms

Experiments were conducted at the Friday Harbor Laboratories on San Juan Island, Washington, USA. The rockweed, *Fucus distichus*, is a habitat forming seaweed in the upper-middle zone of the rocky intertidal and is distributed along the northeast Pacific coast from central California to Alaska. (Dethier et al. 2005, Wahl et al. 2011). Mesograzers that feed on *F. distichus* include snails (*Littorina sitkana*, *Littorina scutulata*, and *Lacuna vincta*), limpets, and the isopod *Idotea wosnesenskii* (Dethier and Williams 2009). We used *L. sitkana* for our study because it is patchily distributed in the intertidal (Dethier and Williams 2009) and is a potential source of

waterborne chemical cues even in the absence of direct feeding on *F. distichus*. In addition, *L. sitkana* has been used in previous studies of phlorotannin production in *F. distichus* in response to feeding by herbivores (Van Alstyne 1990).

Waterborne Cue Experiment

This experiment consisted of two treatments: 1) waterborne cues from the herbivorous snail *L. sitkana* feeding on adult *F. distichus* (snail cue), or 2) waterborne cues from adult *F. distichus* alone (control). Sixteen juvenile *F. distichus* 1-2 cm in length with no visible herbivore damage were collected from the field and grown for 70 days in mesocosm tanks and exposed to one of the two treatments (N = 8 per treatment).

Juvenile *F. distichus* were collected on 12 Aug 2006 from the middle of the vertical *Fucus* zone at Cattle Point on San Juan Island (N48°27.0', W122°57.8'). To avoid disturbing the holdfast, pieces of rock were chiseled from the substratum with *F. distichus* attached. Following collection, thalli were brushed lightly with a toothbrush to remove herbivores or epiphytes and then held in a flow-through seawater tank until the start of the experiment. On 14 Aug each juvenile *F. distichus* was assigned a unique identification number and photographed flat against a white background. Juvenile plants were attached in an upright position by super gluing each rock to the center of a ceramic tile. On 15 Aug tiles with juvenile *F. distichus* were placed in 18.5 L outdoor mesocosms with one tile per tank. At this time eight additional juvenile *F. distichus* collected from the same site on the same day using identical criteria were freeze dried and stored at -80°C for future measurement of baseline phlorotannin levels in the field.

Flow-through seawater was delivered to each tank individually through cue boxes containing either 25 *L. sitkana* feeding on adult *F. distichus* (snail cue), or adult *F. distichus* alone (control) (Fig. 2). Cue boxes were plastic containers with mesh openings at the bottom. This design allowed water to circulate within the cue box and then exit carrying any chemicals from the cue box into the tank, but herbivores were prevented from feeding directly on the juvenile *F. distichus*. The rate of water flow into the cue box was 42 ml/s and dye tests showed that water in each mesocosm tank was replaced in 15 minutes. Juvenile *F. distichus* were brushed lightly with a toothbrush twice a week to reduce diatom epiphytes. Tanks were cleaned and cue boxes were rinsed every week. During the weekly tank cleaning, tiles with juvenile *F. distichus* were removed from the tanks and dried in the shade for one hour to simulate drying that occurs during low tide in the intertidal zone. Each week the juvenile *F. distichus* were rotated among the tanks for each treatment to control for any unknown within treatment variation in tank conditions. Adult *F. distichus* used in cue boxes for both treatments were collected every two weeks from the field and brushed clean before being placed in the cue boxes. The snail treatment cue boxes contained 25 *L. sitkana* snails (5 large at 9-12 mm, 20 medium at 5-9 mm) that fed on the adult *F. distichus* in the cue boxes. Snails remained in snail treatment cue boxes for the duration of the experiment.

After 70 days, each juvenile *F. distichus* was re-photographed, weighed, and scored for reproductive status (receptacles present or absent). Tissue from non-reproductive tips of each individual was collected for analyses of carbon and nitrogen (CHN) and phlorotannins, except for two individuals that were excluded from CHN analysis due to tissue decay. Tissue for CHN analysis was frozen at -80°C after collection and processed (Marine Chemistry Lab, University

of Washington School of Oceanography). Tissue for phlorotannin analysis was flash frozen in liquid nitrogen, placed at -80°C and freeze-dried. Freeze dried samples were stored at -80°C until processed (Van Alstyne 1988, Shannon Point Marine Center, WA, USA). Data obtained from the chemical analyses were tested for normality and homogeneity of variance, and a one-way analysis of variance was used to compare mean levels of tissue chemicals (C, N, C:N, phlorotannins) between treatments (R, R Core Development Team 2012).

Morphological responses of *F. distichus* to waterborne cues from *L. sitkana* were quantified from the initial and final photographs of each individual using ImageJ v.1.37 (National Institutes of Health, USA). The following morphological response variables were measured: growth in surface area, final length from holdfast to tip of longest part of thallus, average blade width between second and third dichotomy, number of dichotomies, and blade length between second and third dichotomy. Morphological response variables were compared between treatments with a Multivariate Analysis of Variance (R, R Development Core Team 2012).

Feeding Experiment

In addition to carrying out chemical analyses of *F. distichus* tissue at the close of the cue experiment, tissue approximately 2 cm in length was collected from the thallus tips of individuals in each treatment and used these in a choice feeding trial. Thallus tips from control and snail cue treatments were weighed, threaded with a colored string to identify source treatment, and then one tip from each treatment was placed in each feeding trial container. Feeding trial containers were plastic petri dishes drilled on the bottom and top to allow water exchange between the dish and the tank. Each replicate contained either ten *L. sitkana* snails

(feeding treatment, N = 10) or no snails (control treatment, N = 4). To stimulate feeding, containers were placed in a flowing seawater tank on a shaker table and exposed to a three-hour low tide every three hours. After 36 hours snails were removed and all tissue was weighed. Amount of tissue consumed was calculated using the standard formula for choice feeding trials (Peterson and Renaud 1989, Cronin and Hay 1996,). The mean amount of tissue consumed from the snail cue control and snail treatments was compared with a one-way ANOVA (R, R Development Core Team 2012). Results from one feeding container were excluded because the snails failed to eat. A multiple regression analysis (R, R Development Core Team 2012) was done to determine whether differences in feeding were related to differences in tissue phlorotannin or nitrogen levels.

Results

Waterborne Cue Experiment

Over the 70-day experiment, juvenile *F. distichus* grew 2.5-fold in length and 7-fold in biomass. There were no significant differences between treatments for any response variable of growth or morphology (Table 1). Phlorotannin production is correlated with chemical defenses against herbivory in *F. distichus*, however we found no significant difference in phlorotannin production between control and snail cue treatments. Additionally, the concentrations measured were not different from phlorotannin concentrations in the field (Table 2). Results for growth, morphology and chemistry suggest that *F. distichus* either cannot detect or does not respond to waterborne cues from *L. sitkana*. Carbon to nitrogen ratios differed between treatments and were lower in the snail cue treatment than in the controls (P = 0.05, Table 2). This reflects a trend of higher %N and lower %C in snail cue treatments relative to controls (Table 2, Fig. 3) and suggests that *F.*

distichus exposed to waterborne cues in the snail cue treatment may have incorporated nitrogen from the metabolic wastes produced by the snails.

Feeding Experiment

If morphological or chemical defense responses are induced by waterborne snail cues, snails should consume more of the control *F. distichus*. However, since *F. distichus* did not exhibit a defense response to the experimental treatments, we expected to see no difference in feeding between tissues from each treatment. Instead we found that *L. sitkana* consumed about twice as much tissue when *F. distichus* had been exposed to waterborne cues from snails (Fig. 4, $P = 0.02$). Based on multiple regression, amount consumed by snails was positively related to %N (Fig. 5b, $P = 0.028$) and not related to phlorotannin concentrations in the tissue of *F. distichus* (Fig. 5a, $P = 0.21$). The significant positive relationship between tissue nitrogen and consumption suggests that *L. sitkana* fed preferentially on *F. distichus* tissue with higher nitrogen content.

Discussion

Morphological and defensive chemistry results

Waterborne cues from *L. sitkana* had no effect on the growth or morphology of *F. distichus* and did not induce a phlorotannin defense response (Table 1). The lack of a defense response was further supported by the feeding trial results, where snails strongly preferred plants that had been exposed to effluent from feeding snails (Fig. 4). These results suggest that *F. distichus* either cannot detect or does not respond defensively to waterborne cues from *L. sitkana* feeding on conspecifics.

Tests of induced defenses to herbivory in seaweeds have rapidly expanded in recent years (Toth and Pavia 2007). Across many studies, seaweeds frequently become less palatable following direct damage from herbivores. Variation in results may stem from different induction cues, time scales, and bioassays as well as intra- or interspecific variation in the seaweeds themselves (Toth and Pavia 2007). Previous research in which *F. distichus* has responded to herbivory with increased phlorotannins was approximately 14 days in length (Van Alstyne 1988). In order to test the effect of waterborne cues from feeding snails on morphology as well as chemistry, our study was conducted over a relatively long time scale and we cannot rule out the possibility that phlorotannins may have been induced earlier in our experiment before measurements were taken (Dethier et al. 2005). The induction of defenses in brown algae, especially when the cue is waterborne, is species specific and idiosyncratic (Amsler and Fairhead 2006, Long and Trussell 2007, Toth and Pavia 2007, Wahl and Rohde 2008). Species may only induce defenses in response to specific herbivores and not all herbivores respond to those induced defenses (Rhode et al. 2004, Amsler and Fairhead 2006, Molis et al. 2006, Long et al. 2007, Molis et al. 2008). Phlorotannins have been correlated with deterring feeding (Van Alstyne 1988, Toth and Pavia, 2000) but this result is not consistent among studies and phlorotannins may not in fact be the chemical that is deterring herbivory in brown algae (Kubaneck et al. 2004, Long et al. 2007). Our study design cannot rule out the possibility that there was a non-phlorotannin chemical defense response. If such an unknown defense was induced, it was overwhelmed by the response of *L. sitkana* to nutritional differences between the tissues in our feeding experiment (Van Alstyne et al. 2009).

In our experiment, nitrogen in the snail cue treatment was apparently allocated to tissue concentration, rather than to growth. That *F. distichus* did not increase growth in response to nitrogenous wastes from the snails is consistent with previous nutrient addition studies on this species (Britton-Simmons, unpublished data) and other taxa within the genus *Fucus* (Hemmi et al. 2005). Our region is relatively nutrient rich and seaweeds are generally not nutrient limited, which may explain why there was no difference in growth between the snail cue and control treatment in *F. distichus* (Wootton 1991, Pfister and Van Alstyne 2003).

Delivery of the waterborne cue

Of concern in any waterborne cue induction study with negative results is whether the potential cue substances were effectively delivered to the target species. We are confident that our experimental design did deliver snail and snail feeding effluent to *Fucus distichus* for two reasons. First, prior to initiating the experiment we carefully examined the water flow patterns in our mesocosms using dye to ensure that effluent from the feeding chamber flowed directly over the target *F. distichus*. Second, plants exposed to snail effluent had higher carbon to nitrogen ratio, driven by higher levels of tissue nitrogen (Fig. 3), indicating that there was delivery of waterborne nitrogen from the wastes produced by feeding *L. sitkana*. Nevertheless, though the distance between the cue box and the target *F. distichus* was short, we cannot exclude the possibility that some potential cues became diluted and ineffective in our mesocosm setup.

Tissue Nitrogen increase and positive effect on feeding

Although the focus of feeding theory and experimentation has been on the role that defensive chemicals play in mediating herbivory, nutrients may be equally important in regulating feeding

choices. *F. distichus* that had been exposed to waterborne cues from feeding *L. sitkana* had increased tissue nitrogen levels compared to the control thalli (Fig. 3), suggesting that *F. distichus* absorbed nitrogen from the snail's nitrogenous wastes. This result is consistent with work showing that fucoid algae can uptake and store excess nitrogen (Rosenberg and Ramus 1984, Svennsson et al. 2007). In the subsequent feeding trial, *L. sitkana* preferred to feed on *F. distichus* exposed to snail wastes and feeding preference was positively correlated with tissue nitrogen levels. These results indicate that *L. sitkana* is capable of assessing relative N levels of seaweed tissues and selecting tissue with higher N.

Preferential feeding on nitrogen-rich thalli makes sense given that herbivores tend to be nitrogen limited. Previous studies have demonstrated that invertebrate (Cruz-Rivera and Hay 2000, Van Alstyne 2009) and vertebrate (Boyer et al. 2004, Goecker et al. 2005) marine herbivores preferentially feed on nutrient rich foods. The unique aspect of our result is that the small difference in nitrogen translated to such a strong a feeding preference. Consistent with our results, Van Alstyne et al. (2009) demonstrated that *L. sitkana* preferentially feed on seaweeds of higher N content even when chemical defenses are present. Overall, although *L. sitkana* can induce phlorotannins in *F. distichus* through direct feeding, and phlorotannins are correlated with the reduction of subsequent herbivory (Van Alstyne 1990), our study indicates that *L. sitkana* feeding on neighboring *F. distichus* can enhance nutrient content of neighbors and increase susceptibility to herbivory (Fig. 5). These two processes are likely occurring simultaneously in the field, and, to the extent that snails select individual algal thalli to feed on based on information about both defensive chemistry and nutrients, a spatially and temporally complex landscape of food choices exists for *L. sitkana*.

The interplay between nutrient availability, chemical defenses and feeding preference is complex (Van Alstyne et al. 2009). It is likely that the net effect of nutrient enrichment on feeding depends on the extent to which the nutrients are stored by the alga, converted to growth or used to produce chemical defenses. When nutrients are stored rather than converted or utilized, the result may be preferential feeding on nutrient enriched tissues.

Feedback Loop

The capacity for *Fucus* to uptake herbivore derived nutrients combined with the potential for those nutrients to influence subsequent feeding preferences has implications for algal-herbivore interactions in the field. Our data suggest the potential for a feedback loop, in which nitrogen is passed back and forth between herbivores and their algal prey and plays an important role in regulating their interactions. Although this hypothesis remains to be tested, it is consistent with recent field studies suggesting that nutrient dynamics in benthic marine ecosystems may be more localized than previously thought (Plagányi and Branch 2000, Bracken 2004, Pfister 2007, Pfister et al. 2007).

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Tables

Table 1. Morphological measurements of *F. distichus* thalli at the beginning and end of the experiment and MANOVA results.

	Initial (n=13)	Final control (n=7)	Final snail (n=6)	F ratio	P value
Surface area (cm ²)	2.50 ± 0.34	18.64 ± 2.6	17.39 ± 1.9	0.27	0.61
Wet mass (g)	nd	1.16 ± 0.14	1.09 ± 0.10	0.15	0.70
Thallus Length (cm)	1.82 ± 0.09	4.73 ± 0.37	4.60 ± 0.16	0.53	0.48
Mean thallus width (cm)	nd	1.04 ± 0.075	1.08 ± 0.12	0.11	0.75
Branching levels	1.85 ± 0.10	4.42 ± 0.30	4.00 ± 0.26	0.89	0.37
Dichotomy number	2.54 ± 0.22	12.43 ± 1.53	12.50 ± 1.73	0.00	0.95
Length between 2nd and 3rd dichotomy (cm)	nd	1.50 ± 0.14	1.46 ± 0.07	0.07	0.80

MANOVA Pillai test: Pillai value = 0.2313, P = 0.9657

Table 2. *F. distichus* tissue chemistry and ANOVA results.

	Field baseline (n=8)	Final control (n=7)	Final snail (n=7)	F ratio	P value
Phlorotannin concentration (mg g ⁻¹ dry mass)	1.30 ± 0.11	1.21 ± 0.09	1.10 ± 0.07	1.39	0.27
N (% dry mass)	-	2.04 ± 0.06	1.93 ± 0.04	3.36	0.09
C (% dry mass)	-	35.6 ± 0.4	35.7 ± 0.3	0.11	0.75
C:N ratio	-	17.5 ± 0.4	18.6 ± 0.3	4.65	0.05

Figures



Figure 1. *F. distichus* from the same site and tidal height showing large variation in morphology.

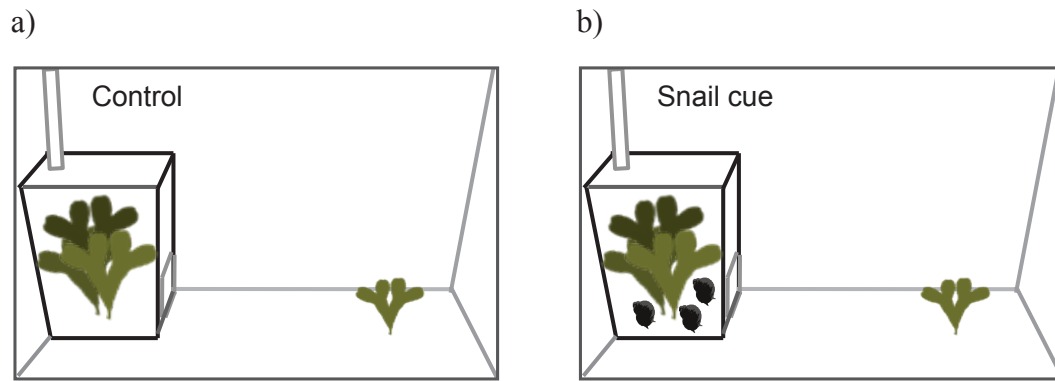


Figure 2. Diagram of waterborne cue tanks showing the two treatments with cue boxes containing a) *F. distichus* alone (control) and b) *L. sitkana* feeding on *F. distichus* (snail cue). Measurements, chemical analyses, and feeding trial were done using the small *F. distichus* located outside of the cue box in each tank.

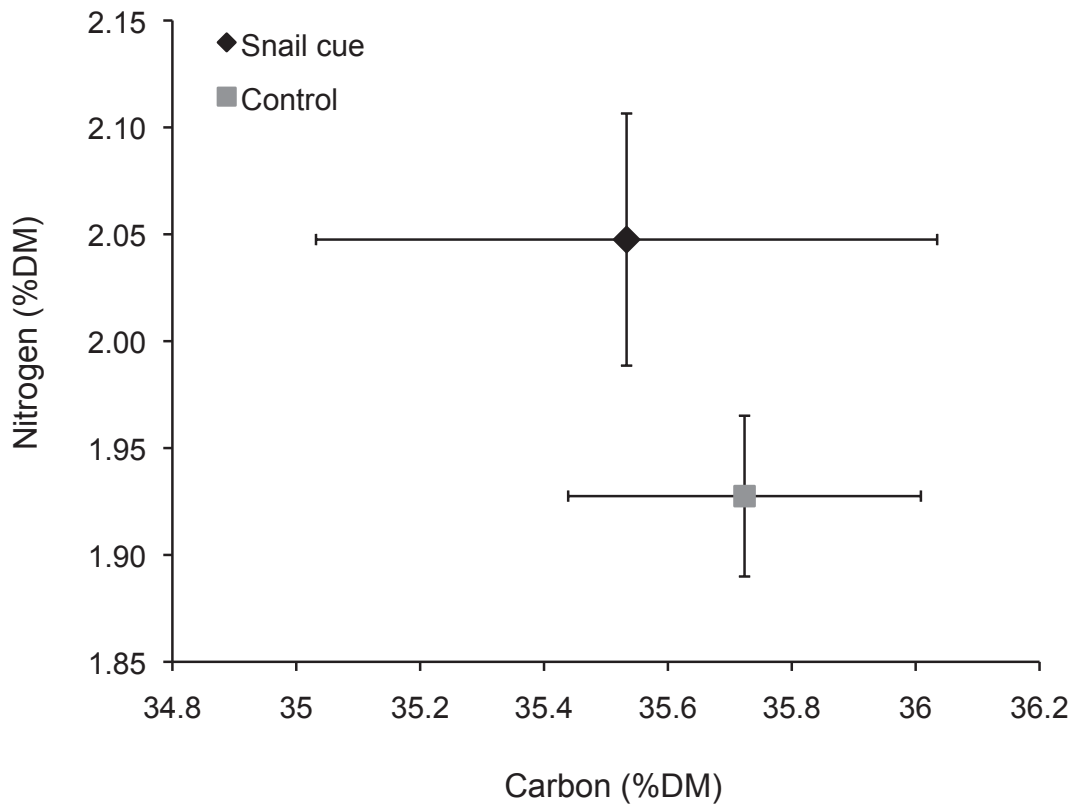


Figure 3. Carbon to nitrogen ratios differed between treatments and were lower in the snail cue treatment than in the controls ($P = 0.05$). Although not statistically significant, there was higher mean tissue nitrogen in *F. distichus* from the snail treatment ($P = 0.09$). There was no difference in mean tissue carbon between the control and snail treatments ($P = 0.75$). Data are means and error bars are SE.

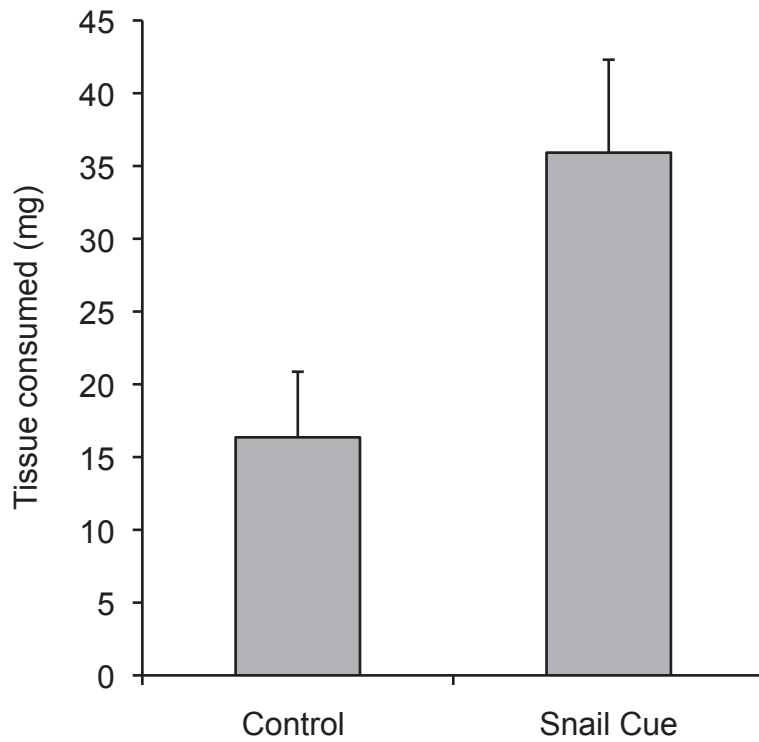


Figure 4. *L. sitkana* consumed more of the *F. distichus* tissue that had been grown in waterborne cues from snails ($P = 0.02$). Data area means and error bars are SE.

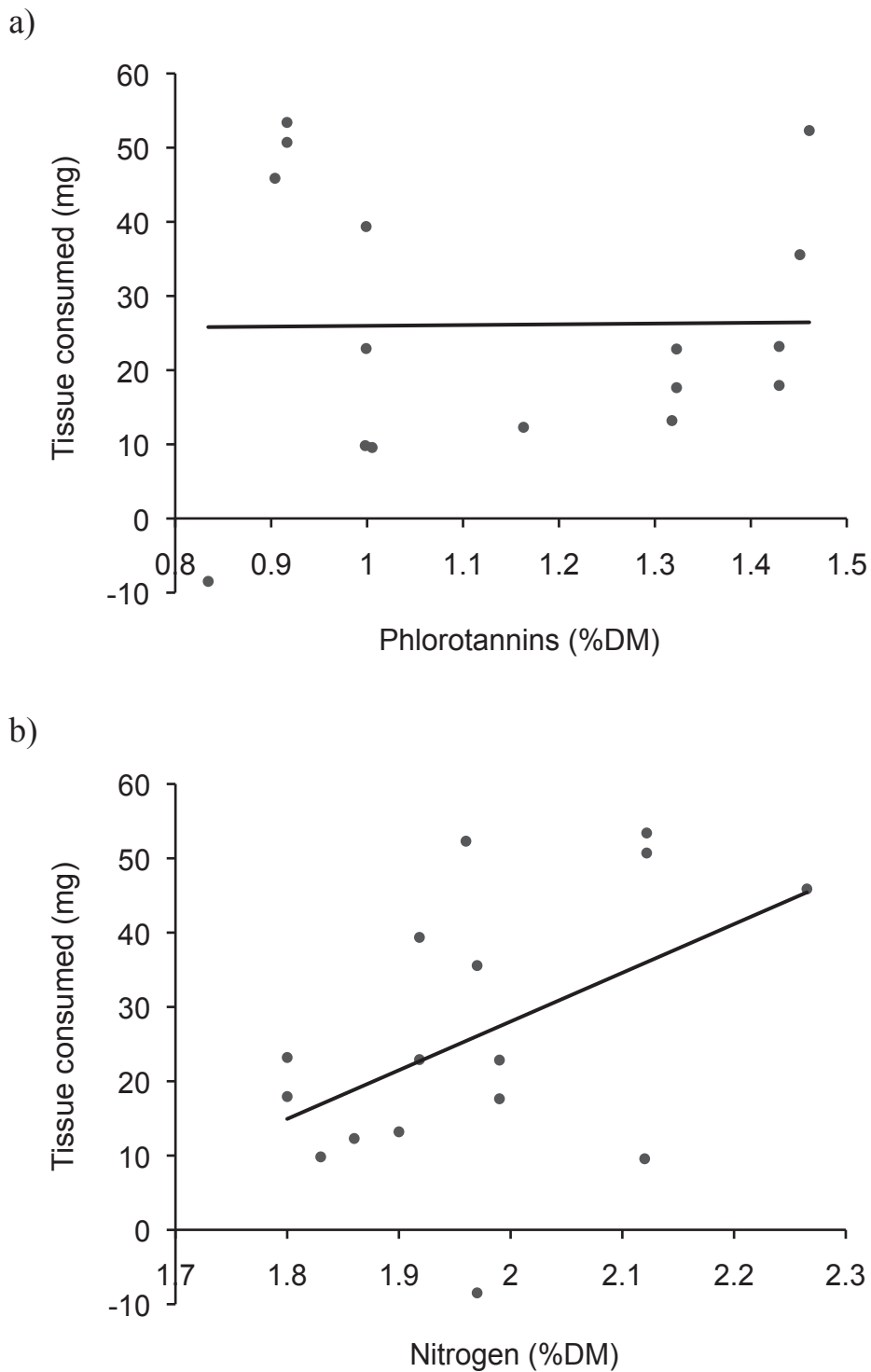


Figure 5. Results of regression analysis showing a) no relationship between phlorotannins and tissue consumed ($P = 0.21$) and b) a positive relationship between tissue nitrogen and the amount of tissue consumed ($P = 0.03$). Data are individual thalli.

CHAPTER 2

Multiple-stressor effects of herbivory, competition and immersion on the performance of the intertidal rockweed *Fucus distichus*

Abstract

The rockweed *Fucus distichus* experiences gradients of tidal immersion, competition, and herbivory across its vertical range in the rocky intertidal zone. I designed two experiments to separate the effects of these stressors and examine their interactions. In a test of *F. distichus* growth under simulated intertidal and subtidal conditions, growth was slower when always immersed, snail herbivory reduced growth, and the combined effects were additive. Herbivory had a greater effect in reducing epiphyte load than immersion. In a test of growth across immersion time in the absence of herbivory, *F. distichus* had the lowest growth when always immersed, and manual removal of epiphytes increased growth. The reduced growth of *F. distichus* in subtidal conditions occurred regardless of experimental treatment, indicating that immersion itself may be a stress for *F. distichus*. Immersion stress may be an important factor determining the lower limit of *F. distichus* in the intertidal zone.

Introduction

Species experience a variety of environmental stressors and understanding how these stressors interact to affect abundance and distribution is critical for making accurate predictions about the impact of environmental change. Traditionally, experimental ecology has measured the effect of each stressor alone with the assumption that their effects are additive. However, stressors do not

always interact additively and may instead be synergistic, when their combined effect is greater than predicted by additivity, or antagonistic, when their combined effect is less than predicted (Folt et al. 1999, Crain et al. 2008, Darling and Cote 2008). Non-linear effects of multiple stressors make it difficult to predict the outcome of ecological interactions and more studies of multiple stressors are needed to determine how they influence ecological interactions (Crain et al. 2008).

The rocky intertidal is an excellent system in which to study the effects of multiple stressors on the growth and reproduction of an organism. Intertidal species experience strong gradients of biotic and abiotic stressors across a relatively small geographic scale (Dayton 1971, Menge and Branch 2001). The rockweed *Fucus distichus* is a habitat forming species on protected to semi-protected rocky temperate coasts in the NE Pacific. *F. distichus* forms dense canopies and is responsible for a significant amount of primary production in the intertidal zone (Wahl et al. 2011). Therefore it is important to understand how multiple stressors affect *F. distichus* across its vertical range.

The main stressors that determine the distribution of *F. distichus* in the intertidal zone are immersion time, competition, and herbivory. Upper limits of *F. distichus* may not be driven by desiccation per se, but by lower net photosynthesis in air compared to water, thus during the summer months, upper-elevation individuals grow slower than those at mid-elevations in the intertidal zone (Williams and Dethier 2005, Dethier and Williams 2009). Herbivory reduces the growth of *F. distichus* and interacts additively with desiccation stress at the upper end of its range (Dethier and Williams 2005, Dethier and Williams 2009). Biotic stress is expected to

determine the lower limits of intertidal species ranges (Connell 1961). In locations on the Western Atlantic *F. distichus* and *Fucus vesiculosus* are limited by competition with *Chondrus crispus* in the low intertidal (Lubchenco 1980). *C. crispus* is not present in the San Juan Islands and the extent to which this interaction occurs in other locations and with other species that might compete with *Fucus* has not been established. Epiphyte growth has been measured and observed on *F. distichus* in the mid-low intertidal zone (Hart unpublished data, Wright et al. 2004). Epiphytes reduce seaweed growth by shading and restricting photosynthesis (D'Antonio 1995), but the effects of epiphytes on *F. distichus* growth have not been well studied. In the Baltic Sea, epiphytes reduce the growth of *F. vesiculosus* in the subtidal and interact with light limitation stress at the lower end of its range (Rohde et al. 2008). For *F. distichus*, little research has been carried out to address its lower limit and factors such as light or species interactions that might matter, particularly in the context of multiple stressors.

I designed two experiments to examine factors that could determine the lower limit of *F. distichus*. Immersion time and herbivory were manipulated in a fully crossed design and responses measured in terms of *F. distichus* growth, reproduction, and epiphyte load. To manipulate immersion time and epiphyte load, *F. distichus* was isolated from herbivores and growth was compared at high, low, and subtidal tide heights, with and without epiphytes.

Methods

Location and study species

Experiments were conducted at the Friday Harbor Laboratories (FHL) on San Juan Island, WA, USA. *F. distichus* was collected from the middle of the *Fucus* zone (~0.7 m MLLW) near the

Friday Harbor Laboratories (48°32'41.35"N, 123° 0'49.36"W). Juvenile thalli with no visible damage were carefully removed from the rock and transferred to a flow-through seawater aquarium at FHL until the start of the experiments. The herbivore used in the immersion x herbivory experiment was the snail *Littorina sitkana*, which is an important consumer of *F. distichus* and has been used in previous *F. distichus* herbivory studies (Van Alstyne 1990, Hart Chapter 1).

Immersion x herbivory experiment

Two potential stressors were examined in a crossed experimental design: immersion time and herbivory. *F. distichus* were grown in mesocosm tanks with either a subtidal or intertidal tide cycle and in the presence or absence of herbivorous littorine snails. Each mesocosm tank contained 6 juvenile *Fucus* thalli (< 10 cm in length) attached to bricks (Fig. 1, N = 6 tanks for each tide treatment). Subtidal thalli were always submerged and intertidal thalli were exposed to a 3 hour low tide from 9am – 12pm. Within each tank three *F. distichus* thalli were assigned to a pulsed snail herbivory treatment and three were no-herbivory controls. Every 4 days all *F. distichus* thalli were placed in containers that held either 10 *L. sitkana* snails (+ herbivore treatment) or were empty (– herbivore treatment). Thalli were held in these containers for between 4 and 7 hours and then were returned to their bricks. Treatments were maintained for 80 days between Aug and Nov 2007. Tanks were cleaned at the time of the herbivory treatment and all *F. distichus* thalli were carefully brushed to remove epiphytes. Although brushing was effective at removing diatom epiphytes, ulvoid epiphytes persisted on the thalli in the – herbivore treatments and these were removed on 29 Oct, filtered through a 149 µm nitex mesh, patted dry and weighed. The experiment was ended on 18 Nov. *F. distichus* thalli were photographed

before and after the experiment and initial area, final area, and receptacle number and area were measured using ImageJ (National Institutes of Health, Bethesda, MA, USA). Epiphytes were carefully removed, filtered, patted dry, and weighed. Since the experiment was a split-plot design, data were analyzed using a linear mixed effects model looking at the effect of immersion and herbivory on growth or epiphyte load with tank as a random effect. Data for receptacle number and area were log transformed to account for increasing variance with mean. Data analysis was done in R (R development core team 2012).

Immersion x Epiphyte experiment

The two potential stressors manipulated in this experiment were immersion time and competition from epiphytes. Herbivores were excluded by attaching *F. distichus* to ropes suspended vertically in the water from overhanging fixed points on a pier. 36 *F. distichus* thalli (< 7 cm) were attached to the rope at three tidal heights (0.5 m, -0.3 m, and -1.2 m MLLW) representing the upper (“high”) tidal height and lower (“low”) tidal height of its range and (“subtidal”) subtidal conditions. During the time period of the experiment these tide heights corresponded to average exposure time per day of 306, 78, and 0 minutes respectively as measured from tide data for the experimental period (Mr. Tides, Version 4, Hahn Software). Desiccation stress is higher on ropes than in the intertidal zone so tide heights on the ropes are approximately 0.5 m lower than the actual upper and lower tide heights of *F. distichus* in the intertidal zone. Thalli were secured to the three-ply polypropylene rope by placing their holdfast between the rope strands. Half of the individuals at each tidal height (N = 6) were assigned to the epiphyte removal treatment and epiphytes were removed by careful brushing every three days. Epiphytes were allowed to colonize naturally on the remaining 6 thalli at each tidal height. The experiment was

maintained for 45 days from May-Jul 2008. *F. distichus* growth was measured from initial and final photographs of individual thalli using ImageJ. Epiphytes were carefully removed from thalli at the end of the experiment, filtered through a 1.5 μm filter, dried at 60°C, and weighed. Light availability at each depth was measured at noon in full sun with PAR underwater spherical quantum sensor (LI-COR model LI-193). PAR measurements were taken from the surface down to 6 m in 1 m increments and these data were used to determine the percent reduction of light with depth. *F. distichus* growth was calculated as the rate of change in area from initial size to final size, per day. *F. distichus* growth between epiphyte treatment and tide height was compared by Analysis of Variance with main effects of epiphyte load scaled to thallus area, tidal elevation, and their interaction. Data was analyzed in R (R Core Development Team 2012).

Results

Immersion x herbivory

There was a significant effect of tide height on the growth of *F. distichus* thalli ($F_{1,8} = 74.6$, $P < 0.0001$), and a significant effect of herbivory ($F_{1,8} = 8.09$, $P = 0.0217$), but there was no interaction between these two factors ($F_{1,8} = 0.38$, $P = 0.5528$). Growth of *F. distichus* was slower when continuously immersed, snail feeding reduced growth, and the combined effects were additive (Fig. 2). Epiphyte loads on *F. distichus* thalli were measured once after 61 days (29 Oct) and again 19 days later at end of the experiment (18 Nov). Epiphytes were a mix of diatoms, *Ulva*, and red algae. Herbivory had a strong negative effect on the epiphyte load of *F. disticus* thalli on 29 Oct and on 18 Nov (29 Oct, $F_{1,8} = 63.1$, $P < 0.001$, Fig. 3; 18 Nov, $F_{1,8} = 88.6$, $P < 0.001$, Fig. 4). There was no effect of immersion treatment on the 29 Oct epiphyte load ($F_{1,8} = 0.25$, $P = 0.63$), nor an interaction between immersion and herbivory ($F_{1,8} = 0.25$, $P = 0.63$,

Fig. 3), which is consistent with herbivory having a greater effect than emersion on epiphyte load. However, during the shorter time period from 29 Oct to the end of the experiment, there was an effect of immersion treatment on epiphyte load ($F_{1,8} = 24.5$, $P < 0.0011$) and an interaction between immersion and herbivory ($F_{1,8} = 22.9$, $P < 0.0014$). This indicates that over shorter growth periods, the combined effect of immersion and herbivory on epiphyte load are non-additive. Snails consumed all of the epiphytes in the + herbivore treatment, regardless of immersion treatment, but in the absence of herbivores final epiphyte load was larger in the subtidal conditions indicating that epiphytes grew faster with longer immersion time.

Reproduction was higher in the intertidal treatment than the subtidal treatment (receptacle area, $F_{1,8} = 5.6$, $P < 0.05$, Fig. 5; receptacle number, $F_{1,8} = 5.5$, $P = 0.047$). Herbivory had no effect on receptacle area ($F_{1,8} = 2.1$, $P = 0.18$) or number ($F_{1,8} = 0.83$, $P < 0.39$). There was no interaction between tide and herbivory for either receptacle area ($F_{1,8} = 3.2$, $P = 0.11$) or number ($F_{1,8} = 1.5$, $P = 0.26$).

Immersion x epiphytes

Tide height had a significant effect on *F. distichus* growth ($F_2 = 10.2$, $P = 0.0005$) with the slowest growth occurring when thalli were always immersed (subtidal tide height) and the fastest growth occurring at the low tide height (Fig. 6). Epiphyte removal increased growth, especially at the low and subtidal tide heights ($F_1 = 7.3$, $P = 0.012$). The larger effect of epiphyte removal on growth at the subtidal tide height compared to the high tide height suggests a trend towards non-additivity, however, the effect of epiphyte load and tide height on *F. distichus* growth was statistically additive (tide height x epiphyte load, $F_2 = 1.6$, $P = 0.23$).

Manual removal of epiphytes was effective at reducing the epiphyte load on *F. distichus* thalli ($F_1 = 68.3$, $P < 0.0001$, Fig. 7). Epiphyte load increased as tidal height decreased and was highest at the lowest tidal height ($F_2 = 40.5$, $P < 0.0001$). The interactive effect of tide height and epiphyte removal on epiphyte load was non-additive ($F_2 = 27.8$, $P < 0.0001$). Epiphyte removal had a greater effect on epiphyte load at lower tide elevations because unmanipulated epiphyte loads increased by approximately 40% with each 0.8 m decrease in tide height.

Light decreased exponentially with depth and even in peak sunlight conditions, light decreased to 70% of surface irradiance at 1 m depth and 45% at 2 m depth. *F. distichus* photosynthesis is reduced when light levels are below $480 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Dethier and Williams 2005) and at peak midday sun this light limit was reached by 3 m depth.

Discussion

The effect of multiple stressors can be characterized by three types of interactions: additive, synergistic, or antagonistic (Crain et al. 2008). My results showed that the interactive effects of immersion with herbivory, and immersion with epiphyte load on *F. distichus* growth and reproductive tissue, were mostly additive. Meta-analyses of other multiple stressor experiments in marine systems, including those with fucoids, show that additive interactions are the most common outcome of two factor experiments (Crain et al. 2008, Darling and Cote 2008, Wahl et al. 2011). However, in many cases the addition of a third factor created a synergism or antagonism (Crain et al. 2008, Darling and Cote 2008). I did not explicitly test the interaction of

herbivory, epiphytes, and immersion in a three-factor design, however, it is possible that when these three stressors occur together, the interaction is non-additive.

In the immersion x herbivory experiment *F. distichus* grew faster and reproduced more in the intertidal treatment. Continuous immersion had a greater negative effect on growth than pulsed herbivory, which also reduced growth through tissue removal. The immersion x herbivory experiment was designed as a two-factor (abiotic stress x biotic stress) experiment, but the unexpected growth of epiphytes, despite weekly cleaning, introduced a third factor (biotic stress). Pulsed herbivory removed all epiphytes on the *F. distichus* thalli in + herbivore treatment, but ephemeral algal epiphytes (primarily *Ulva*) accumulated on *F. distichus* thalli in the – herbivore treatment. This large difference in epiphyte load between the + herbivory treatment and – herbivore treatment likely caused the non-additive result for epiphyte load because *L. sitkana* was so effective at removing epiphytes. The experiment was not designed to test the interaction of three stressors, however epiphytes are known to decrease *Fucus* performance by shading and reducing photosynthesis (Pavia et al. 1999, Jormalainen 2003, Jormalainen 2008, Rohde et al. 2008). Therefore, the presence of epiphytes in the – herbivore treatment suggests that the reductions in *F. distichus* growth between the – herbivore and + herbivore treatments in both intertidal and subtidal conditions were likely conservative. There was no difference in epiphyte load after 61 days in the – herbivore subtidal and intertidal treatment, however, the larger epiphyte load in the subtidal treatment at the end of the experiment confirms that epiphytes grow faster with longer immersion.

The complete removal of epiphytes in the + herbivore treatment was accomplished even with pulsed herbivory where *L. sitkana* fed on the *F. distichus* thalli for a few hours every five days. Grazing on epiphytes can either facilitate the host alga if grazers remove just epiphytes, or harm the host alga when the presence of epiphytes attract grazers that the preferentially consume host tissue (Wahl and Hay 1995, Karez et al. 2000). In the Baltic Sea, *F. vesiculosus* experiences greater damage by *Idotea baltica* when epiphytes are present (Karez et al. 2000, Råberg and Kautsky 2008). However, there are many examples of grazers, especially snails, indirectly facilitating *Fucus* and other seaweeds by removing epiphytes (Lubchenco 1983, Williams 1990, Karez et al. 2000, Stachowicz and Whitlatch 2005, Korpinen et al. 2007, Rohde et al. 2008). *L. sitkana* and other snail mesograzers preferentially consume epiphytes over *F. distichus* tissue (Dethier and Williams 2005, Hart Chapter 3). The large difference in epiphyte load between the + herbivore and – herbivore treatments suggests that mesograzers can remove epiphytes from *F. distichus* even when their distribution is patchy and their feeding on any individual thalli is sporadic. The strong effect that *L. sitkana* had on epiphyte load suggests that mesograzers may be important for reducing epiphytes on *F. distichus* in the field.

In the immersion x epiphyte experiment, epiphyte load increased exponentially as tide height decreased and was highest in the subtidal treatment. This pattern is consistent with *F. distichus* experiencing longer immersion time at lower tide heights because epiphyte growth responds negatively to desiccation. The interaction between removal treatment and tide height on epiphyte load was non-additive because epiphyte removal had a larger effect on epiphyte load at the subtidal tide height than the high tide height due to positive effect of immersion on epiphytes. Epiphyte removal in the immersion x epiphyte experiment had the greatest effect on *F. distichus*

growth in the subtidal tide height treatment where removal of epiphytes increased the mean growth rate by 49%. Even with this increased growth at the subtidal tide height was still 37% slower than at the low tide height. This reduction in growth even when epiphytes were removed may have been partly due to light limitation at lower depth (Dethier and Williams 2005, Rohde et al. 2008), but is also consistent with the result from the immersion x herbivory experiment which showed that *F. distichus* growth is reduced in subtidal conditions, regardless of light. At the low tide height, epiphyte removal increased the mean growth by 20% and growth with or without epiphytes was the highest for all tide heights. Epiphyte removal had the least effect, only 8%, on growth at the high tide height. Desiccation stress reduces *F. distichus* growth, as does its lower photosynthesis rate in air (Williams and Dethier 2005) and these factors are likely what caused the slower *F. distichus* growth and low epiphyte load at the high tide height.

In both experiments, *F. distichus* grew poorly in subtidal conditions regardless of other stressors that could covary with tidal height. It was predicted that herbivory and/or epiphytes would have a greater effect on *F. distichus* at low tidal elevations, providing a mechanism for the lower limit of the *Fucus* zone. However, the results of both the immersion x herbivory and immersion x epiphyte experiments, indicate that complete immersion itself is a stress that reduces performance of *F. distichus*, independent of biotic factors. The immersion x herbivory results are particularly important because light was equivalent in all tanks and since photosynthesis is lower in air than water (Williams and Dethier 2005), this should have caused faster growth in the absence of other stressors.

Marine algae are generally thought to grow faster with longer immersion times and although epiphytes showed this pattern, *F. distichus* did not. This result for *F. distichus* was surprising because the lower portion of the intertidal zone is considered to be a physiologically optimal location for *Fucus* (Lubchenco 1980). Exceptions to this are very high intertidal fucoids such as *F. spiralis* and *Pelvetia canaliculata* (Schonbeck and Norton 1980), which experience decay when grown subtidally, but are not found as low as *F. distichus* in the intertidal zone.

Factors other than those investigated here (light, herbivory, and epiphytes) that could have reduced *F. distichus* growth in the subtidal are temperature, physiology, and bacterial or fungal decay. Short-term temperature pulses can increase growth rate in *F. distichus* (Strömngren 1977a) and photosynthesis increases with temperature up to 30°C (Williams and Dethier 2005). However, this response occurred in water and the morning low tide in the immersion x herbivory experiment meant that *F. distichus* in all tanks were immersed in the afternoon during peak solar heating, so the thalli in both treatments should have experienced similar water temperature regimes. There is evidence that *F. spiralis* and *Ascophyllum nodosum*, but not *F. vesiculosus*, respond physiologically to exposure to air with a short-term increase in growth rate (Strömngren 1977b). This response has not been tested in *F. distichus* but might account for the faster growth in the intertidal treatment of the immersion x herbivory experiment. Given the timing and length of the low tide treatment, however, reduced photosynthesis from exposure to air should have limited growth in the intertidal treatment (Dethier et al. 2005). Reduced growth with longer immersion due to tissue decay is seen in high intertidal fucoids (Schonbeck and Norton 1980) and this could be another factor limiting *F. distichus* performance in the subtidal. Although thalli in these experiments were examined for decay, there was no evidence of greater decay for *F.*

distichus in the immersion x herbivory subtidal treatment or the immersion x epiphyte subtidal tide height treatment. Tissue decay has been observed in some individuals in another experiment where juvenile *F. distichus* were grown primarily in subtidal conditions (Hart Chapter 1). Tissue decay has also been reported for adult *F. distichus* grown subtidally at depths of 1 m (Gail 1918), although this was attributed to light limitation.

How biotic and abiotic interact to determine the lower limit of *F. distichus* in the NE Pacific has not been well established. Studies of *Fucus* range limits in other locations and with other *Fucus* species have come to different conclusions depending on the species, geographic location, and experimental design (Gail 1918, Lubchenco 1980, Rohde et al. 2008). Biotic interactions are thought to set the lower limits of species ranges (Connell 1961). For *Fucus* there are examples of competition with other macroalgae determining the lower limits (Lubchenco 1980) but epiphytes have not been as widely suggested as a mechanism, although my results suggest that it may be an important factor. Abiotic stress from immersion reduces *F. distichus* growth in subtidal conditions and needs to be considered when determining how the interaction of multiple stressors influences the distribution of *F. distichus* in the intertidal zone.

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Figures

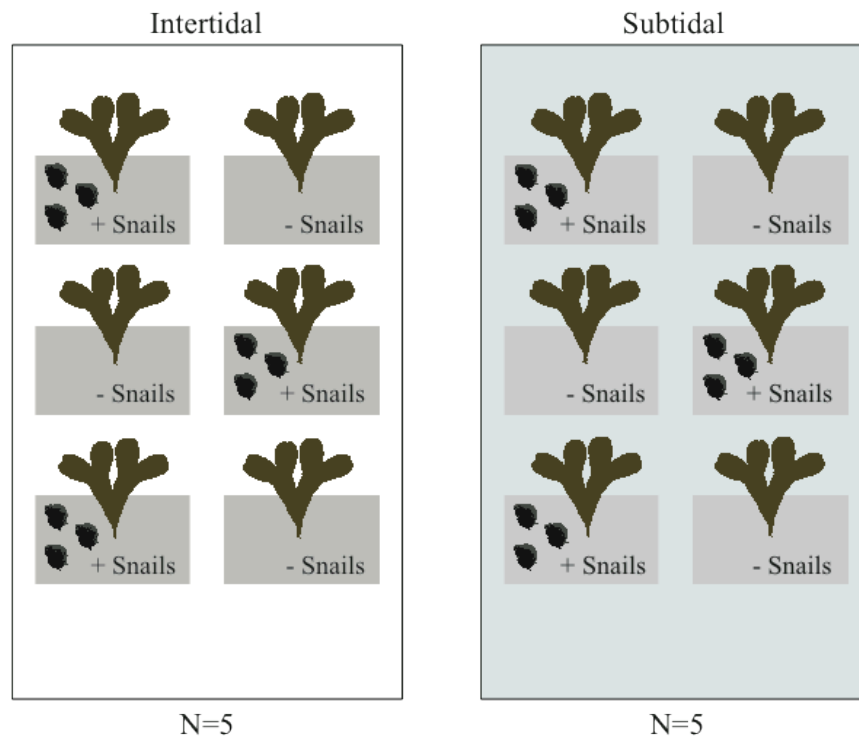


Figure 1. Experimental set-up for the immersion x herbivory experiment showing the split-plot design. Each subtidal and intertidal mesocosm tank was replicated 5 times. Each tank contained 6 juvenile *F. distichus* thalli attached to bricks and of these, three thalli were exposed to pulsed snail herbivory, and three were no-herbivore controls.

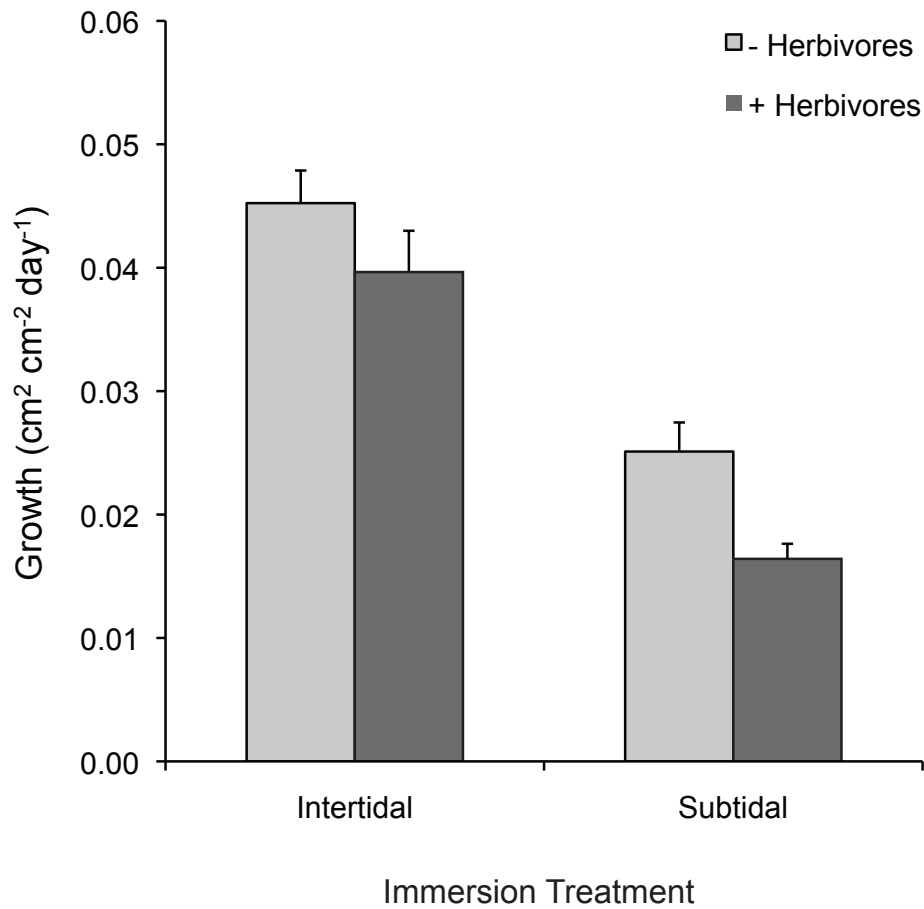


Figure 2. Growth, increase in area per day, of *F. distichus* in the immersion x herbivory experiment. Growth was slower in the subtidal treatment ($P < 0.001$) and in response to herbivory ($P = 0.022$). Herbivory and tidal immersion reduced growth additively ($P = 0.55$). Data are means and error bars are SE.



Figure 3. Epiphyte load on 29 Oct scaled to *F. distichus* area in intertidal and subtidal treatments. Epiphyte load on the snail treatments was zero for both tidal treatments. Herbivory had a strong effect on epiphyte load ($P < 0.001$) but there was no effect of tide ($P = 0.63$) and no interaction ($P = 0.63$). Data are means and error bars are SE.

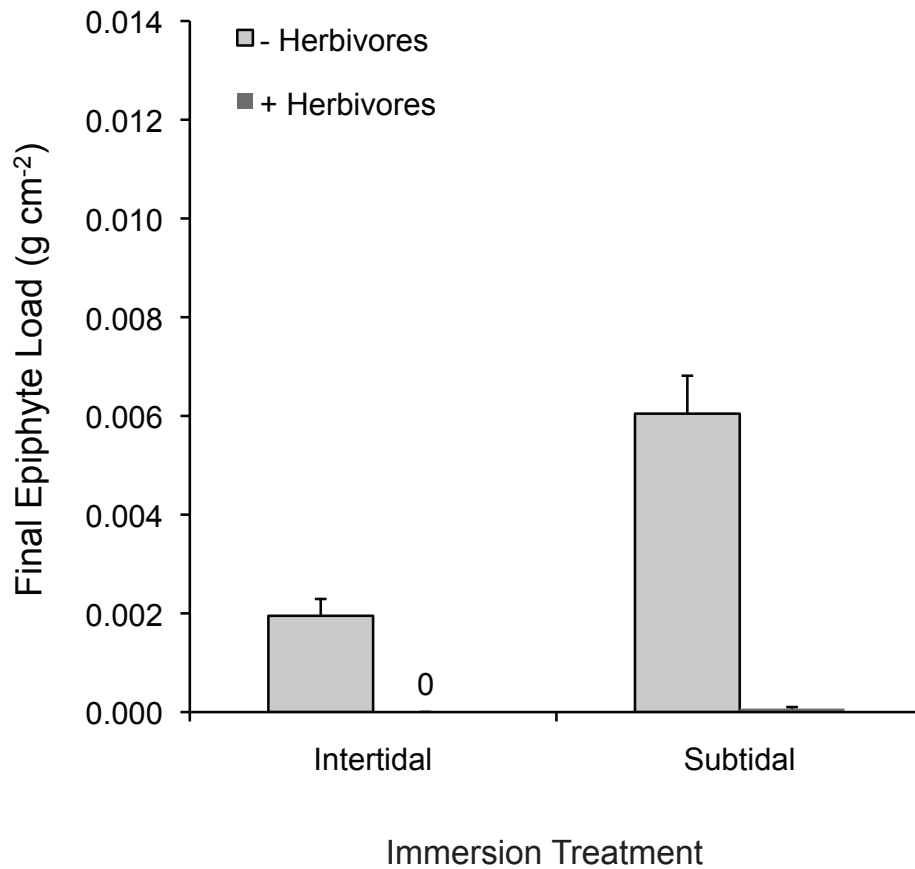


Figure 4. Epiphyte load scaled to *F. distichus* area in intertidal and subtidal treatments at the end of the experiment. Epiphytes grew for 19 days. Epiphyte load was significantly lower in the intertidal treatment than the subtidal treatment after 61 days of growth ($P < 0.0001$). Herbivory had a negative effect on final epiphyte load ($P = 0.0011$). There was an interactive effect of tide and herbivory on epiphyte load ($P = 0.0014$). Data are means and error bars are SE.

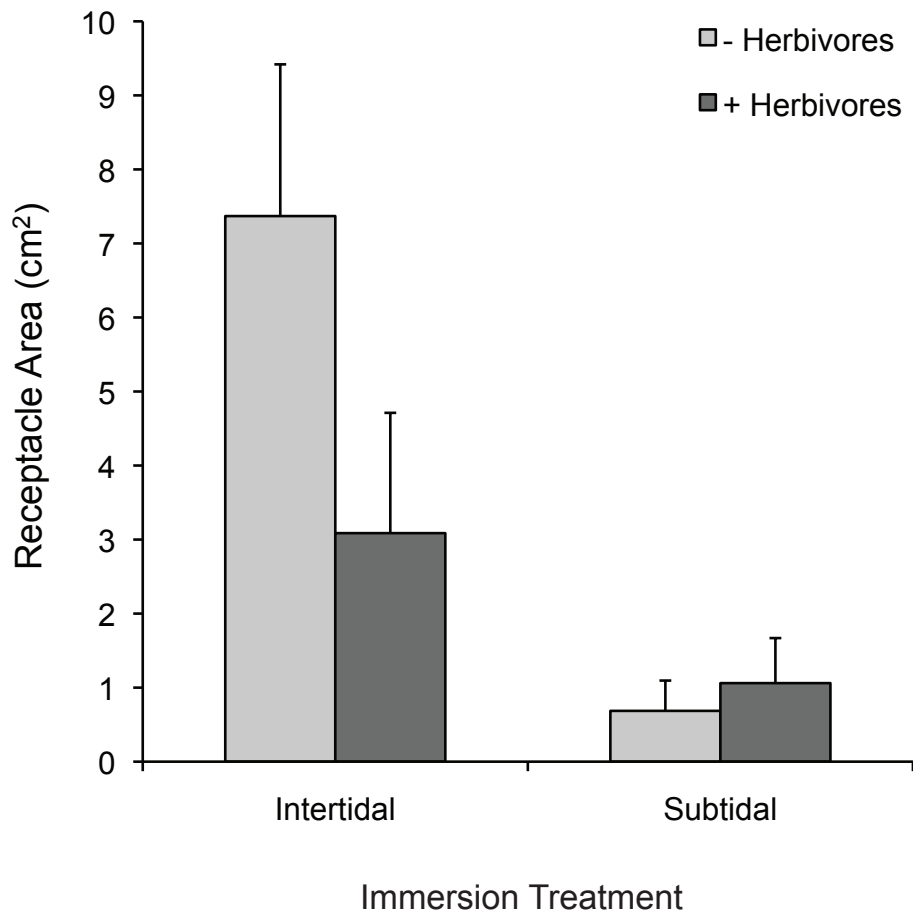


Figure 5. Total area of receptacles in the presence and absence of pulsed snail herbivory. Receptacle area was significantly larger in the intertidal treatment ($P = 0.045$). There was no effect of herbivory ($P = 0.18$) and no interaction between treatments ($P = 0.11$). Data are means and error bars are SE.

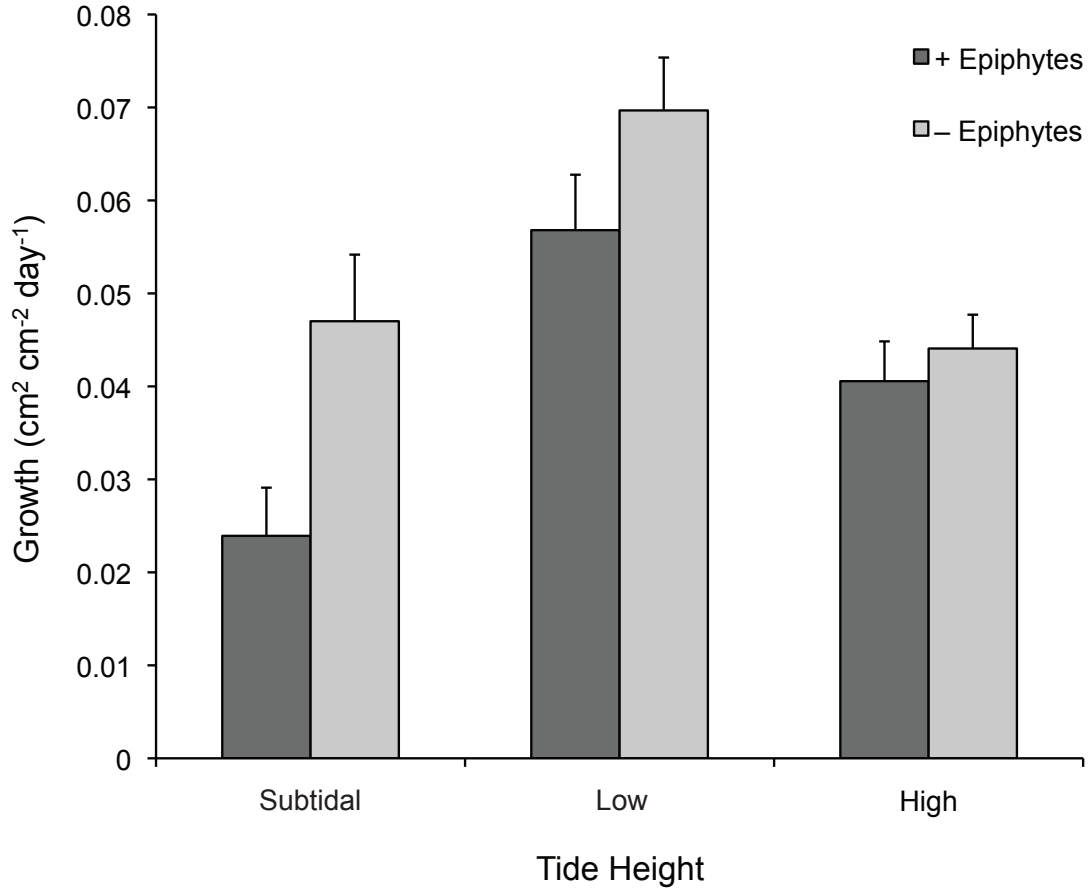


Figure 6. Growth, increase in area per day, of *F. distichus* across tide height in the presence and absence of epiphytes. Tide height had a significant effect on growth, which was highest at the low tide height ($P < 0.0005$). Removal of epiphytes improved *F. distichus* growth ($P = 0.012$). The interaction between tide and epiphyte removal was additive. Data are means and error bars are SE.

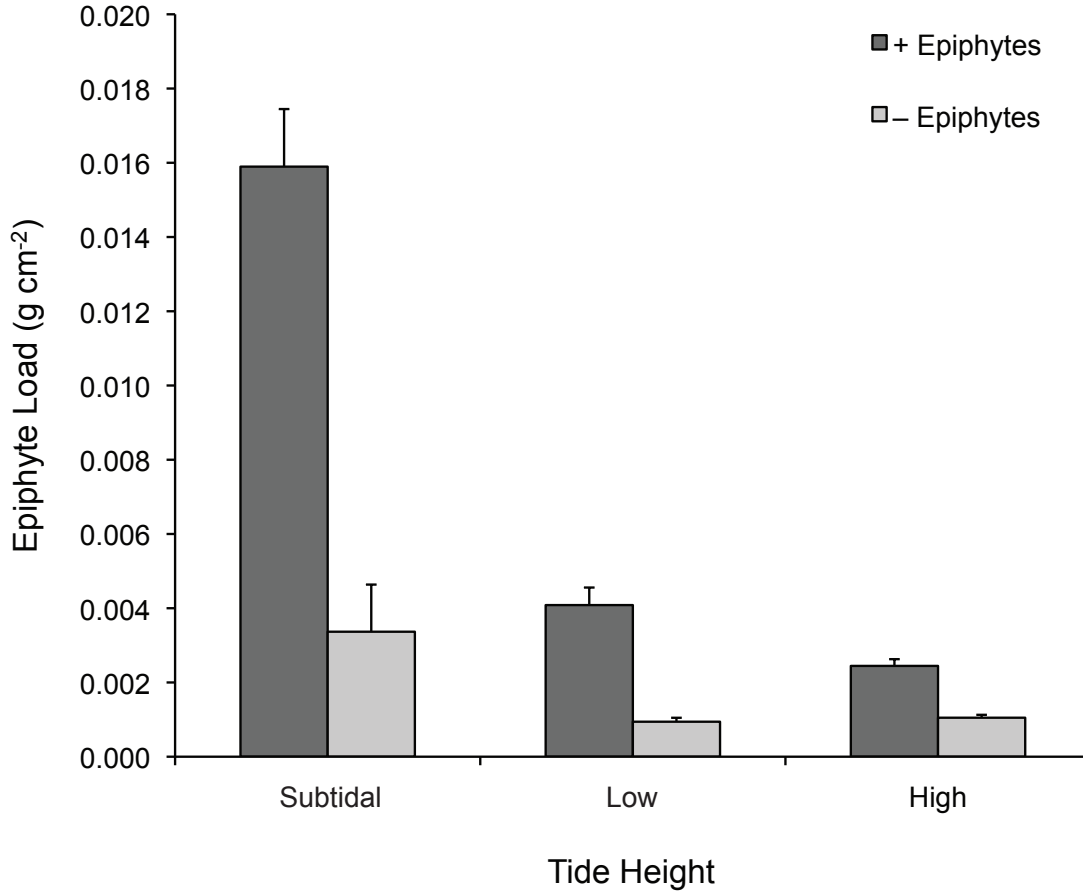


Figure 7. Epiphyte load scaled to *F. distichus* area across tide heights. Tide height ($P < 0.00001$) and epiphyte removal ($P < 0.00001$) had a significant effect on epiphyte load. The interaction was non-additive ($P < 0.00001$). Data are means and error bars are SE.

CHAPTER 3

Context-dependent interactions between the rockweed, *Fucus distichus*, and its snail herbivore are mediated by snail density and epiphyte load

Abstract

A major challenge for predicting the outcome of species interactions is determining the relative strengths of different interaction pathways that change with environmental context or density. The marine snail, *Littorina sitkana*, consumes the intertidal rockweed, *Fucus distichus*, directly but also feeds on epiphytes that could interfere with light capture and growth of *F. distichus*. The arrangement of species in this interaction web generates opportunities for direct and indirect interactions to exert opposing effects. I predicted that the net effect of snails on rockweed would switch from positive indirect facilitation to negative direct herbivory across the range of snail densities commonly encountered in the field. I grew *F. distichus* in mesocosm tanks with 0-80 snails (0-1280 m⁻²) and measured growth, tissue loss due to consumption, and epiphyte load after one month. Epiphyte load decreased as snail density increased, and the loss of *F. distichus* tissue due to consumption increased with snail density. *F. distichus* growth was maximized at intermediate snail densities, where both epiphyte load and herbivory on *F. distichus* were low. The net effect of *L. sitkana* on *F. distichus* switched from positive to negative with increased snail density, and this was mediated by epiphyte load. Results indicate that context dependence in species interactions can arise when consumer density changes the relative strengths of direct and indirect effects.

Introduction

Experimental tests of species interactions often fail to adequately characterize the relative contributions of direct and indirect pathways under different biotic and abiotic conditions. Species interactions can be positive or negative and consist of direct and indirect effects (Wootton 1994, Paine 1980). Empirical tests of interaction strength are frequently carried out in ways that cannot distinguish the contributions of direct and indirect effects, and therefore must assume, for instance, that positive effects of an herbivore on a primary producer are transmitted indirectly (Paine 1992). For food web modeling, interaction strengths are drawn from empirical distributions of few weak and many strong effects, but variation in species-specific interaction strength is rarely acknowledged (McCann et al. 1998).

It is increasingly recognized, however, that the direction and strengths of direct and indirect interactions can switch in response to changes in context caused by dynamic traits (Abrams 1995, Peacor and Werner 2001, Křivan and Schmitz 2004, Schmitz et al. 2004), variation in abiotic conditions (Bracken et al. 2011), or consumer density (Berlow 1999, Křivan and Schmitz 2004, Ruesink 1998). Context dependent species interactions are important because there is evidence that they stabilize community structure and buffer disturbance (Navarrete and Berlow 2006). Understanding the extent to which the outcome of species interactions is context dependent is crucial for creating a predictive framework for community ecology (Agrawal et al. 2007).

The rocky intertidal zone is highly variable in both abiotic and biotic stress (Menge and Branch 2001, Dayton 1971). Gradients of physical stress, patchy distribution of herbivores, and spatial competition (Menge and Branch 2001, Dayton 1971, Dethier and Williams 2009, Lubchenco 1980) across small geographical scales make this an excellent system to explore the relative strength of species interactions in varying contexts. Here, I focus on an interaction in which a generalist herbivore, *Littorina sitkana*, consumes the rockweed, *Fucus distichus*, directly but also feeds on epiphytes that colonize *F. distichus* thalli. Epiphytes exert a negative effect on macroalgal growth, primarily by shading the host thallus and reducing its photosynthesis (Jormalianen et al. 2008, D'Antonio 1985, Hart Chapter 2). The arrangement of species in the interaction web between *Littorina sitkana*, *F. distichus*, and epiphytes generates opportunities for direct and indirect interactions to exert opposing effects (Fig. 1).

In this experiment, I tested whether for a given epiphyte load there is a snail density at which the interactions between an intertidal rockweed and a marine herbivorous snail switch from indirect positive facilitation to direct negative herbivory across the range of snail densities commonly encountered in the field.

Methods

Natural History

Fucus distichus is an important habitat forming species in the mid-upper rocky intertidal zone in the NE Pacific and experiences gradients of immersion, herbivory, and competition from epiphytes across its vertical range (Wright et al. 2004, Dethier and Williams 2009, Wahl et al. 2011, Hart unpublished data). *F. distichus* is consumed by a suite of mollusc and crustacean

mesograzers that remove *F. distichus* tissue by biting and scraping (Van Alstyne 1990, Dethier and Williams 2009). Epiphyte communities that colonize *F. distichus* thalli are dominated by diatoms, but *Ulva* and other ephemeral algae may also be present (Hart Chapter 2). The littorine snails *L. sitkana*, *L. scutulata* and *L. plena*, are the most numerically dominant consumers of *F. distichus* and co-occur in the mid-upper portion of the vertical range that it occupies. Littorines are patchy in their distribution but abundances can reach over 1500 snails m⁻² (Dethier and Williams 2009, Hart unpublished data). Littorines are generalist herbivores that feed on *F. distichus* (Personal observation, Dethier and Williams 2009, Van Alstyne 1990), however they are thought to prefer diatoms (Dethier et al. 2005, Lubchenco 1983). *L. sitkana* was chosen for this experiment because it has been used previously for *F. distichus* herbivory studies (Hart Chapter 1 and 2, Van Alstyne 1990) and because it does not exhibit variable radular phenotypes in response to different food types (Dittman 1998, Padilla 1998), which could otherwise affect feeding rates and preferences.

Experimental Design

The interactions among *F. distichus*, epiphytes, and littorine snails were established on tiles within 120 cm x 60 cm 173 L flow-through seawater mesocosm aquaria at the Friday Harbor Laboratories, San Juan Island, Washington, USA. Overall, eight treatments (N = 7) included different snail numbers (0, 5, 10, 20, 40, 60, 80 snails, 0-2000 m⁻²) and a control treatment with no snails and regular manual removal of epiphytes. Snail densities encompass the range of snail densities experienced by *F. distichus* in the field (Dethier and Williams 2009, Hart unpublished data).

Juvenile *F. distichus* thalli were collected from the middle of the *Fucus* zone at Colin's Cove (48°33'0.66"N, 123° 0'20.50"W) on 22 Jun 2009. Individuals between 6 and 8 cm in length with minimal herbivore damage were carefully removed from the rock to avoid damaging the holdfast and transported to the lab where they were checked for herbivores or epiphytes and held in a flow through seawater aquarium. On 23 June 2009, five individual thalli were assigned to each tile, patted dry, weighed for initial biomass and photographed flat on a light table to determine initial area. Thalli were attached to each tile by placing their holdfasts between strands of polypropylene rope that were glued to each tile within the interaction arena. Diatom epiphytes, which are naturally present in the seawater, were allowed to colonize *F. distichus* thalli for one month. During this period, mesocosm aquaria were on a two-hour early morning (6-8 am) low tide cycle for two weeks and then a no emersion period for two weeks to facilitate faster establishment of epiphytes.

Snails were collected at the same site as *F. distichus* on 4 July, and held in containers with adult *F. distichus* in flow through seawater aquaria prior to being added to the experiment. Snails were added to the tiles on 23 July, and were contained within the 20 cm x 20 cm interaction arena by 2 cm tall copper strips glued to each tile. Snail density treatments were randomly assigned to each tile and tiles were placed in flow-through seawater in outdoor mesocosm aquaria in a randomized block design with eight treatments per block and seven blocks arranged across 14 tanks. During the snail density treatments, the mesocosm tanks were returned to the two-hour early morning low tide cycle. Tanks and tiles were cleaned weekly to remove diatoms growing on substrates other than *F. distichus* and tiles were rotated within tanks. Epiphytic diatoms on *F. distichus* were not disturbed except for on the no-snail-no-epiphyte tiles on which the *F. distichus* thalli

were brushed lightly with a toothbrush every three days. Snails on each tile were counted weekly and missing snails were replaced to maintain treatment densities. Snail density treatments were applied for one month and the experiment was ended on 23 August 2009.

The no-snail-no-epiphyte controls were treated identically to all other tiles during the four weeks that epiphytes accumulated prior to the addition of snails. The thalli in this treatment were used to measure initial epiphyte load. Epiphytes were first removed from the no-snail-no-epiphyte control thalli on 23 Jul 2009 by gentle scraping into filtered seawater with a razor blade and toothbrush. Epiphytes were dried at 60°C and weighed. The five cleaned thalli per tile were patted dry, weighed and photographed before being reattached to the tile. *F. distichus* in this treatment were subsequently cleaned of epiphytes every three days for the remainder of the experiment.

At the end of the experiment, epiphytes were removed as previously for the no-snail-no-epiphyte controls, dried and weighed. *F. distichus* on each tile were patted dry, photographed flat on a light table, weighed, and dried at 60°C and weighed for dry biomass. Area and midrib length measurements were done for initial and final photographs of the thalli using ImageJ (National Institutes of Health, Bethesda, Maryland, USA) and Adobe Photoshop (Adobe Systems Incorporated, San Jose, California, USA).

Epiphyte load, necrotic tissue and growth responses were pooled for the five thalli on each tile. Epiphyte load was measured by dry weight scaled to thallus area. Necrotic tissue was identified by notable discolorations each thallus. *F. distichus* growth was calculated using the final and

initial areas measured from photographs. Tissue consumed was estimated from the proportional difference of the measured final area of *F. distichus* thalli and the predicted final area based on midrib length in the absence of herbivory.

Data Analysis

Snail density was considered a continuous predictor variable in all analyses. The primary response variable was growth of *F. distichus*. Growth as increase over time from initial area, $(\text{final area} - \text{initial area}) / \text{initial area} / \text{day}$, was calculated from 23 June to 23 August, necrotic tissue was excluded from the final area. A likelihood ratio test and ΔAICc model selection were used to determine if a linear or quadratic fit was a better model for the relationship between growth and snail density. Linear and exponential fits for the relationship between epiphyte load and snail density were compared by Root Mean Square Error (RMSE). The same was done for necrotic tissue and snail density. A linear model of the relationship between final midrib length and final area of the no-snail-no-epiphyte controls ($N = 36$), which had not been exposed to herbivory or epiphytes and represented maximum growth during the experiment, provided an equation to calculate final predicted area based on midrib length for all thalli. The difference in actual and predicted final area was an estimate of the amount of tissue removed by snail herbivory.

Herbivory across snail density was tested by linear regression. All analyses were done in R (R Development Core 2012).

Results

At the time the snails were added the mean epiphyte load on the no-snail-no-epiphyte control thalli was $0.0078 \text{ g cm}^{-2} \pm 0.00072$ (mean \pm SE). At the end of the experiment epiphyte load was

highest in the 0 snail treatment with $0.014 \text{ g cm}^{-2} \pm 0.0013$ and decreased to $0.002 \text{ g cm}^{-2} \pm 0.0027$ in the 80 snail treatment (Fig. 2, Fig. 3a). An exponential fit of the data was only slightly better than linear and both models showed the trend of epiphyte load decreasing with snail density ($R^2 = 0.7883$, $F_{1,47} = 179.7$, $P < 0.001$, $\text{RMSE} = 0.00276$). Necrotic tissue showed the same pattern as epiphyte load and was highest on thalli in the 0 snail treatment, $13.81 \text{ cm}^2 \pm 4.24$, and decreased to 0 cm^2 at the highest snail densities (Fig. 3b). The negative exponential fit was used because it was slightly better than linear ($R^2 = 0.5996$, $F_{1,47} = 72.88$, $P < 0.0001$, $\text{RMSE} = 5.432$).

The linear relationship between midrib length and final area in the no-snail-no-epiphyte controls generated the equation Predicted Area = $0.7548 * \text{Midrib length} + 1.3453$ ($R^2 = 0.82$, $F_{1,33} = 151.2$, $P = 0.0001$). *F. distichus* tissue lost to herbivory increased linearly with snail density ($R^2 = 0.66$, $F_{1,47} = 94.29$, $P < 0.0001$). Herbivory had a negative effect on the growth of *F. distichus* at densities higher than 40 snails (Fig. 2, Fig. 4) as indicated by the positive values for tissue consumed. Loss of *F. distichus* tissue to herbivory increased from 0% to 25% at densities of 40 to 80 snails, respectively.

F. distichus in the no-snail-no epiphyte control were $167.9 \text{ cm}^2 \pm 9.1$ when snails were added, and increased to $189.8 \text{ cm}^2 \pm 12.6$ at the end of one month. Growth of *F. distichus* peaked at intermediate snail density, 40 snails per tile, when epiphyte load and herbivory were low (Fig. 2, Fig. 5). Growth data was fit with a quadratic equation ($R^2 = 0.093$, $F_{2,46} = 3.47$, $P < 0.395$). Model comparisons revealed that a quadratic fit was the best model for growth across snail

density based on maximum likelihood and ΔAICc comparisons ($F_1 = 6.906$, $P = 0.1164$, $\Delta\text{AICc} = 4.5$).

Discussion

I found a non-linear effect of *L. sitkana* on *F. distichus* growth in which the snails indirectly facilitate *F. distichus* at low densities by removing epiphytes, but switch to negative direct herbivory on *F. distichus* at high densities. The mechanism for this switch seems to be that *L. sitkana* feeds preferentially on epiphytes when they are present but switches to feeding on *F. distichus* when epiphytes are absent or have been consumed. The interaction between the productivity of epiphytes and density of snails is an example of interaction strength differing in both magnitude and direction in a context dependent manner. For the initial epiphyte load in this experiment the switch occurred when snail density reached 40 snails per tile, which corresponds to a field density of 1000 snails m^{-2} . The initial epiphyte load at the time of snail addition was within the range of diatom epiphyte loads measured in the field at the middle-low part of the *F. distichus* range (Hart unpublished data), suggesting that when herbivore densities are high this switch from indirect facilitation to direct herbivory could occur in the field.

Epiphyte load decreased with snail density indicating that *L. sitkana* has a direct negative effect on epiphytes. The negative exponential relationship was driven by epiphytes being completely consumed at snail densities over 40 per tile and was only a slightly better fit than linear. This suggests that *L. sitkana* exerts a relatively constant per capita interaction strength on epiphytes with density (Ruesink 1998). Necrotic tissue showed the same pattern as epiphytes and was highest in the treatments with 0 snails. Like epiphyte load, the area of necrotic tissue decreased

with snail density and was zero at snail densities over 40. This suggests that epiphytes may reduce *F. distichus* growth by inducing tissue decay. The mechanism of this decay is not known, but it may occur from epiphyte shading since decay has been reported for light limited *F. distichus* grown at depth (Gail, 1918).

Many mesograzers feed on epiphytes in marine systems and the abundance and diversity of mesograzers can affect productivity of macrophytes through indirect effects of epiphyte removal (Duffy and Harvilicz 2001, Jormalainen et al. 2008, Karez et al. 2000, Lubchenco 1983, D'Antonio 1985). The role of epiphytes in the interactions between herbivores and macroalgae can be positive or negative depending on the species and feeding preference of the herbivore (Karez et al. 2000, Wahl and Hay 1995). Positive interactions occur when the herbivore consumes epiphytes but not the host alga until the epiphyte resource has been depleted. Negative interactions occur when herbivores feed on both the epiphytes and host at the same time (Wahl and Hay 1995, Karez et al. 2000). Epiphyte load decreased with snail density and was zero at 40 snails. There was little to no feeding damage on *F. distichus* thalli in the 0-40 snail treatments, which suggests that the interaction between *L. sitkana* and *F. distichus* is positive when epiphytes are present.

When snail densities reached levels at which all epiphytes were consumed, *L. sitkana* had a negative effect on *F. distichus* through direct herbivory. At the highest snail density there was on average a 25% loss of tissue to herbivory. This estimate of herbivory was conservative because some damage occurred to the no-snail no-epiphyte control thalli during the regular removal of

epiphytes by brushing. Consequently the thalli in some treatments were actually larger for their midrib length than those in the no-snail-no-epiphyte controls.

Variance in herbivory increased with snail density, and even among thalli on a single tile herbivory was not consistent in the high density snail treatments. One reason for this might have been differences in defensive chemistry among thalli and tiles. *F. distichus* is able to induce a chemical defense response against direct herbivory (Van Alstyne 1988). Phlorotannin levels are correlated with this defense, and there is genetic variation in phlorotannin levels among thalli of a related species *Fucus vesiculosus* (Honkanen and Jormalainen 2005). If some *F. distichus* thalli were better defended than others then they may have been avoided by snails. A second reason for variation in feeding might be variation in tissue nitrogen among individual thalli and tiles due to differences in local uptake of nitrogen from snail excretions (Bracken 2004, Pfister 2007). Snails have a strong preference for nitrogen rich tissue and small differences in tissue nitrogen can lead to large differences in feeding, which may have caused some thalli to be consumed more than others in the higher snail density treatments (Hart Chapter 1, Van Alstyne et al. 2009). Thirdly, there may have been interactions between the snails themselves when they were at high densities that led to variation in feeding on some tiles and thalli.

The net effect of *L. sitkana* on *F. distichus* switches from indirect positive to direct negative with increased snail density and this is mediated by epiphyte load. This result indicates that context dependence in species interactions can arise from consumer density when this changes the relative strengths of direct and indirect effects. This type of context dependence mediated by consumer density may be common in interactions where a consumer has both a direct and

indirect effect on a prey species, mediated by a second prey species that exploits or competes with the first prey species. The signs and relative importance of direct and indirect effects in species interaction are not static, however they can be made predictable when we understand how these interactions switch in response to changing context.

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Figures

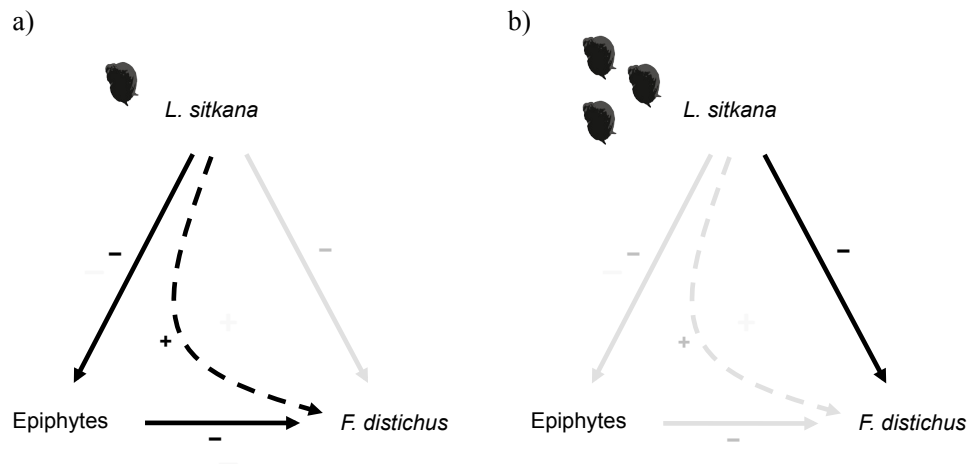


Figure 1. Diagram showing the interaction between *L. sitkana*, *F. distichus*, and epiphytes switching from a) a net positive indirect interaction to b) a net negative direct interaction with increased snail density.

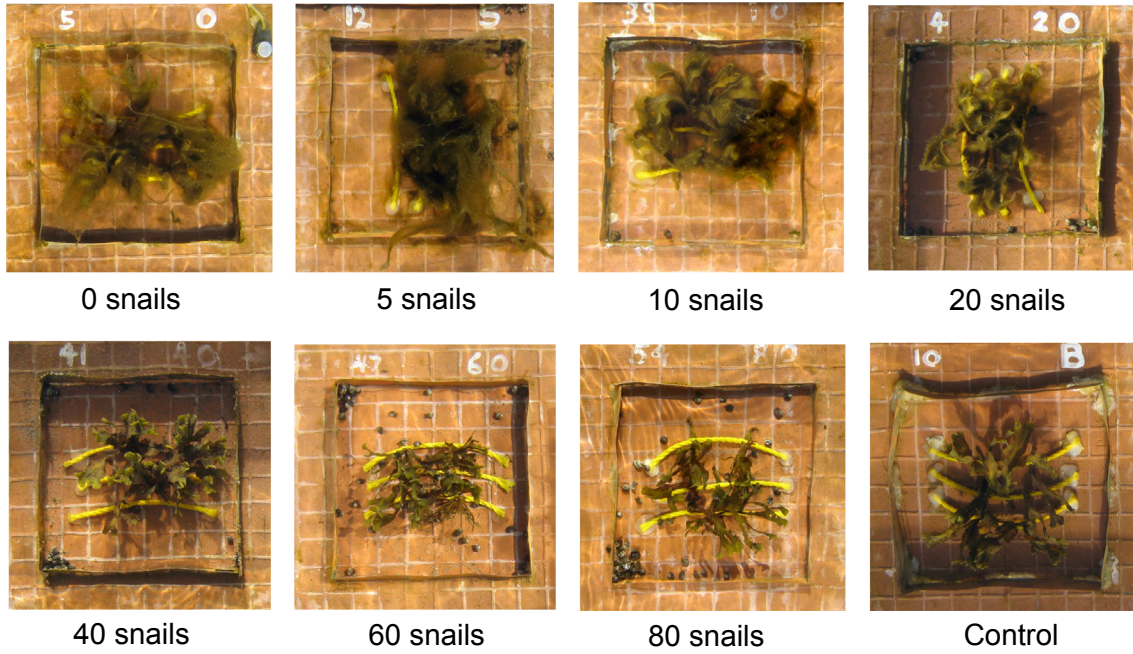
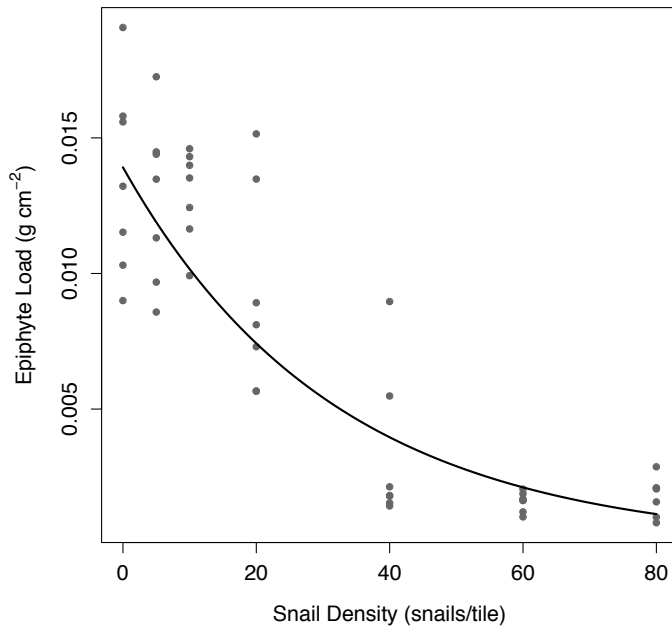


Figure 2. Photographs of representative tiles from each treatment at the end of the experiment showing the effect of snail density on epiphyte load and herbivore damage.

a)



b)

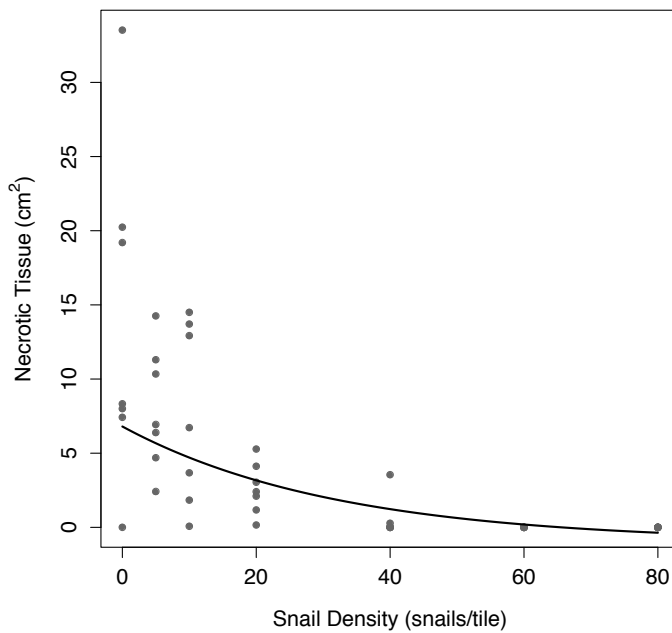


Figure 3. a) Final epiphyte load on *F. distichus* across snail density. Each point represents the total epiphytes removed from the five *F. distichus* thalli on one tile, scaled to thallus area. Line shows best-fit exponential relationship ($R^2 = 0.79$). b) Necrotic *F. distichus* tissue across snail density. Necrotic tissue was identified by notable discolorations in the *F. distichus* thalli. Line shows negative exponential relationship ($R^2 = 0.60$).

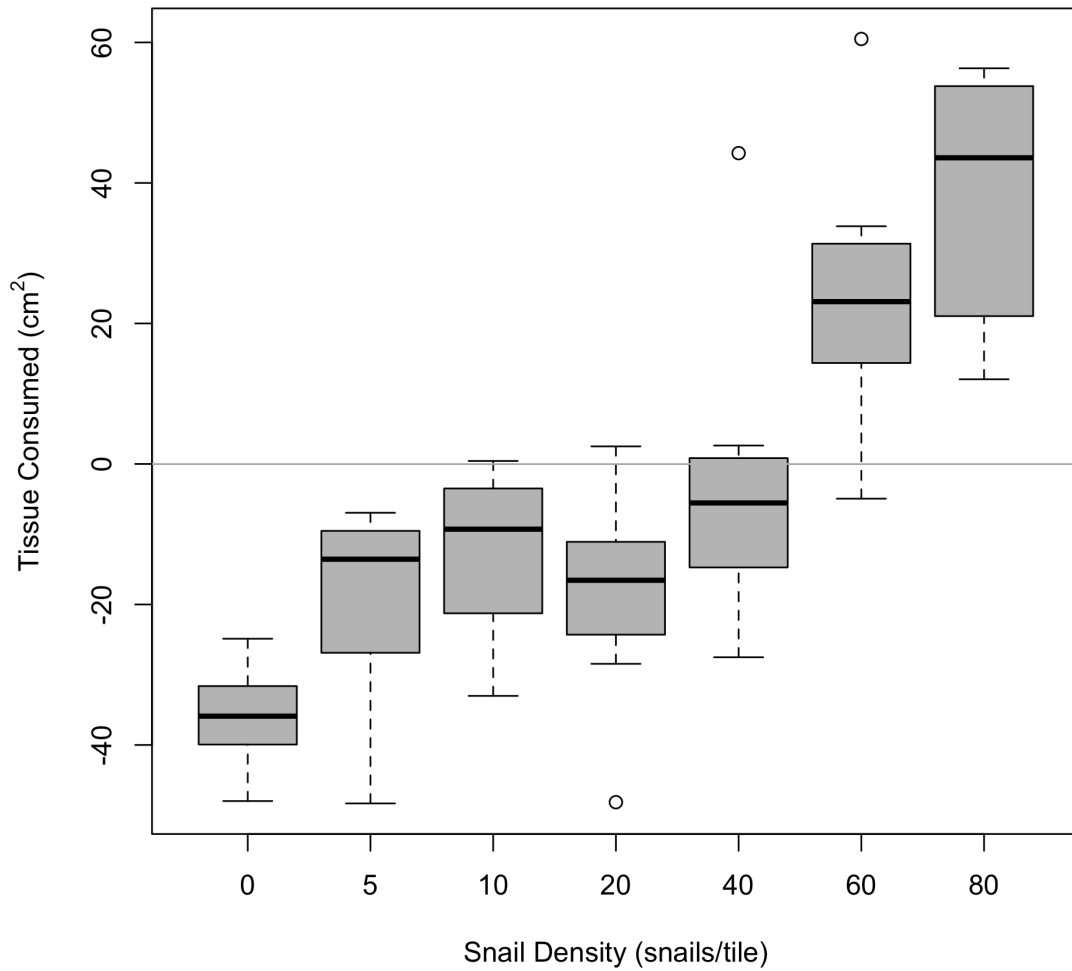


Figure 4. *F. distichus* tissue consumed as a function of snail density. Positive values represent tissue eaten by snails. Tissue consumed was estimated from the proportional difference of the measured final area of *F. distichus* thalli and the estimated final area based on midrib length.

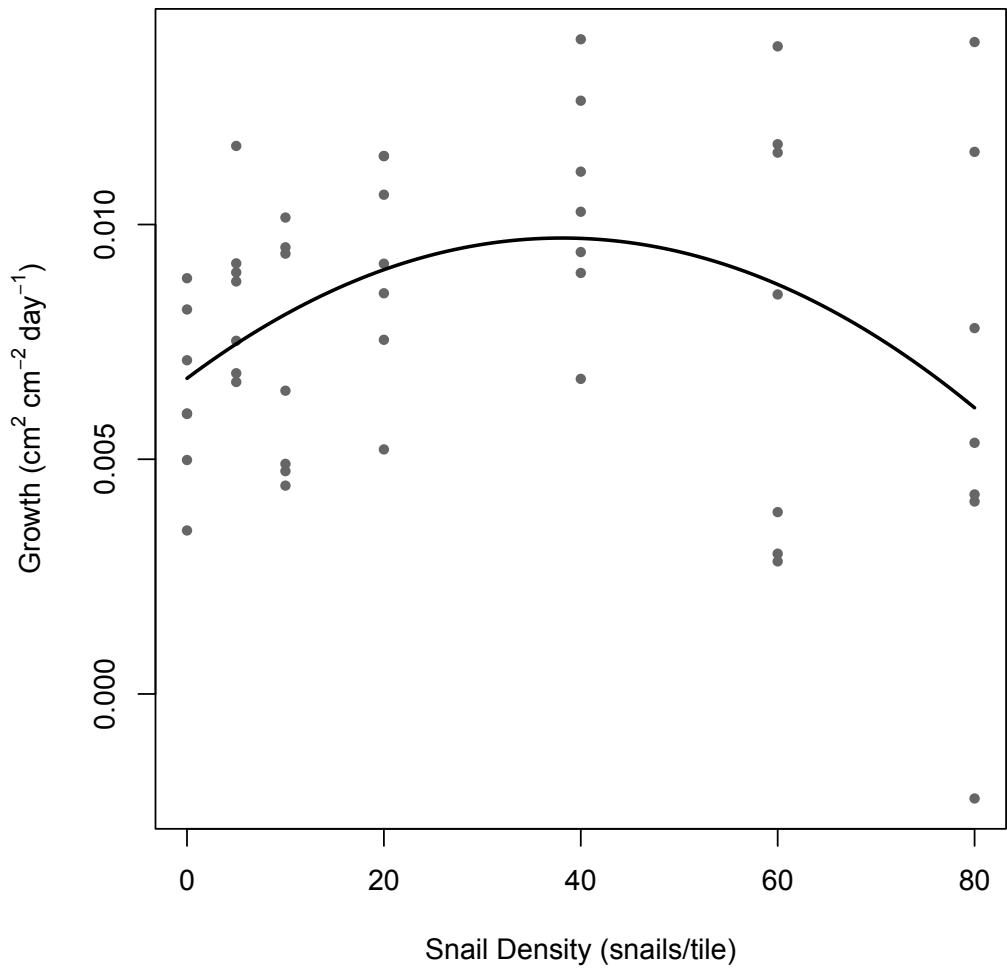


Figure 5. Growth, increase in area per day, relative to initial size of *F. distichus* across snail density. Necrotic tissue was excluded from final area. Each point represents the total surface area of five *F. distichus* thalli on one tile. Line shows best-fit quadratic relationship ($R^2 = 0.093$), which was significantly better than linear.