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Title: Impacts of Ocean Acidification on Coralline Algae: From Species to Community Consequences.

Abstract approved:

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Bruce A. Menge

Oceanic uptake of rising anthropogenic CO_2 emissions has caused the emergence of ocean acidification as a major threat to marine ecosystems worldwide. Along eastern boundary current systems, seawater is naturally acidified due to coastal upwelling of low pH seawater from depth. Compounded by ocean acidification, upwelling regions are expected to become increasingly corrosive to calcifying organisms, potentially forcing them beyond their physiological tolerance windows. In my dissertation, I focused on the impacts of ocean acidification on calcareous coralline algae in the California Current System. Using coralline algae in rocky intertidal habitats as model organisms, I extend the implications of ocean acidification from the organismal level to the broader community level.

Global environmental change implies not only gradual changes in the mean values of environmental variables but also an increase in variability and the likelihood of rare, extreme events. In Chapter 2, I conducted a laboratory experiment to explore

potential interactions between two different types of environmental stressors. Specifically, I tested the effects of elevated pCO_2, including variable pCO_2 treatments, and a severe desiccation event on the coralline species, *Corallina vancouveriensis*. I found that *C. vancouveriensis* growth was negatively impacted by both elevated pCO_2 and desiccation stress, although their combined effects were approximately additive rather than synergistic. Furthermore, while high pCO_2 at constant levels only caused small reductions in algal growth over a two-week period, these effects were exacerbated by pCO_2 variability.

One criticism of laboratory experiments testing species responses to environmental change is that they isolate organisms under simplified conditions. The potential of overlooking important biotic or abiotic factors present in the natural environment limits the inferences that can be made from laboratory studies. In Chapter 3, I conducted a reciprocal removal experiment at two field sites and two wave exposures to investigate potential changes in the interactions between coralline and fleshy turf-forming algae since the 1980s. I used as a baseline the results from a similar study conducted nearly 30 years ago that failed to detect spatial competition between coralline and fleshy algae. Despite the progression of ocean acidification over the last three decades, my results indicated that the lack of competition between coralline and fleshy algae persists to this day, with results consistent across both sites and wave exposures.

The findings in Chapter 3 refer to present-day interactions, but in the future, ocean acidification is expected to be detrimental to coralline algae while potentially benefitting fleshy algae. Both coralline and fleshy algae form turf habitats that shelter

diverse epifaunal communities. Thus, changes in the algal composition of turf habitats may lead to broader changes encompassing epifaunal communities, depending on the degree of specialization displayed in epifaunal habitat associations. In Chapter 4, I compared the abundance, richness, and community composition of epifauna between coralline and fleshy turf habitats at four sites along the Oregon-California coast. I found that epifauna were more abundant in coralline turfs due to higher turf density. However, epifaunal richness and community composition were similar between turf types, indicating high levels of redundancy in habitat provision between coralline and fleshy algae. Since most species of epifauna tended to be turf generalists, they may be resistant to the potential indirect effects of ocean acidification involving declines in coralline turf habitat.

My dissertation combined a variety of standard ecological methodologies to help translate ocean acidification impacts from the organismal level to the community level. Overall, while I found that elevated pCO_2 decreased coralline growth in the laboratory, evidence from the field suggested a capacity for communities to resist the effects of ocean acidification and remain resilient. In the natural environment, ocean acidification impacts may be moderated by multiple environmental variables working in different directions, the temporal dynamics of stressors allowing for periods of recovery, and species interactions having dampening effects. One way forward to unite theories of change with those of resistance is to identify ecosystem indicators and critical thresholds that may help provide a more comprehensive view of ecosystem functioning and stability in the face of global change.

Impacts of Ocean Acidification on Coralline Algae: From Species to Community Consequences

by
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APPROVED:

_____

Major Professor, representing Zoology

_____

Chair of the Department of Integrative Biology

_____

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

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Chenchen Shen, Author

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IMPACTS OF OCEAN ACIDIFICATION ON CORALLINE ALGAE: FROM SPECIES TO COMMUNITY CONSEQUENCES

CHAPTER 1 – GENERAL INTRODUCTION

With the acceleration of anthropogenic climate change, the work of ecologists in identifying patterns in nature and determining the mechanisms driving these patterns has gained a new critical application. However, predicting the effects of climate change on ecological communities is a major challenge. While it can be straightforward to test species responses to constant levels of one environmental factor subject to future change, these responses can be several degrees removed from those in natural ecosystems. First, environmental parameters can vary on multiple temporal scales and may not be well-represented at constant levels (Hofmann et al. 2011, Reum et al. 2014). Second, environmental factors are not isolated from one another and can interact to produce unanticipated consequences (Harley et al. 2006, Boyd and Hutchins 2012). Similarly, species are not isolated from one another and can interact in ways that either dampen or intensify individual responses to environmental change (Tylianakis et al. 2008, Gilman et al. 2010). Finally, effects on one influential species can reverberate through a much larger community to influence biodiversity (Harley 2011). My dissertation aims to advance our understanding of the ecological responses to climate change by addressing each of these elements that may contribute to a more holistic perspective of future community change.

My research is embedded in the dynamics of rocky intertidal ecosystems of the U.S. west coast, a major component of the California Current System (CCS). Rocky intertidal environments have inspired a long and rich history of ecological investigation

and serve as model ecosystems for the study of abiotic-biotic linkages (Connell 1972, Menge and Sutherland 1987, Paine et al. 1994). Steep environmental gradients exist at small spatial scales, with strong zonation patterns corresponding to tidal height and wide-ranging variation in wave intensity along coastal contours. Intertidal organisms are also small and abundant, and many are sessile, facilitating manipulation. Additionally, my field sites are exposed to relatively low levels of anthropogenic influences, i.e. pollution, harvesting, invasive species, and coastal development.

As an eastern boundary upwelling system, however, the CCS is at the leading edge for impacts from ocean acidification (OA) (Feely et al. 2008, Hauri et al. 2009, Gruber et al. 2012). Coastal upwelling creates naturally acidic waters because upwelling-favorable winds help to deliver high pCO_2/low pH water from depth to the surface. CO_2 concentrations are higher at depth due to the respiration of bacteria associated with sinking organic matter. OA will further decrease pH as increasing concentrations of CO_2 in the atmosphere are absorbed into the ocean, raising concerns for coastal organisms that may already be living at the edge of their physiological limits (Hofmann et al. 2011). Acidified waters may become corrosive to calcifying organisms (Orr et al. 2005, Feely et al. 2008) as conditions begin to favor the dissolution of calcium carbonate (Kleypas et al. 1999, Doney et al. 2009). In contrast, CO_2 enrichment increases the availability of an essential resource for photosynthesis, potentially benefitting primary producers (Connell et al. 2013).

While coralline algae calcify and photosynthesize simultaneously, they are considered to be one of the most vulnerable organisms to OA due to the higher solubility

of their high-Mg calcite mineralogy (Andersson et al. 2008, Ragazzola et al. 2013). Coralline algae are important ecosystem engineers worldwide, forming habitats through reef-building activities or turf aggregations, which support high levels of coastal biodiversity (Adey 1998, Liuzzi and Gappa 2008). Macroalgal turfs, however, can be composed of either calcifying coralline algae or non-calcifying fleshy algae. Although OA is expected to negatively impact coralline algae, its effects on fleshy algae will likely be more benign (Hofmann et al. 2012a, Johnson et al. 2014). These contrasting effects of OA on coralline and fleshy algae have the potential to shift the composition of algal communities toward increasing dominance by fleshy algae (Porzio et al. 2011, Brodie et al. 2014). In my dissertation, I focused on the effects of ocean acidification on coralline turfs in rocky intertidal environments along the CCS and extended this theme to include multiple stressors (Chapter 2), competition with fleshy turfs (Chapter 3), and implications for turf-associated epifauna communities (Chapter 4).

In Chapter 2, I tested the effects of both elevated pCO_2 and a severe desiccation event on the coralline species, *Corallina vancouveriensis*. Desiccation during low tides on windy, warm, and/or sunny days can lead to coralline bleaching, the most visible present-day indication of stress in coralline algae (Martone et al. 2010). Stress from both elevated pCO_2 and desiccation is expected to increase with climate change due to stronger winds during the spring-fall upwelling season (Bakun 1990). While the peaks of these two stressors coincide in the same season, their temporal patterns are not perfectly coupled. Covarying with pCO_2, upwelling along the coast of Washington and Oregon to about Cape Blanco occurs intermittently, with alternations between upwelling and

relaxation or downwelling on a scale of 3-10 days (Menge et al. 2004, Menge and Menge 2013). Desiccation stress leading to substantial amounts of bleaching, however, requires a collision of multiple environmental factors. It is an extreme event that occurs semi-regularly but to a limited extent. Oftentimes, the extreme but rare deviations in abiotic factors are the drivers of ecological change rather than continuous but gradual shifts in mean conditions (Thompson et al. 2013). Combining both these elements, I designed a laboratory experiment to investigate the cumulative effects of elevated pCO_2 and desiccation on *C. vancouveriensis*, entailing constant and changing pCO_2 treatments and a severe desiccation event leading to coralline bleaching.

My goal in Chapter 3 was to connect the results from laboratory studies on the effects of elevated pCO_2 on coralline algae with evidence for change in the field. In the natural environment, species may be influenced by multiple abiotic factors directly as well as indirectly, mediated through species interactions. Many researchers have predicted that OA, in addition to having a direct impact on coralline algae, will influence its interactions with fleshy algae by conferring a disadvantage compared to fleshy algae in competition (Hofmann et al. 2012a, Kroeker et al. 2013b). Drawing from a manipulative field experiment conducted in the 1980s that failed to detect competition between coralline and fleshy algae (van Tamelen 1996), I re-examined the potential for algal competition after nearly 30 years of environmental change. If the impacts of OA on algal physiology have started to translate into changes at the community level, fleshy algae may be observed to outcompete coralline algae. I conducted a reciprocal removal experiment on coralline and fleshy algal turfs in the low intertidal zone to determine

whether changes in algal interactions have emerged over the last three decades in a manner consistent with predictions under OA.

In Chapter 4, I extended the implications of ocean acidification on coralline algae beyond the algal community. Both coralline and fleshy turf-forming algae provide habitat to a diverse community of invertebrate epifauna (Davenport et al. 1999, Bracken et al. 2007, Bates 2009). However, habitat associations between algae and epifauna are unclear. Epifauna may be turf generalists or associate more closely with a particular species or type of turf algae. Since OA is expected to negatively impact coralline algae while potentially benefiting fleshy algae (Hofmann et al. 2012a, Johnson et al. 2014), similarity in epifaunal communities between the two turf types might indicate relative insensitivity to habitat shifts resulting from OA. However, epifaunal communities in coralline and fleshy turfs that are dissimilar suggests that these habitats are perceived distinctly, and declines in the abundance of either turf habitat could be accompanied by losses in epifaunal diversity. In order to determine the degree of similarity between epifaunal communities in coralline and fleshy turfs, I sampled both types of algae at four sites located at four capes along the Oregon-California coast and quantified the epifauna collected in each sample.

My dissertation builds upon the general finding that ocean acidification will be detrimental to coralline algae by expanding this theme to include the consideration of multiple stressors, environmental variation, and both negative and positive species interactions. These research extensions extrapolate from single-species and single-factor experiments to larger scales of community organization. By embracing the dynamics of

nature and its interdependencies, I explore the potential pathways that can either operate as catalysts for climate change or buffers against it.

CHAPTER 2 – MULTIPLE STRESSORS AND BEYOND: THE EFFECTS OF ELEVATED PCO_2 AND DESICCATION ON CORALLINE ALGAE

Abstract

Climate change will amplify multiple environmental factors that may interact to affect organisms in ways that are unpredictable based on our knowledge of single-factor impacts. Of greatest concern are synergistic interactions that generate disproportionately large effects compared to those of their independent components combined. While studies evaluating species responses to multiple environmental stressors are common, most employ experimental designs that apply constant levels of each stressor and test the effects of two stressors acting simultaneously. In this study, we examined the effects of elevated pCO_2 on an intertidal coralline alga species, *Corallina vancouveriensis*, punctuated by a bleaching event caused by prolonged emersion and desiccation. Algae were exposed to either constant pCO_2 levels or transferred between two pCO_2 levels to account for the variation in seawater carbon chemistry experienced by organisms in an intermittent upwelling regime. Key results included: (1) growth rates in *C. vancouveriensis* decreased from both elevated pCO_2 and desiccation, (2) the extent of bleaching was only affected by desiccation and not pCO_2, (3) net photosynthesis rates increased from elevated pCO_2 but decreased from bleaching, and (4) net calcification rates were not clearly affected by either stressor. Treatments including pCO_2 change further depressed growth rates compared to constant pCO_2 treatments. The combined effects of elevated pCO_2 and desiccation tended to be additive when both stressors were relevant to the measured response. However, the classification of multiple stressor

interactions as additive, antagonistic, or synergistic is scaled to the effects of individual stressors, thereby obscuring the magnitude of their effects. *C. vancouveriensis* growth rates were more sensitive to desiccation than to high pCO_2 levels, while net photosynthesis rates were more sensitive to high pCO_2 levels than to desiccation.

Introduction

With the acceleration of climate change, marine ecosystems will face a rise in global mean temperatures, ocean acidification, and deoxygenation, among a variety of other associated and unassociated anthropogenic stressors (Harley et al. 2006, Halpern et al. 2007, Gruber 2011, Doney et al. 2012, Somero et al. 2016). Many abiotic factors will deviate from their natural range of variability, and organisms will face a set of new environmental challenges. The potential for interactive effects among multiple stressors contributes considerably to the uncertainty in predicting future ecological change (Sala et al. 2000). Within the last two decades, researchers have recognized the importance of testing the cumulative effects of multiple stressors (Breitburg et al. 1998, Paine et al. 1998, Folt et al. 1999), and among marine climate change experiments conducted from 2000-2009, over one-third manipulated two or more climate variables (Wernberg et al. 2012).

Without studies directly addressing multiple stressors, the assumption is that the combined effects of two environmental factors will be additive, i.e. the sum of their individual effects (Crain et al. 2008). However, synergistic or antagonistic effects that are larger or smaller, respectively, than those expected from an additive model are common.

An additive model underestimates impacts if effects are synergistic and overestimates impacts if effects are antagonistic. Several meta-analyses reviewing multi-stressor studies in marine ecosystems have found that synergisms were the most prevalent type of interaction or that overall interaction effects were synergistic (Crain et al. 2008, Harvey et al. 2013, Ban et al. 2014, Przeslawski et al. 2015). Furthermore, synergisms became more common when more than two stressors were included (Crain et al. 2008). However, interaction type was found to vary within and between stressor pairs, response types, trophic levels, life history stages, habitat types, and experiment duration (Crain et al. 2008, Przeslawski et al. 2015). Even with several meta-analyses and a sizeable number of studies evaluating the effects of multiple stressors, the interaction between two or more stressors in relation to a particular biological response remains difficult to predict. One criticism in this research area is that experimental designs that expose organisms to simultaneous, constant levels of stressors, i.e. most multi-stressor studies, do not represent typical field conditions and are biased toward finding synergistic interactive effects, or the most negative outcome (Gunderson et al. 2016). Thus, while it is difficult to make generalizations about multi-stressor effects, studies should strive to design experiments that reflect more realistic temporal dynamics of stressors relevant to the ecosystem and organisms under study.

Eastern boundary upwelling systems, e.g. the Benguela, California, Canary, and Humboldt Currents (Chavez and Messié 2009), serve as an ideal backdrop for studying species responses to multiple climate change stressors, as environmental covariation abounds (Gruber 2011). Wind-driven coastal upwelling transports deep, nutrient-rich

waters to the surface, giving rise to some of the ocean's most productive regions (Reum et al. 2016). Upwelled water from depth is cold, low in O_2, and high in CO_2 due to vertical stratification and respiration of organic material exported from the surface. Since upwelling regions are naturally high in CO_2 and low in O_2, further acidification and deoxygenation expected with climate change may drive conditions past critical thresholds (Gruber 2011, Reum et al. 2016). Global mean land and ocean surface temperatures are also expected to increase with climate change (IPCC 2013), but paradoxically, this leads to uncertain predictions about changes in sea surface temperature in coastal upwelling systems. Uneven atmospheric heating over continents and oceans is predicted to intensify atmospheric pressure gradients, alongshore winds, and upwelling, which is associated with cooler water (Bakun 1990, Schwing and Mendelssohn 1997, Snyder et al. 2003). These competing forces, i.e., both warming and cooling surface waters in upwelling systems, may be reconciled via increased temperature variability, since upwelling fluctuates at multiple temporal scales (Checkley Jr. and Barth 2009). Perhaps most relevant to experimental manipulation is intra-seasonal variation due to intermittent upwelling, which alternates between upwelling and relaxation or downwelling on a scale of 3-10 days (Menge et al. 2004, Menge and Menge 2013). In the California Current, seasonal upwelling in the spring-fall is characterized by an intermittent pattern along the coasts of Washington and Oregon to about Cape Blanco (Menge et al. 2004).

One of the most sensitive types of organisms to ocean acidification in the California Current System (CCS) are calcareous coralline algae (Hauri et al. 2009, Kroeker et al. 2010). Coralline algae in the CCS can be classified into either crustose

(strictly prostrate) or articulated (upright) growth forms, although articulated species have crustose holdfasts (Padilla 1984). They are abundant and widespread not only in the CCS but also worldwide in coastal waters from tropical to polar regions (Steneck 1986, Nelson 2009). Coralline algae play important ecological roles by providing habitat (Adey 1998, Kelaher and Castilla 2005, Liuzzi and Gappa 2008), acting as a food source (Paine 1980, Steneck 1982, 1983), and inducing propagule settlement and recruitment (Turner 1983, Morse and Morse 1984, Rodriguez et al. 1993, Heyward and Negri 1999, Harrington et al. 2004, Barner et al. 2016). In some areas, coralline algae also contribute significantly to carbon and carbonate budgets (Martin et al. 2007, Basso 2012). Coralline algae precipitate high-Mg calcite, which is the most soluble natural form of calcium carbonate (50% more soluble than calcite and 20% more soluble than aragonite) (Andersson et al. 2008, Smith et al. 2012, Ragazzola et al. 2013). As calcium carbonate saturation states (termed Ω) decrease with the acceleration of OA, net calcification in coralline algae is expected to decrease, with the risk of dissolution (Orr et al. 2005, Roleda et al. 2012a, Koch et al. 2013). Many experiments testing the effects of elevated pCO_2 levels on coralline algae have demonstrated negative impacts (Kroeker et al. 2010, Harley et al. 2012, Koch et al. 2013), including reductions in growth (Jokiel et al. 2008, Hofmann et al. 2012b, Ragazzola et al. 2013), calcification (Gao et al. 1993, Anthony et al. 2008, Büdenbender et al. 2011a, Price et al. 2011, Johnson et al. 2014), photosynthesis (Anthony et al. 2008), and recruitment (Kuffner et al. 2007).

Research investigating the effects of elevated pCO_2 combined with an additional stressor on coralline algae have yielded variable results. Multiple studies have examined

the combined effects of elevated pCO_2 and temperature on coralline algae, with some demonstrating a negative synergistic effect of these two stressors on survival and the proportion of healthy tissue in coralline algae (Anthony et al. 2008, Martin and Gattuso 2009, Diaz-Pulido et al. 2012). However, in all these cases, one stressor was dominant, having a relatively large negative impact, while the other stressor was less influential. In a contrasting study, while both elevated pCO_2 and temperature reduced coralline growth independently, their combined effect matched that of elevated pCO_2 alone, the dominant stressor (Kram et al. 2016). This is best described by the comparative effects model, where a single stressor takes precedence over the other in determining their combined effect, although it also qualifies as an antagonistic stressor interaction (Folt et al. 1999). In another example, elevated pCO_2 and temperature did not interact at all, rather separately influencing different coralline responses (Noisette et al. 2013a). Only pCO_2 affected calcification and photosynthesis, and only temperature affected pigmentation. Studies testing the combined effects of elevated pCO_2 and another stressor, besides warming, on coralline algae have revealed synergistic (+ ultraviolet radiation) (Gao and Zheng 2010), additive (+ nutrient enrichment) (Russell et al. 2009), and antagonistic/comparative effects (+ light exposure) (Russell et al. 2011).

In this study, we designed a laboratory experiment to test the combined effects of elevated pCO_2 and desiccation on coralline algae. Desiccation is the primary cause of bleaching in intertidal coralline species, which occurs when hot/sunny/windy days coincide with daytime low tides (Padilla 1984, Ortlieb et al. 1996, Martone et al. 2010). In contrast, subtidal understory corallines primarily bleach due to increased light intensity

following the disturbance of algal canopies (Figueiredo et al. 2000, Irving et al. 2004). Elevated temperatures (Martin and Gattuso 2009) and ocean acidification (Anthony et al. 2008, Noisette et al. 2013b) have also been associated with coralline bleaching. Bleaching is one of the most striking signs of stress in coralline algae, indicating the degradation or loss of photosynthetic pigments, which results in tissues turning white (Martone et al. 2010, McCoy and Kamenos 2015). Bleaching reduces photosynthetic activity (Irving et al. 2004), inhibits growth (Figueiredo et al. 2000), and may indicate tissue necrosis (Littler 1972, 1973, Martin and Gattuso 2009). If bleaching is limited, bleached portions of algal thalli may flake off to allow for new growth (McCoy and Kamenos 2015). Bleaching may also be reversible, depending on the duration and severity of environmental stress. Bleached crustose coralline algae have been observed to regain color and recover from bleaching following stress amelioration (Littler 1972, Figueiredo et al. 2000). Furthermore, bleached coralline tissue can still maintain low levels of photosynthesis, suggesting the preservation of light-harvesting pigments below the level for visual detection (Irving et al. 2004). Coralline populations can recover from bleaching via restoration of pigments, new growth, or recruitment once stressors abate. However, bleaching events may increase in the future with increasing temperatures, winds, and storms that can remove algal canopies (Harley et al. 2006, Doney et al. 2012). Furthermore, in the CCS, desiccation stress is highest in spring and summer, which coincides with elevated pCO_2 caused by upwelling and increasingly, ocean acidification. Both desiccation and elevated pCO_2 have been linked to coralline bleaching and may be

exacerbated in the future, as atmospheric pressure gradients and winds are expected to intensify with climate change (Bakun 1990).

Despite the sensitivity of coralline algae to both elevated pCO_2 and desiccation stress, we believe that this is the first study evaluating their combined effects. We investigated growth, bleaching, photosynthesis, and calcification in the intertidal coralline species, *Corallina vancouveriensis*, in response to both elevated pCO_2 and desiccation stress. We also assessed whether coralline bleaching is affected by exposure to elevated pCO_2 levels both before and after prolonged emersion (aerial exposure). By varying pre- and post-emersion pCO_2 treatments, we were able to incorporate short-term pCO_2 variability in our experiment to reflect sudden swings in seawater chemistry characteristic of intermittent upwelling. While we predict that both elevated pCO_2 and desiccation will negatively impact coralline algae, with the exception that elevated pCO_2 may enhance photosynthesis, the type of interactive effect (i.e. additive, synergistic, or antagonistic) these two stressors will produce is unpredictable.

Methods

Algae collection

All *Corallina vancouveriensis* samples in the experiment were collected from the low intertidal zone at Fogarty Creek (44.84ºN, 124.06 ºW) located on the central Oregon coast on May 6, 2015. At low tide, algae were chiseled from the rock substrate to which they were attached, retaining crustose holdfasts. Samples were transported in a cooler filled with seawater to Hatfield Marine Science Center (HMSC) in Newport,

Oregon within half an hour. Algae were cleaned of epifauna and epiphytes and placed in a flow-through seawater table under a 12-hour light cycle for two days to adjust to laboratory conditions before the start of the experiment.

Experimental system

At HMSC, an ocean acidification mesocosm system modeled after Fangue et al. (Fangue et al. 2010) enabled controlled additions of CO_2 to incoming seawater. CO_2-free air and pure CO_2 were mixed to create three pCO_2 treatments using three pairs of mass flow controllers (MFCs) managed by Flow Vision software (Alicat Scientific, MFC models MCP-50 SLPM-D/5M and MC-500 SCCM-D/5M). CO_2-free air was produced by scrubbing CO_2 from compressed air using a CO_2 adsorber (Twin Tower Engineering Inc., CAS2-11), and CO_2 cylinders (Airgas®) provided the pure CO_2. Both gas streams were filtered before blending, and the CO_2 concentrations of the resulting gas mixtures were monitored using a CO_2 sensor coupled with Data Acquisition Software (CO2Meter Inc., sensor model K-30 1%). These custom-blended gas mixtures were added to incoming seawater in three reservoir tanks using a system of Venturi injectors (Mazzei Injector Company LLC, ¾" 0584 and 1" 0878) and water pumps (Danner Manufacturing Inc., Supreme 1800 gph and 2400 gph), with airflow controlled by three rotameters (OMEGA Engineering Inc., FL-2021-NV). Each reservoir tank delivered manipulated seawater at a gravity-driven flow rate of approximately 3.5-4 L/min to 8 clear 10 L flow-through chambers contained in a seawater table. Incoming seawater in each seawater table surrounded the lower half of the chambers to regulate temperatures. Above each

seawater table, a light fixture held four 54 W T5 fluorescent light bulbs, which were kept on a 12-hour cycle.

Experimental design

We conducted a 2-week experiment in the ocean acidification (OA) mesocosm system to investigate the combined effects of OA and desiccation stress on *Corallina vancouveriensis*, and in particular, the impact of OA on coralline bleaching events. We tested the effects of ocean acidification on *C. vancouveriensis* before and after a long emersion period causing extreme desiccation stress. Experimental conditions varied by: 1) pre-emersion pCO_2 level (10 days), 2) emersion on Day 10 (4 hours), and 3) post-emersion pCO_2 level (4 days). Pre-emersion and post-emersion time allocations were informed by pilot experiments indicating that bleaching in *C. vancouveriensis* occurs over 3-4 days of gradual pigment loss, which is also supported in the literature for other coralline species (Littler 1973). pCO_2 treatments were set at ambient or low (500 µatm), mid (1000 µatm), and high (1700 µatm) levels. The low and mid pCO_2 treatments lie within the present-day range of pCO_2 variation experienced by organisms along the Oregon coast, with *in situ* pCO_2 levels occasionally rising to 1100 µatm (Evans et al. 2011), while the high pCO_2 treatment represents future acidification. At the beginning of the experiment, 10 *C. vancouveriensis* samples were placed in low pCO_2, 20 were placed in mid pCO_2, and 20 were placed in high pCO_2. Each experimental chamber contained 1-3 algae samples, which were individually labeled by securing color-coded zip-ties to their bases. After 10 days, half the samples from each pCO_2 treatment were removed from the water, blotted to minimize and standardize water retention (larger, bushier thalli retain

more water), and emersed above water for 4 hours to simulate desiccation stress during an extreme low tide. Following emersion, half of the emersed samples originating from the mid and high pCO_2 treatments were returned to their previous treatment water, while the other half were transferred to low pCO_2 water. All samples originating from the low pCO_2 treatment were returned to low pCO_2. During the last 4 days of the experiment, there were 30 *C. vancouveriensis* samples in low pCO_2, 10 in mid pCO_2, and 10 in high pCO_2, with each experimental chamber containing 1-4 algae samples. All treatments and chambers were assigned randomly, and no two algae samples shared the same two experimental chambers over the course of the experiment. In total, there were 5 pCO_2 treatment configurations (low-low, mid-low, mid-mid, high-low, and high-high, abbreviated L-L, M-L, M-M, H-L, and H-H, respectively) crossed with 2 emersion treatments (control, emersed), resulting in 10 different treatment combinations with five replicates each.

Experimental conditions

Experimental conditions were monitored daily with *in situ* sensors, and further analysis was performed on daily seawater samples taken from each reservoir tank. Temperature, salinity, and pH of seawater were measured once daily in each chamber and 3-6 times daily in each reservoir tank using a multiparameter instrument for temperature and salinity (YSI Inc., Pro 2030) and a pH probe (Honeywell, Durafet pH Sensor). Light levels above each chamber were measured daily using a light meter and quantum sensor (LI-COR Inc., LI-250 and LI-190SA). Seawater samples from each reservoir tank were collected daily in acid-washed brown glass bottles and preserved with $HgCl_2$ to allow for

subsequent determination of alkalinity and validation of *in situ* pH measurements. Total alkalinity of seawater in bottle samples was determined via spectrophotometric titration (Yao and Byrne 1998), using a light source and SpectraSuite spectrometer operating software (Ocean Optics Inc., light source model DH-2000-DUV) for absorbance measurements. The accuracy of total alkalinity measurements was verified by certified seawater reference material (Batch 145; http://cdiac.ornl.gov/oceans/Dickson_CRM/batches.html) supplied by Andrew Dickson (Scripps Institution of Oceanography). Reference material was tested between about every 16 bottle samples, and total alkalinity measurements were always within 2 μmol/L of the reference value. Spectrophotometric pH measurements were also conducted on seawater from bottle samples using a SAMI-pH sensor (Sunburst Sensors LLC, Ocean pH Sensor) (Martz et al. 2003). To increase precision, pH measurements for each bottle sample were repeated until two measurements were within 0.005 units of one another. The average of these two values was taken and corrected for temperature differences between the time of collection and the time of measurement using the seacarb package in R (Gattuso et al. 2016). These empirical measurements of pH, alkalinity, and temperature allowed for calculation of other carbonate system parameters, including pCO_2, DIC, and $\Omega_{aragonite}$, using the seacarb package in R (Gattuso et al. 2016). As recommended best practices (Dickson et al. 2007), we used the constants K_1 and K_2 from Lueker et al. (Lueker et al. 2000), K_f from Perez and Fraga (Perez and Fraga 1987), and K_s from Dickson (Dickson 1990).

Algal growth

Prior to the start of the experiment, all *C. vancouveriensis* samples were submersed in a 0.02% solution of a nontoxic fluorescent brightener, Calcofluor white (Sigma-Aldrich, Fluorescent Brightener 28), for about 5 minutes (Martone 2010). Calcofluor white stains the meristems at the tips of algal fronds, leaving a fluorescent mark that persists as new tissue develops and extends past it, enabling the measurement of algal growth rates. At the end of the experiment, six fronds of all *C. vancouveriensis* samples were photographed with a long exposure (15-25 s) under a dissecting scope in ultraviolet light to reveal fluorescent marks. For algae in the emersion treatment, 3 pigmented fronds and 3 bleached fronds were selected. Algal growth was evaluated by measuring the length of newly deposited tissue from the fluorescent stain mark to the tip of each algal frond along its primary growth axis using ImageJ 1.46r image processing software (Rasband 1997). Algal growth was taken as the mean growth length of the six photographed fronds from each sample.

Algal bleaching

To evaluate algal bleaching, all *C. vancouveriensis* samples were photographed at the end of the experiment in a light box on a black background. Algae samples were photographed twice, once on each opposing side, and the total surface area and bleached area on each side were determined using ImageJ 1.46r (Rasband 1997). Photographs were converted to a grayscale with pixels ranging in brightness from 0 (black) to 255 (white). Surface area was automatically traced by setting the minimum brightness threshold to 50, which filtered out the black background and selected only the algae for area

measurements. To measure only the bleached portions of algae, the minimum brightness threshold was raised to 150. The proportion of algal thalli bleached was calculated by dividing the mean bleached area by the mean algal surface area. However, this method overestimated the extent of bleaching, especially in algae that were always submersed, due to reflections of light on algal thalli appearing white. These areas were measured as being bleached, despite obscuring the actual presence or absence of algal coloration. Thus, even algae that were always submersed and did not bleach were recorded with 7-22% bleaching (Figure 2.2B), though this measurement error is likely much lower in emersed algae that had considerable amounts of bleaching.

Algal calcification and photosynthesis

Net calcification and photosynthesis rates in *C. vancouveriensis* were evaluated using the alkalinity anomaly technique (Smith and Key 1975, Chisholm and Gattuso 1991). Net calcification rate can be estimated from changes in total alkalinity (1), and net photosynthesis rate can be estimated from changes in inorganic C not attributable to calcification (2):

$$\frac{\Delta TA/_2 * V}{time/_{60}} \qquad (1)$$

$$\frac{(\Delta DIC - \Delta TA/_2) * V}{time/_{60}} \qquad (2)$$

in which ΔTA is the difference in total alkalinity in μmol/L between the start and end of a trial ($TA_{initial}$-TA_{final}), ΔDIC is the difference in dissolved inorganic carbon in μmol/L between the start and end of a trial ($DIC_{initial}$-DIC_{final}), V is the volume of seawater in liters, and time is the duration of the trial in minutes.

At the end of the experiment, two 45-minute trials were conducted to assay net calcification and photosynthesis rates in all *C. vancouveriensis* samples. In each trial, 25 clear 4 L chambers were placed in a seawater table with incoming seawater surrounding the outside of the chambers to regulate temperatures. Algae samples from the experiment were randomly assigned to a trial and a chamber. Chambers were filled with 2 L of water from the reservoir tank corresponding to the post-emersion pCO_2 treatment of each sample, and water was circulated in each chamber using an aquarium pump (Hydor, Pico 400). To document starting conditions in each trial, we measured the temperature and salinity of seawater from each reservoir tank using a multiparameter instrument (YSI Inc., Pro 2030). In addition, we collected seawater samples from each reservoir tank in acid-washed brown glass bottles before each trial and added $HgCl_2$ to preserve samples for later spectrophotometric assessment of pH and alkalinity. Trials began with the placement of *C. vancouveriensis* into chambers under saturating lights, ~300-1,000 μmol m^{-2} s^{-1} (Tait 2014, Guenther and Martone 2014). At the start and end of each trial, seawater temperature and salinity were measured in each chamber, and light levels above each chamber were measured using a light meter and quantum sensor (LI-COR Inc., LI-250 and LI-190SA). At the end of each trial, *C. vancouveriensis* was removed from the chambers, and chamber water was collected and preserved for later assessment of pH and alkalinity in comparison with starting conditions. Net calcification rates were calculated using direct measurements of changes in alkalinity. Net photosynthesis rates required additional calculations in the seacarb package in R (Gattuso et al. 2016) to derive DIC from pH, alkalinity, and temperature measurements. After all algal responses were

measured, *C. vancouveriensis* samples were dried overnight at 70°C and weighed following removal of attached rock substrate. Dry coralline biomass was used to standardize net calcification and photosynthesis rates.

Data analysis

Analysis of variance (ANOVA) was used to compare experimental conditions and validate differences between the three pCO_2 treatments. Linear regression models were used to analyze algal responses, including growth, bleaching, photosynthesis, and calcification, to emersion and pCO_2 treatments. Algal biomass and light levels during the experiment were also included as explanatory variables in algal response models. Temperature in the experiment was strongly correlated with pCO_2 (r_s = -0.953, $P <$ 0.001) and excluded from models to reduce multicollinearity, since there is less evidence to support the effects of small temperature differences on *C. vancouveriensis* than large pCO_2 differences (Tait 2014, Guenther 2016). In net photosynthesis and calcification models, light and temperature conditions during the assay were included instead of those during the experiment, and both physiological rates were standardized by algal biomass rather than including biomass as a predictor. Bleaching was only assessed in algae that were emersed, since bleaching did not occur otherwise. Data were checked for normality and homogeneity of variance using normal probability plots and plots of residuals against predicted values. Kruskal-Wallis tests were performed in lieu of ANOVAs when data failed to satisfy normality assumptions, even after transformation. Robust regression using M-estimation with Huber weighting was applied to model net photosynthesis in order to reduce the weight of a strong outlier. All analyses were performed in R version

3.2.3 (R Core Team 2015), with robust regression implemented in the MASS package (Venables and Ripley 2002).

Results

Experimental conditions

Target pCO_2 treatments were achieved and successfully maintained over the course of the experiment. As expected, pCO_2, pH, $\Omega_{aragonite}$, and DIC all differed between pCO_2 treatments (Kruskal-Wallis tests, all $\chi^2 = 39.13$, all df = 2, all $P < 0.001$) (Table 2.1, Figure 2.1). Addition of CO_2 to seawater increased pCO_2 and DIC while decreasing pH and $\Omega_{aragonite}$. CO_2 additions were not expected to affect total alkalinity, which remained similar between pCO_2 treatments (ANOVA, $F_{2,42} = 0.269$, $P = 0.766$) (Table 2.1). However, unintentional temperature differences were revealed between pCO_2 treatments (ANOVA, $F_{2,42} = 16.59$, $P < 0.001$), with mean temperatures in the high pCO_2 treatment about 0.6-0.7 °C lower than in the low or mid pCO_2 treatments (Tukey HSD, H-L: $P < 0.001$, H-M: $P < 0.001$) (Table 2.1, Figure 2.1). Although this resulted in a strong correlation between temperature and pCO_2, temperature differences between treatments were relatively small compared to differences in pCO_2. Based on previous studies, temperature differences between about 11 °C and 12 °C would not be expected to have significant or consistent effects on *C. vancouveriensis* growth or productivity (Tait 2014, Guenther 2016).

Treatment effects

The effects of the emersion treatment on *Corallina vancouveriensis* were very strong. On average, emersed corallines bleached 58% (Figure 2.2B), ranging from 39-80%. Emersion reduced algal growth and net photosynthesis rates (Tables 2.2, 2.4). While the mean growth rate of fully pigmented algae was 1.4 mm over 14 days, partially bleached algae in the emersion treatment only grew 0.9 mm (Figure 2.2A). Emersed algae likely stopped growing after the emersion treatment on Day 10 of the experiment, given a mean growth rate of 0.1 mm/day. Emersed algae also tended to have lower net photosynthesis rates, with slightly negative mean photosynthesis rates in partially bleached algae compared to generally positive mean photosynthesis rates in fully pigmented algae (Figure 2.2C). The effect of emersion on net calcification was less pronounced, interacting with pCO_2 treatments (Table 2.5, Figure 2.2D). While emersed algae had lower mean calcification rates than controls across most pCO_2 treatments, particularly ML and HH, emersed algae had higher mean calcification rates than controls in the LL pCO_2 treatment. Thus, there was a lack of consistency in the effect of emersion on net calcification rates in *C. vancouveriensis*, although it is worth noting that in most cases, net calcification rates were neutral to negative (mean ± SE: -3 ± 1 μmol C g^{-1} h^{-1}).

pCO_2 treatments impacted *C. vancouveriensis* growth and net photosynthesis but had less influence on bleaching and net calcification. Neither pre- nor post-emersion pCO_2 levels affected the extent of coralline bleaching (Table 2.3, Figure 2.2B), and the effects of pCO_2 on net calcification rates were difficult to discern, as they depended on emersion in an inconsistent manner (Table 2.5, Figure 2.2D). Impacts to coralline growth

from the 2-week exposure to elevated pCO_2 were modest but significant. Higher pCO_2 reduced coralline growth, particularly in ML, HL, and HH treatments, indicating an effect of both high pCO_2 and pCO_2 variability (Table 2.2, Figure 2.2A). Despite transfer from more stressful to less stressful pCO_2 conditions, mean coralline growth was lower in ML and HL treatments than in MM and HH treatments, respectively. Growth reductions in the ML treatment were similar to those in the HH treatment, suggesting that near-future ocean acidification (OA), when accounting for pCO_2 variability, can have as much impact as far-future OA at constant pCO_2. Although elevated pCO_2 reduced coralline growth, at high pCO_2 levels, net photosynthesis rates increased substantially (Table 2.4, Figure 2.2C). Assays were conducted in seawater corresponding to post-emersion pCO_2 levels, providing a snapshot view of physiological performance at the end of the experiment. Assay results revealed that net photosynthesis rates were only influenced by post-emersion pCO_2 treatments, with no apparent carryover effects from pre-emersion treatments.

Besides the treatment effects of emersion and pCO_2, additional peripheral variables were measured and accounted for in our models, including algae biomass, light and temperature. Of these, only algae biomass had a significant effect on any response variable. Algae biomass affected the degree of coralline bleaching, with larger thalli bleaching less (Table 2.3). Algae were blotted at the beginning of the 4-hour emersion to standardize water retention between samples of varying biomass, but larger algal thalli still have less surface area exposed to air, providing some resistance against desiccation. Algae biomass did not affect growth rates (Table 2.2), since growth was measured on

individual fronds, and net photosynthesis and calcification rates were already standardized by biomass.

Discussion

In our study, elevated pCO_2 and emersion treatments both reduced *Corallina vancouveriensis* growth, although their independent effects on other coralline responses were less coordinated. As predicted, desiccation stress caused by prolonged emersion of *C. vancouveriensis* led to substantial bleaching, reduced net photosynthesis rates, and hindered growth. The effects of elevated pCO_2 were less severe than those of desiccation, with no influence on bleaching, either pre- or post-emersion, and positive effects on net photosynthesis rates. However, elevated pCO_2 reduced growth, especially in corallines that received different pCO_2 treatments pre- and post-emersion, despite the change involving transfer from mid and high pCO_2 to less stressful low pCO_2 conditions. Surprisingly, there were no clear effects of elevated pCO_2 or desiccation on net calcification rates in *C. vancouveriensis*. Since the independent effects of elevated pCO_2 and desiccation varied between coralline responses, the determination of their combined effects was difficult to generalize.

With ocean acidification, $[CO_2]$ and $[HCO_3^-]$ both increase while $[CO_3^{2-}]$ decreases (Fabry et al. 2008, Doney et al. 2009). Coralline algae use HCO_3^- for both photosynthesis and calcification (McCoy and Kamenos 2015). In our experiment, elevated pCO_2 increased net photosynthesis rates in *C. vancouveriensis*, suggesting that DIC limitation of photosynthesis at ambient conditions was at least partially alleviated at

higher pCO_2. Elevated pCO_2 may also increase the energetic efficiency of photosynthesis in coralline algae, as they can switch to increasing diffusive use of CO_2 for photosynthesis when it is available, which is less energetically costly than using HCO_3^- (Cornwall et al. 2012a). In contrast to net photosynthesis rates, net calcification rates in *C. vancouveriensis* seemed unaffected by elevated pCO_2 in our experiment. While we anticipated a decline in net calcification rates with increasing pCO_2 due to lower calcium carbonate saturation states (Ω), net calcification accounts for both calcification and dissolution. In our mid and high pCO_2 treatments, $\Omega_{aragonite} < 1$, which indicates conditions favorable for dissolution (Feely et al. 2008, Doney et al. 2009), especially for high-Mg calcite in coralline algae. However, *C. vancouveriensis* in elevated pCO_2 treatments may be able to regulate carbonate chemistry microenvironments at the site of calcification, particularly as higher photosynthesis rates increased energy availability (Ries et al. 2009). This explanation is consistent with the observation that although photosynthesis increased at high pCO_2, growth rates declined, suggesting that the extra energy was used for tissue maintenance rather than for growth. Increased energy availability has been observed to offset the negative effects of elevated pCO_2 on calcification in mussels (Thomsen et al. 2013, Kroeker et al. 2016). Even when energy budgets are constant, energetic trade-offs can allow calcification to increase under elevated pCO_2 (Wood et al. 2008). Thus, it is likely that increased photosynthesis in *C. vancouveriensis* enabled calcification rates to keep pace with dissolution rates under high pCO_2 conditions. Interestingly, while net calcification rates varied around 0 in most treatment combinations, *C. vancouveriensis* still managed to grow in all treatments,

perhaps increasing their ratio of non-calcareous:calcareous biomass. A shift toward negative net calcification was only observed in emersed coralline samples assayed in high pCO_2 water, suggesting that the maintenance of favorable microenvironments for calcification was compromised in bleached tissues.

Growth and bleaching were more integrative measures of coralline performance over the course of the experiment than either net photosynthesis or calcification rates, which were measured in the assays only at the end of the experiment. Elevated pCO_2 reduced *C. vancouveriensis* growth rates, corroborating other studies, but the magnitude of the response was only moderate. One recent study found that *C. vancouveriensis* from naturally variable and high pCO_2 environments, such as upwelling regions, while negatively impacted by elevated pCO_2, were more resistant to its effects than corallines from more stable and lower pCO_2 environments (Padilla-Gamiño et al. 2016). *C. vancouveriensis* in our experiment, sampled from an upwelling region, only experienced moderate growth reductions in elevated pCO_2, but variable pCO_2 further reduced growth, similar to findings in other coralline species (Cornwall et al. 2013, Roleda et al. 2015). Recent experiments have demonstrated that diurnal and semidiurnal changes in pCO_2 negatively affect *C. vancouveriensis* (A. Hettinger, unpublished data). However, in our experiment, we showed that even less frequent changes in pCO_2, once over a two-week period, significantly reduced *C. vancouveriensis* growth. Furthermore, even when samples were transferred from more stressful mid and high pCO_2 levels to less stressful low pCO_2 levels, growth was reduced compared to samples in constant mid and high pCO_2, suggesting an energetic cost to acclimating to variable conditions. Nevertheless,

elevated and variable pCO_2 were not severe enough to cause coralline bleaching in our experiment. While coralline algae have bleached in response to high pCO_2 in other studies (Anthony et al. 2008, Noisette et al. 2013b), *C. vancouveriensis* in our study appeared to be less sensitive to pCO_2 stress, perhaps reflecting adaptation to coastal upwelling. Emersed samples were the only ones to bleach, and neither pre- nor post-emersion exposure to high pCO_2 exacerbated bleaching. High pCO_2 before emersion did not increase susceptibility to bleaching, and high pCO_2 after emersion did not increase subsequent pigment loss over the last 3-4 days of the experiment.

Desiccation in coralline algae increases oxidative stress, which causes intercellular damage and is directly linked to bleaching (Martone et al. 2010). Additionally, as cells shrink from water loss, plasma membranes may pull away from surrounding calcified cell walls and tear. The emersion treatment caused substantial bleaching in *C. vancouveriensis*, as 4-hour aerial exposure induced severe desiccation stress. Although *C. vancouveriensis* is known as one of the most desiccation-resistant intertidal coralline species due to its bushy structure that traps water during emersion (Padilla 1984), samples in our experiment were blotted prior to emersion to minimize and standardize water retention between samples varying in biomass. Still, larger samples bleached less, likely because they have less surface area exposed to air relative to their volume. Since bleaching began at the tips of coralline fronds where apical meristems are situated, it is unsurprising that bleaching seemed to have halted algal growth in our experiment, at least in the short-term. We also expected that bleached apical meristems would hinder calcification, but net calcification rates varied around 0 in both emersed and

control coralline samples. Only in high pCO_2 water did emersed *C. vancouveriensis* gravitate more strongly toward dissolution, while those in the control treatment maintained similar net calcification rates as in other pCO_2 conditions. Presumably, bleached corallines have a reduced capacity to increase calcification rates in response to increasing dissolution rates. With fewer algal pigments, bleached *C. vancouveriensis* samples demonstrated negative net photosynthesis rates in low and mid pCO_2 water (mean ± SE: -7 ± 5 μmol C g^{-1} h^{-1}), although in high pCO_2, positive net photosynthesis rates were maintained (mean ± SE: 29 ± 2 μmol C g^{-1} h^{-1}). Studies have shown that bleached coralline tissue is not necessarily dead and can still photosynthesize at low levels (Irving et al. 2004, 2005).

In our experiment, the effects of elevated pCO_2 and desiccation on *C. vancouveriensis* were assessed both independently and in combination. However, there were no combined effects of elevated pCO_2 and desiccation on net calcification or bleaching in *C. vancouveriensis* because neither stressor had a clear effect on net calcification and only desiccation affected bleaching. Combined effects are only relevant when both stressors have an impact, as they did for net photosynthesis and growth. The interaction between elevated pCO_2 and desiccation on net photosynthesis in *C. vancouveriensis* may be classified as additive. While desiccation negatively affected net photosynthesis, the magnitude of its effects was comparable between low and high pCO_2 levels. Although, elevated pCO_2 increased net photosynthesis rather than decreasing it, and thus may be better described as an “enhancer” rather than a “stressor” (Boyd and Hutchins 2012). Since elevated pCO_2 and desiccation affected net photosynthesis in

opposite ways, designating their interactive effects as additive is somewhat unclear (Piggott et al. 2015). For *C. vancouveriensis* growth, the combined effects of elevated pCO_2 and desiccation stress could be described under either the additive or comparative models. Both interpretations are reasonable since the effects of elevated pCO_2 on growth were small enough to allow for some ambiguity. Desiccation was the dominant stressor, and emersed *C. vancouveriensis* had similar growth rates in all pCO_2 treatments, indicating a comparative effect where desiccation takes precedence. However, mean growth rates in emersed samples were slightly lower in the most stressful pCO_2 treatment than in the least stressful pCO_2 treatment, suggesting an additive effect of the two stressors, since the influence of pCO_2 on control samples was also modest. To reconcile these views, the comparative model may better explain interactions between elevated pCO_2 and desiccation on *C. vancouveriensis* growth when bleaching is frequent enough to swamp the effects of pCO_2, while the additive model may better explain their interaction when bleaching is infrequent and the effects of pCO_2 have time to compound.

When elevated pCO_2 and desiccation both affected the response of *C. vancouveriensis*, the effects tended to be additive rather than synergistic or antagonistic in our experiment. This result is consistent with meta-analyses revealing that for autotrophs and macroalgae, multiple stressors are more commonly found to interact additively or antagonistically rather than synergistically (Crain et al. 2008, Strain et al. 2014). While elevated pCO_2 may prompt coralline algae to expend additional energy to increase calcification, it also provided additional energy via increased photosynthesis, leading to relatively minor reductions in growth rate. Severe bleaching resulted from

desiccation, which subsequently reduced photosynthesis and growth rates, but our results suggest that coralline bleaching will not be amplified by ocean acidification, hence the additive effects. However, it should be noted that in our experiment, samples were only exposed to pCO_2 treatments for two weeks. In a 10-month experiment, researchers found that the negative effect of elevated pCO_2 on coralline growth rates had increased over time (Ragazzola et al. 2013). Although the short duration of our experiment demonstrated that pCO_2 effects can impact the performance of *C. vancouveriensis* relatively quickly, had we made observations over a longer period of time, our results may have shown larger reductions in *C. vancouveriensis* growth from exposure to elevated pCO_2.

While individual stressors can vary in their temporal expression, so too can the interaction between multiple stressors. However, most multi-stressor experiments (80%) expose organisms to constant levels of each stressor, and the overwhelming majority of experiments (96%) expose organisms to stressors simultaneously (Gunderson et al. 2016). In our study, a severe desiccation event was sandwiched between pCO_2 treatments that either remained constant or varied before and after desiccation. Thus, we included variable levels of one stressor, pCO_2, and sequential application of stressors, since pCO_2 stress occurs at high tide while desiccation stress occurs at low tide. Many more temporal configurations of single and combined stressor levels are possible and worthwhile to explore. By fine-tuning our understanding of the temporal dynamics of multiple environmental stressors and their interactions, we will improve our ability to assess the limits of ecosystem resistance and resilience, the potential for recovery, and critical thresholds that threaten stability.

There is concern that interactions between multiple environmental stressors will have synergistic effects on organisms, thereby amplifying the negative effects of climate change and other anthropogenic stressors (Harley et al. 2006, Darling and Côté 2008). While synergisms are common, and perhaps increase in prevalence with the addition of more than two simultaneous stressors (Crain et al. 2008), they are not always the predominant outcome of environmental interactions (Darling and Côté 2008, Strain et al. 2014). Furthermore, there may be a bias toward experimental designs that favor synergistic interactive effects (Gunderson et al. 2016). We also highlight the possibility of non-interacting stressors that could contribute to publication bias. Interaction type varies by trophic level and response level, with results trending toward antagonistic in autotrophs and community level responses, while stressors affecting heterotrophs and population level responses are more synergistic (Crain et al. 2008). Energy acquisition via photosynthesis may help buffer against compounding stressors, and species interactions within communities may dilute the effects of multiple stressors on particular organisms. In an effort to predict the nature of stressor interactions, researchers have called for a better understanding of the mechanisms driving the responses to single stressors and how combinations of these pathways may align or otherwise interact both physiologically and temporally (Crain et al. 2008, Gunderson et al. 2016).

Here, we would like to offer another suggestion, which is to take into consideration the direction and magnitude of effects from single stressors in cumulative impact assessment. For example, if the effects of two environmental variables are both negative and similarly large, they may be less likely to produce a synergism, which

would require an extreme response to the combined stressors. If on the other hand, the negative effect of one stressor is dominant while the other stressor has a relatively minor negative effect, their combined impact may be more likely to be expressed as a synergism because of the lower requirements, only having to surpass the effects of the dominant stressor by a small amount. This hypothesis is speculative but warrants additional review. Furthermore, it hints at the notion that synergistic cumulative effects in one stressor pair are not inherently worse than additive or antagonistic cumulative effects in another stressor pair, depending on the overall impact. These classifications of stressor interactions are scaled by the magnitude of each individual stressor involved, shifting emphasis away from inherent effect sizes. Future research on the combined effects of multiple environmental stressors should emphasize the magnitude of individual and total effects in addition to classifying interaction types.

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Figures

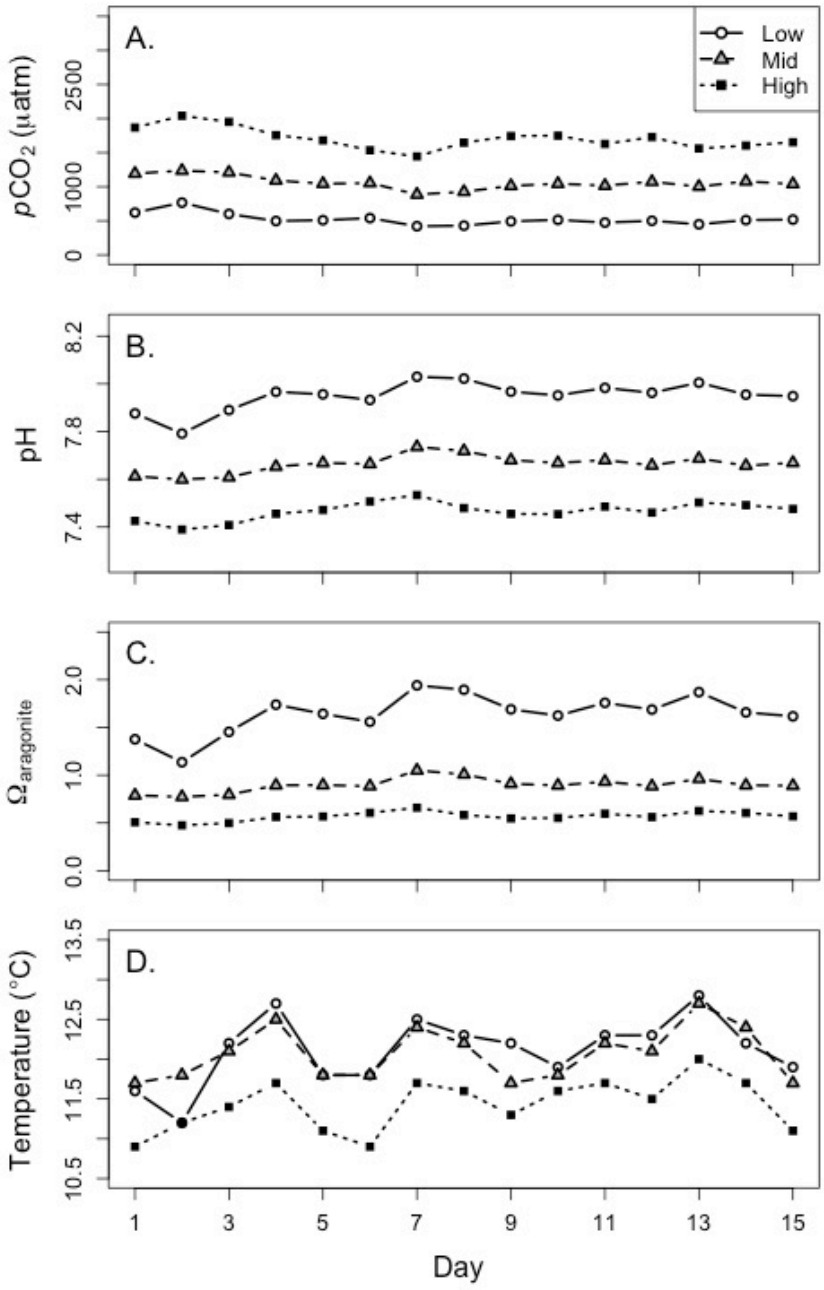

Figure 2.1. Seawater parameters in the pCO_2 treatment reservoir tanks over the course of the 2-week experiment, including A) pCO_2, B) pH, C) $\Omega_{aragonite}$, and D) temperature.

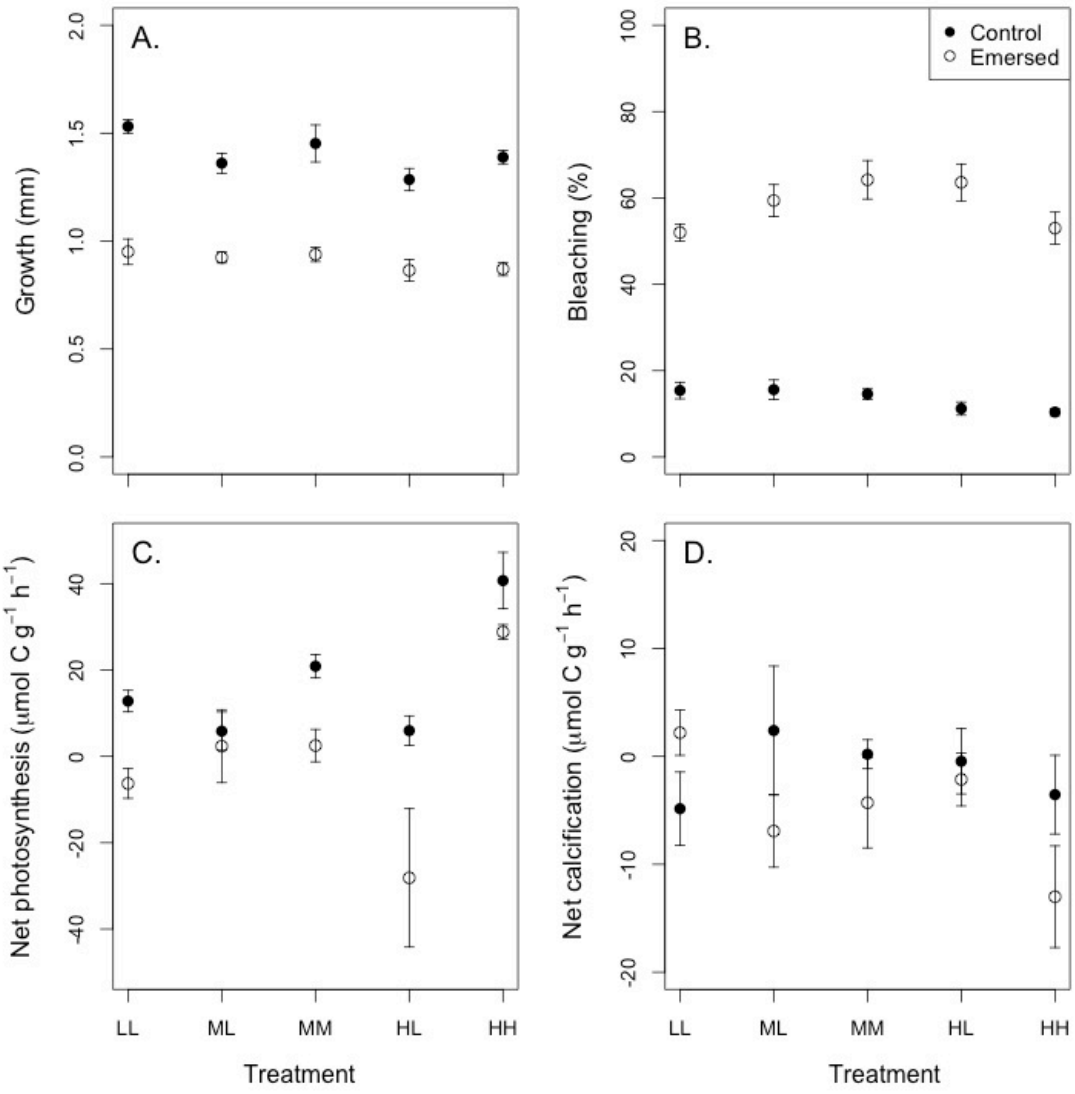

Figure 2.2. *C. vancouveriensis* responses across pCO_2 and emersion treatment combinations, including A) growth, B) bleaching, C) net photosynthesis rate, and D) net calcification rate. Growth and bleaching were assessed after the 2-week experiment. Net photosynthesis and calcification rates were assessed in physiological assays conducted immediately following the 2-week experiment.

Tables

Table 2.1. Carbonate system parameters (mean ± SE) for each pCO_2 treatment in the experiment.

Treatment	Measured parameters			Calculated parameters		
	pH	**TA (µmol/L)**	**Temp (°C)**	**pCO_2 (µatm)**	**$\Omega_{aragonite}$**	**DIC (µmol/L)**
Low	7.95 ± 0.02	2245 ± 1	12.11 ± 0.11	524 ± 22	1.64 ± 0.05	2112 ± 6
Mid	7.66 ± 0.01	2244 ± 1	12.06 ± 0.09	1062 ± 25	0.90 ± 0.02	2204 ± 3
High	7.47 ± 0.01	2243 ± 1	11.43 ± 0.09	1707 ± 41	0.57 ± 0.01	2263 ± 4

Table 2.2. Linear regression model coefficients and standard errors for the effects of pCO_2, emersion, algae biomass, and light on *C. vancouveriensis* growth (mm). The reference group is LL pCO_2 with no emersion. Model adjusted $R^2 = 0.84$. Significant *P*-values are shown in boldface.

	Estimate	**Std. Error**	***t* value**	***P* value**
Intercept	1.635	0.153	10.663	**< 0.001**
pCO_2 = ML	-0.155	0.073	-2.134	**0.039**
pCO_2 = MM	-0.067	0.071	-0.94	0.353
pCO_2 = HL	-0.245	0.069	-3.561	**0.001**
pCO_2 = HH	-0.144	0.069	-2.095	**0.043**
Emersion	-0.575	0.069	-8.315	**< 0.001**
Algae biomass	-0.005	0.047	-0.109	0.914
Chamber light	-0.001	0.001	-0.825	0.414
pCO_2 = ML × emersion	0.142	0.097	1.457	0.153
pCO_2 = MM × emersion	0.058	0.098	0.594	0.556
pCO_2 = HL × emersion	0.153	0.101	1.515	0.138
pCO_2 = HH × emersion	0.064	0.097	0.662	0.512

Table 2.3. Linear regression model coefficients and standard errors for the effects of pCO_2, algae biomass, and light on *C. vancouveriensis* bleaching (%) in the emersion treatment. The reference group is LL pCO_2. Model adjusted $R^2 = 0.36$. Significant *P*-values are shown in boldface.

	Estimate	**Std. Error**	***t* value**	***P* value**
Intercept	0.595	0.134	4.427	**< 0.001**
pCO_2 = ML	0.035	0.052	0.677	0.507
pCO_2 = MM	0.076	0.050	1.513	0.148
pCO_2 = HL	0.057	0.052	1.091	0.290
pCO_2 = HH	-0.003	0.047	-0.068	0.947
Algae biomass	-0.137	0.051	-2.710	**0.014**
Chamber light	0.001	0.001	0.726	0.477

Table 2.4. Robust regression linear model coefficients and standard errors for the effects of pCO_2, emersion, light, and temperature on net photosynthesis rates (μmol C $h^{-1}g^{-1}$) in *C. vancouveriensis*. The reference group is LL pCO_2 with no emersion. Significant *P*-values are shown in boldface.

	Estimate	**Std. Error**	***t* value**	***P* value**
Intercept	-73.132	84.045	-0.870	0.390
pCO_2 = ML	-7.184	5.903	-1.217	0.231
pCO_2 = MM	9.418	6.143	1.533	0.134
pCO_2 = HL	-7.223	5.816	-1.242	0.222
pCO_2 = HH	27.662	5.909	4.681	**< 0.001**
Emersion	-20.607	5.946	-3.466	**0.001**
Assay light	0.007	0.009	0.791	0.434
Assay temperature	6.071	6.089	0.997	0.325
pCO_2 = ML × emersion	11.732	8.214	1.428	0.161
pCO_2 = MM × emersion	0.198	8.195	0.024	0.981
pCO_2 = HL × emersion	0.361	8.446	0.043	0.966
pCO_2 = HH × emersion	9.533	8.345	1.142	0.260

Table 2.5. Linear regression model coefficients and standard errors for the effects of pCO_2, emersion, light, and temperature on net calcification rates (μmol C $h^{-1}g^{-1}$) in *C. vancouveriensis*. The reference group is LL pCO_2 with no emersion. Model adjusted R^2 = 0.13. Significant *P*-values are shown in boldface.

	Estimate	**Std. Error**	***t* value**	***P* value**
Intercept	59.536	73.928	0.805	0.426
pCO_2 = ML	5.434	5.192	1.047	0.302
pCO_2 = MM	2.237	5.403	0.414	0.681
pCO_2 = HL	3.715	5.116	0.726	0.472
pCO_2 = HH	-0.553	5.198	-0.106	0.916
Emersion	7.363	5.230	1.408	0.167
Assay light	0.008	0.008	1.021	0.314
Assay temperature	-5.157	5.356	-0.963	0.342
pCO_2 = ML × emersion	-15.487	7.225	-2.143	**0.039**
pCO_2 = MM × emersion	-11.147	7.208	-1.546	0.130
pCO_2 = HL × emersion	-9.226	7.429	-1.242	0.222
pCO_2 = HH × emersion	-16.841	7.341	-2.294	**0.027**

CHAPTER 3 – RE-EXAMINING COMPETITION BETWEEN CORALLINE AND FLESHY ALGAE AFTER THREE DECADES OF ENVIRONMENTAL CHANGE

Abstract

Ocean acidification is expected to have detrimental effects on calcifying algae, while effects on non-calcifying macrophytes are expected to be neutral or positive. These asymmetrical impacts set the stage for regime shifts within the algal community, but few studies have explicitly investigated changes in algal interactions as ocean acidification intensifies. Following up on studies conducted in the 1980s that demonstrated a lack of competition between coralline and fleshy turf algae, we re-examined this interaction after three decades of environmental change. Due to the acceleration of ocean acidification, we tested the hypothesis that fleshy algae have gained a competitive edge against coralline algae since the 1980s. However, our results indicated that coralline and fleshy turf algae have not yet started to compete. Thus, current levels of ocean acidification have not altered coralline-fleshy interactions in our system. Although the possibility for future change remains, we also acknowledge that the signal of ocean acidification may be obscured by high pH variation in our system and that other environmental influences may counterbalance those of ocean acidification. Consideration for the broader environmental context can improve our understanding of the impacts of ocean acidification on biological communities.

Introduction

As anthropogenic CO_2 emissions increase, about 30% has been taken up by the ocean (Feely et al. 2004, Sabine et al. 2004). The addition of CO_2 to seawater lowers pH and increases acidity, leading to ocean acidification (OA). This historically rapid change in global ocean chemistry has serious ramifications for marine ecosystems. The majority of research on the biological implications of OA utilizes controlled laboratory experiments to compare physiological differences in a single species exposed to ambient and simulated future OA conditions (Wahl et al. 2015). While these experiments are a critical first step to understanding how marine ecosystems will respond to OA, it is difficult to extrapolate results from laboratory to field settings. Conducting climate change research in field settings is better able to account for species interactions and how these might change under spatiotemporal variation in multiple environmental factors, thereby advancing our ability to predict ecosystem change in response to anthropogenic impacts.

Investigating climate change responses in a natural context can be achieved through space-for-time substitutions, e.g. at rare CO_2 vent sites, or retrospective approaches. In this study, we take advantage of previous research examining a species interaction involving one of the most sensitive organisms to OA, coralline algae. In 1986-1989, van Tamelen (1996) investigated algal succession along the Oregon coast in artificially constructed tidepools, including a treatment in which new recruits of both articulated and crustose coralline algae were removed. While the removal of coralline algae did not affect the abundance of erect fleshy algae, crustose fleshy algae increased,

suggesting that corallines pre-empt space from fleshy crusts but not erect fleshy algae. An earlier study conducted in San Diego from 1983-1986 is consistent with these results, as the removal of the dominant coralline species did not affect the abundance of fleshy algae, although transient populations of short-lived algae sometimes occupied the cleared space (Stewart 1989). Using these results as a point of reference, we re-examined competition between coralline and fleshy algae after three decades of environmental change.

Climate change, including ocean acidification, is accelerating in the California Current System (CCS) (Bakun 1990, Snyder et al. 2003, Chan et al. 2008, Feely et al. 2008, Hauri et al. 2009, 2013, Gruber et al. 2012). Since the late 1980s, pCO_2 in seawater has risen about 50 µatm in the North Pacific subtropical gyre (Doney et al. 2009), although along the Oregon coast, pCO_2 is highly variable, currently ranging from < 200 µatm to > 1000 µatm in the spring-fall upwelling season (Evans et al. 2011). Still, the addition of anthropogenic CO_2 has caused upwelled waters reaching shallow coastal ecosystems in some parts of the CCS to become corrosive to aragonite, a form of calcium carbonate found in the shells of mollusks (Feely et al. 2008).

Coralline algae precipitate high Mg-calcite, the most soluble natural form of calcium carbonate, making this form more susceptible to dissolution than either aragonite or low Mg-calcite (Andersson et al. 2008). Numerous studies have demonstrated that coralline algae, both crustose and articulated forms, are highly vulnerable to OA (Gao et al. 1993, Anthony et al. 2008, Jokiel et al. 2008, Kuffner et al. 2008, Martin and Gattuso 2009, Kroeker et al. 2010, 2013a, Büdenbender et al. 2011b, Diaz-Pulido et al. 2012,

Hofmann et al. 2012b, Ragazzola et al. 2012, Bradassi et al. 2013, Kamenos et al. 2013, Johnson et al. 2014, McCoy and Ragazzola 2014, Tait 2014, McCoy and Kamenos 2015). In contrast, non-calcareous fleshy algae are less sensitive to OA and actually may receive a boost in productivity through CO_2 enrichment, depending on their carbon capture strategy (Kübler et al. 1999, Wootton et al. 2008, Hepburn et al. 2011, Cornwall et al. 2012a, Roleda et al. 2012b, Connell et al. 2013, Johnson et al. 2014). These divergent responses to OA could threaten the competitive balance between coralline and fleshy algae (Porzio et al. 2011, Koch et al. 2013, Kroeker et al. 2013b, Brodie et al. 2014).

Few studies, however, have explicitly tested changes in competition between coralline and fleshy algae resulting from OA. Results from two studies confirmed that when grown together under elevated pCO_2, percent cover of coralline algae decreased while fleshy algae increased (Russell et al. 2009, Hofmann et al. 2012a). However, these results were not compared to changes in percent cover when each species was grown separately from the other. Explicitly including ecological interactions in experimental designs can lead to novel findings that are unexpected compared to species' individual responses to climate change. For example, the addition of fleshy filamentous algae sometimes increased calcification in crustose coralline algae under both low and high pCO_2 treatments (Short et al. 2014). Fleshy algae may ameliorate local conditions for coralline algae via CO_2 removal through photosynthesis. These results were variable over time in the 10-week experiment but demonstrate the potential for the continuation of positive interactions between coralline and fleshy algae in spite of OA. In another example, while coralline algae recruited and grew at similar rates in ambient and low pH

conditions at a CO_2 vent site, fleshy algae started to overgrow coralline algae in low pH zones after 4-6 weeks, interfering with corallines' surprising tolerance to low pH (Kroeker et al. 2013b). These results suggest that the outcome of single-species responses to simulated climate change may have little bearing on their responses when occurring in a natural setting, interacting with other species.

In a recent study using historical comparison, McCoy and Pfister (McCoy and Pfister 2014) re-examined competition within a guild of crustose coralline algae following original experiments conducted by Paine (Paine 1980, 1984). In the 30 years between experimental iterations, competitive hierarchies among different species of crustose coralline algae had changed, likely due to the intensification of ocean acidification at their study site on Tatoosh Island in Washington State (Wootton et al. 2008, Wootton and Pfister 2012). Additionally, observations of changes in species abundance in permanently marked plots on Tatoosh Island documented more transitions from calcareous to non-calcareous species over 8 years' gradual decline in mean pH (Wootton et al. 2008).

In this study, we explored whether competition has developed between coralline and fleshy algae in the last 30 years. The current body of research suggests that fleshy algae will outcompete coralline algae as pCO_2 rises, eventually prompting a regime shift (Koch et al. 2013, Kroeker et al. 2013b, Brodie et al. 2014). Early signs of this pattern may first emerge in areas more stressful for intertidal corallines, such as wave-protected areas and locations higher on the shore. Coralline algae are sensitive to desiccation stress (Padilla 1984, Martone et al. 2010), and both wave-protected areas and higher tide

heights receive less wave splash at low tide. By employing a historical comparison to examine changes to species interactions, we were able to take into account natural rates of environmental change over a relatively long time frame, as well as the full suite of *in situ* environmental factors that vary at multiple scales. Limitations include the lack of control and precision, such as regulating the levels of an environmental factor and attributing results to a particular cause. Nevertheless, we focused our attention on OA because of its recent acceleration and demonstrated impacts on coralline algae. We examined our results against the prediction that fleshy algae have gained a competitive advantage over coralline algae since the 1980s and discuss how studies conducted in a natural context can improve, and complicate, our understanding of community-level responses to climate change.

Methods

Study sites

We conducted the study at two sites about 60 km apart along the central Oregon coast: Boiler Bay (44.83ºN, 124.06 ºW; hereafter BB) and Strawberry Hill (44.25ºN, 124.11 ºW; hereafter SH) (Figure 3.1). One important difference between these two sites is their continental shelf bathymetry. The continental shelf at SH is much wider than at BB, contributing to greater retention of larvae and phytoplankton at SH (Menge et al. 1997a, 1997b, 2015, Kavanaugh et al. 2009, Moulton and Hacker 2011). Consequently, sessile invertebrate filter feeders, e.g., mussels and barnacles, are relatively more abundant at SH, while macrophytes are relatively more abundant at BB. Another

important difference between sites is the greater influence of sand at SH (D'Antonio 1986, Menge et al. 1994, 1997a, 1997b). While both BB and SH are rocky reef sites, SH experiences massive annual (summer) sand influxes as a consequence of nearby sandy beaches, while BB has no sand accumulation.

We also conducted the study at both wave-exposed and more wave-protected areas within each site. Sessile invertebrate filter feeders tend to be dominant only in wave-exposed areas, and sand influxes to rocky reefs occur primarily in wave-protected areas. Thus, differences in both sand accumulation and the relative abundances of invertebrates and macrophytes between sites vary with wave exposure (Menge et al. 1994). In this experiment, we aimed to test the generality of the interaction between coralline and fleshy algae not only over time but also across space.

While van Tamelen's baseline study (1996) was conducted in artificially constructed tidepools, our experiment manipulated algae growing on low zone emergent surfaces. Competition was measured through recruitment processes in van Tamelen's study, but since we focused on vegetative growth and lateral expansion of algal turfs, the goals of our experiment were best achieved on emergent surfaces rather than in tidepools. Thus, our studies overlap in location at BB, but they did not occur in the same intertidal habitat. Although variation in environmental factors, i.e. temperature, O_2 and CO_2 concentrations, is likely higher in tidepools (Truchot and Duhamel-Jouve 1980, Morris and Taylor 1983), it is unclear how this might differentially affect coralline and fleshy tidepool algae and their relative competitive abilities. Another study conducted in the 1980s (Stewart 1989) yielded results similar to van Tamelen's (1996), demonstrating no

change in the abundance of fleshy algae following removal of coralline algae on emergent rocky intertidal benches in San Diego, CA. These two studies suggest that competition may have been uncommon between coralline and fleshy algae in rocky intertidal environments along the U.S. west coast in the 1980s.

Reciprocal removal experiment

To evaluate spatial competition between coralline and fleshy algae in the low intertidal zone, we initiated a reciprocal removal pulse experiment (Bender et al. 1984) in June 2014 and monitored the experiment for two years in April, June, and August of each year. Monitoring in fall or winter was not feasible because winter storm wave action prevented access to the lower shore tide heights (-0.1 to 1.6 m) of our experiment. At each site and wave exposure, we established fifteen 15 x 15 cm^2 plots, marked at four corners with stainless steel screws, containing both coralline and fleshy algae. The 15 plots accommodated three treatments with five replicates each: 1) removal of coralline algae, 2) removal of fleshy algae, and 3) an unmanipulated control. Algae were removed with a small pry bar and wire brush, leaving bare rock with as little algal crust as possible. The coralline algae consisted of *Corallina vancouveriensis* and *Bossiella plumosa*, which frequently intermingle. The species of fleshy algae in the plots varied between wave exposures, with *Dilsea californica* in wave-exposed areas and *Neorhodomela larix* in wave-protected areas (there were no species of fleshy algae co-occurring with corallines that were abundant at both wave exposures). *D. californica* and *N. larix* were selected because of their relatively high abundances at both BB and SH. *N. larix* was also included in van Tamelen's historical study (1996). Plots were

photographed in June 2014 before and after manipulation, August 2014, April 2015, June 2015, August 2015, April 2016, and June 2016. Percent cover of macrophytes, invertebrates, bare rock, and sand in the photographs was determined using ImageJ 1.46r image processing software (Rasband 1997).

Data analysis

Linear mixed-effects models were used to test for differences in coralline and fleshy algal turf cover through time between treatments at each site and wave exposure combination. We used a random effects term to model repeated measures taken of the same plots through time (Laird and Ware 1982). We included the fixed effects of treatment × time, with a random intercept to account for baseline differences between the 60 experimental plots and a random slope to account for the varying effects of time on each plot. Significant differences in percent cover change between the control and algal removal treatments at each time point were determined with user-defined post-hoc contrasts employing a single-step adjustment for multiple comparisons. Multiple linear regressions were used to assess the influence of site, wave exposure, tide height, and treatment on the overall change in coralline and fleshy algal cover between the beginning and end of the experiment. Data were checked for normality and homogeneity of variance using normal probability plots and plots of residuals against predicted values. Analyses were performed in R version 3.2.3 (R Core Team 2015), using the lme4 package (Bates et al. 2015) for linear mixed-effects models with post-hoc contrasts performed in the multcomp package (Hothorn et al. 2008).

To examine whole-community changes between treatments over time, multivariate analyses were performed on the data from each time point separately and then compared. Data were square-root transformed to compress high values and expand low values, reducing the influence of dominant groups (McCune et al. 2002). Bray-Curtis similarities were calculated between plots, or sample units, based on percent covers of different macrophyte and invertebrate groups, as well as bare rock and sand. We used permutation-based multivariate analyses of variance (PERMANOVAs) (Anderson 2001), with 5000 permutations each, to assess the effects of site, exposure, tide height, and treatment on community composition in the plots. Non-metric multidimensional scaling (NMDS) ordinations were used to help visualize differences in community composition between treatments. To reduce the NMDS to two dimensions while maintaining stress below 0.20 (Clarke 1993), heterogeneity was minimized in the data by averaging values across replicates, collapsing the number of sample units at each time point from 60 to 12. All multivariate analyses were performed in the vegan package in R (Oksanen et al. 2016).

Results

The removal of a potential algal competitor had few effects on the abundance of algae remaining in the plot, and this result was consistent at both sites and wave exposures (Figures 3.2, 3.3). When a potential competitor was removed, changes in the percent cover of the remaining algae mirrored those of the same algae in the control. This applied to both corallines after fleshy algae removal and fleshy algae after coralline

removal. The apparent immediate increase in coralline cover following the removal of fleshy algae (Figure 3.2) was an artifact of percent cover estimation, also observed in van Tamelen's (1996) experiment. Larger species can visually obscure shorter species, so removal of erect fleshy algae can expose coralline algae growing underneath or in between fleshy algal fronds. Longer coralline fronds with adjacent algae removed can also flop over and spread out, covering more area. These artifacts would be expected to fade over time once fleshy algae or other larger species begin to colonize.

After removal, both coralline and fleshy algae tended to grow back over time (Figures 3.2, 3.3), although in most cases, removed species did not return to their original abundances by the end of the experiment. However, algae in many control plots also declined from their original abundances, ultimately resembling algal removal treatments. Recovery of coralline algae and *Dilsea californica* following removals was faster than *Neorhodomela larix*, and the rate of algal recovery also tended to be faster at BB than at SH (Figures 3.2, 3.3).

The changes in percent cover of articulated coralline algae and fleshy algae between the beginning and end of the experiment were not affected by either wave exposure or tide height (Table 3.1, $P >> 0.05$ in all cases). However, the overall decline in articulated coralline algae was greater at SH than at BB (Table 3.1, $P = 0.016$). Also, since *N. larix* recovered more slowly after removals, the overall decline in fleshy algae was greater in the fleshy algae removal treatment than in the control (Table 3.1, $P = 0.006$).

Differences between treatments in community composition decreased over time (Figure 3.4). Prior to treatment applications in June 2014, all plots contained coralline algae and either *D. californica* or *N. larix*, so treatment only accounted for 3% of the variation in community composition (Table 3.2A, $P = 0.386$). Immediately following treatment applications, the control still contained both coralline and fleshy algae, while the other two treatments contained either coralline or fleshy algae, so community composition diverged sharply between treatments (Figure 3.4B; Table 3.2B, $P < 0.001$, $R^2 = 0.672$). Few changes occurred between June and August 2014 (Figure 3.4B-C; Table 3.2C, $P < 0.001$, $R^2 = 0.632$), but between August 2014 and April 2015, differences in community composition between treatments declined drastically (Figure 3.4C-D; Table 3.2D, $R^2 = 0.208$), with further but more gradual declines through the end of the experiment (Figure 3.4D-H; Table 3.2E-H, $R^2 = 0.193$, 0.137, 0.058, and 0.047 in successive samples). At the end of the experiment in June 2016, treatment explained less than 5% of the variation in community composition in the plots, similar to the level explained before treatments were applied. However, treatments occupied more community space at the end of the experiment than at the beginning (Figure 3.4A, H), indicating more variation in community composition within treatments, between sites and wave exposures.

Specific changes in community composition over the course of the experiment included overall declines in articulated coralline algae, *Dilsea californica*, and *Neorhodomela larix* at all sites and exposures, and overall increases in other macrophytes, invertebrates, and bare rock (Figure 3.5). The change in community

composition depended on the interaction between site and wave exposure (PERMANOVA, pseudo-$F_{1,56} = 6.677$, $P < 0.001$). In the wave-exposed area of SH, a large recruitment pulse of the gooseneck barnacle, *Pollicipes polymerus*, displaced both articulated coralline algae and *D. californica*. In the wave-exposed area of BB, *D. californica* underwent a large decline but articulated coralline algae declined less, while other macrophytes, the mussel, *Mytilus californianus*, and coralline crust all increased. Plots in BB's wave-protected area underwent similar changes, except almost no invertebrates recruited to wave-protected areas and much more bare rock was left exposed. In the wave-protected area of SH, articulated coralline algae and *N. larix* declined the least, with modest increases in other macrophytes and bare rock.

Percent cover changes in the plots through time revealed strong seasonal patterns among various community members (Figure 3.6), although some patterns should be interpreted cautiously. *D. californica*/*N. larix* and other non-calcareous erect macrophytes increased over spring/summer, and *D. californica*/*N. larix* also decreased over fall/winter, with patterns mainly driven by *D. californica* (Figure 3.3). In contrast, articulated corallines and both coralline and fleshy algal crusts decreased over spring/summer and increased over fall/winter (Figure 3.6). However, these contrasting patterns to erect macrophytes are likely a direct consequence of concealment when larger algae are present and exposure when larger algae are absent (van Tamelen 1996). In the past, little seasonal variation has been observed for articulated coralline abundance in our region (D'Antonio 1986). Mussels recruited over fall/winter, while gooseneck barnacles increased in cover throughout the year. Bare rock and sand demonstrated opposite

seasonal patterns, in which bare rock decreased while sand increased in spring/summer, and bare rock increased and sand decreased in fall/winter. However, sand was only a prominent factor in the wave-protected area of SH, peaking in August (Figure 3.7).

Discussion

We did not find evidence of competition between articulated coralline algae and either fleshy algae, *Dilsea californica* or *Neorhodomela larix*, in our experiment. When coralline algae were removed, fleshy algae did not gain cover, and when fleshy algae were removed, coralline algae did not gain cover. Algae remaining in both removal treatments mimicked changes in the control. This main result did not differ between sites or wave exposures and did not vary with tide height. Our findings remain consistent with those of van Tamelen (1996) in the late 1980s. Although his results suggested that coralline algae pre-empt space from fleshy crustose algae, we were unable to confirm or contradict this observation since fleshy crusts were rare in our study (Figure 3.5). Thus, the prediction that, in the last 30 years, fleshy algae have gained a competitive advantage over coralline algae was not supported by our results, even in areas regarded as relatively stressful for coralline algae, i.e. wave-protected areas and higher shore levels. Instead, space cleared after algae removals was reclaimed by the same species that was removed, colonized by other macrophytes or invertebrates, or remained bare. As succession progressed, community composition between treatments converged, but community composition between sites and wave exposures diverged, as successional dynamics varied with these factors.

One straightforward explanation for the disparity between our observations and the prediction that fleshy algae would increase in dominance under OA is that present-day levels of OA have not yet reached a critical threshold. Although pCO_2 in the ocean has risen in the three decades since van Tamelen's (1996) and Stewart's (1989) investigations, the magnitude of change may not be significant enough to have made an impact in our study, especially given high levels of pH variability in our system (Hofmann et al. 2011) that might obscure the signal of OA. Although McCoy and Pfister (McCoy and Pfister 2014) detected changes in competitive hierarchies among crustose coralline algae in the last 30 years, we examined different species of algae that did not previously compete. The species we studied thus may be less sensitive to OA and require higher thresholds for change. Despite many studies suggesting the vulnerability of coralline algae to OA, models parameterized from observations on Tatoosh Island predicted that *Corallina vancouveriensis*, one of the coralline species in our study, would not be affected by near-term pH declines (Wootton et al. 2008). However, fleshy algae were predicted to increase in relative abundance. At our sites, no consistent trends have been detected in the population abundances of coralline algae or *Dilsea californica* from 2006-2014, although higher abundances of coralline algae were observed at both BB and SH in 2014 (B. Menge, unpublished data). It is possible that other species of fleshy macrophytes not investigated in our study are competing with coralline algae, or that the species we examined will become more competitive in the future with continued progression of OA.

Another possible explanation for the lack of evidence for competition between coralline and fleshy algae in our study is high environmental stress. The Menge-Sutherland model predicts that the relative importance of competition in community regulation is highest at intermediate levels of environmental stress (Menge and Sutherland 1987). At low levels of stress, consumption is more important, and at high levels of stress, physical factors are more important. Previous work has shown that consumption, or grazing, has little effect on algal recruitment and spatial competition in our study system (Menge et al. 1993a, 2005). Thus, environmental factors may more strongly regulate the algal populations we studied. Wave action and substratum weakness cause frequent disturbances to algae (Menge et al. 2005), and phenological observations of many species of fleshy algae reveal seasonal patterns indicating more favorable conditions in summer than in winter (D'Antonio 1986, van Tamelen 1996). Fluctuations in the abundance of *Dilsea californica* in our experiment were consistent with this seasonal pattern, while *Neorhodomela larix* cover was more persistent but also subjected to seasonal stress (Menge et al. 2005). In winter, lower light intensity and nutrient concentrations reduce algal photosynthesis and growth, and strong wave action from storms can damage and tear away algal fronds. Coralline algae, on the other hand, fare better in winter because they may be tolerant of low light regimes and have the ability to acclimate seasonally to different light intensities (Burdett et al. 2012). Furthermore, the flexible, decalcified genicula (joints) in articulated coralline algae are much stronger and more resistant to wave stress than tissues found in fleshy algae (Martone 2006). However, coralline algae are sensitive to desiccation stress and bleach in spring/summer

when daytime low tides coincide with sunny days (Padilla 1984, Martone et al. 2010). Developing coralline crusts are particularly prone to bleaching without overlying branches to retain water. For this reason, and perhaps also to avoid highly variable pH conditions in the spring and summer, coralline crusts recruit in fall (Padilla 1984). While both coralline and fleshy turf-forming algae are constrained by abiotic conditions, each has different physiological and morphological characteristics that result in susceptibility to different stressors. While climate change could create conditions more favorable to fleshy algae, it could also widen phenological differences and lead to seasonal segregation between coralline and fleshy algae.

Coralline and fleshy algae may also have failed to compete in our experiment because of persistent chemical cues remaining after competitor removals. Oftentimes, clearances created by algal removals were reoccupied by the same species of algae that was removed. By the end of the experiment, 25% of algae removal treatments had recovered to control levels. Although neither coralline nor fleshy turf-forming algae expanded into space cleared from competitor removals, both reclaimed space from which they were removed. This space discrimination suggests the possibility that algal removals left behind residual crusts or chemical cues that promoted algal recovery and restricted colonization by neighboring algae species. Although algae were removed as thoroughly as possible via manual procedures, more exhaustive methods, such as lye application or torching, could have been employed. However, our experiment required a level of precision that may have been compromised using these methods, and even with lye

application and torching, macrophytes can still manage to reclaim disturbances within two years (Barner 2016).

While our main finding, that coralline and fleshy algae evidently have not begun to compete in the last 30 years, was consistent across two sites and wave exposures, community development differed between both site and wave exposure. At BB exposed areas, other macrophytes and mussels increased in abundance, while at SH exposed areas, invertebrate cover surged, primarily due to an unusually high recruitment pulse of gooseneck barnacles. Mussels and gooseneck barnacles may have been particularly successful in colonizing the low zone in our experiment because of release from top-down control. Densities of the keystone predator, *Pisaster ochraceus*, fell dramatically at our sites when an outbreak of sea star wasting disease (SSWD) struck in spring 2014 (Hewson et al. 2014, Eisenlord et al. 2016, Menge et al. 2016). However, the impact of SSWD was probably minimal in protected areas, where few sea stars or sea star prey occur. At BB protected areas, much space remained bare in our plots, while at SH protected areas, declines of coralline and fleshy algae were lower than in other areas, perhaps due to seasonal sand incursions. Sandy areas can provide a refuge for coralline algae and *Neorhodomela larix* because both are resistant to sand burial, while many other organisms, including grazers and potential competitors, are excluded by sand scour and burial (D'Antonio 1986, Stewart 1989). Variation in community structure increased between sites and wave exposures as community dynamics progressed, but neither biotic nor abiotic differences between sites and wave exposures seemed to influence the interaction between coralline and fleshy algae.

Although many studies have suggested that the contrasting effects of ocean acidification (OA) on coralline and fleshy algae will lead to a shift in dominance toward fleshy algae (Porzio et al. 2011, Koch et al. 2013, Kroeker et al. 2013b, Brodie et al. 2014), we did not detect the emergence of competition between coralline and fleshy algae in our study. Furthermore, the absence of spatial competition was consistent across sites and wave exposures characterized by different oceanographic conditions and community dynamics. We suspected that the discrepancy between our results and predictions could be due to a number of factors, including insufficient time for detection of climate change effects between current and historical comparisons, precedence of environmental constraints over competition in structuring algal communities, and interference by chemical cues. Even as OA continues to escalate, it is unclear whether coralline and fleshy algae will begin to compete on our coast, where high variability in pCO_2/pH may detract from slow, systematic change (Hofmann et al. 2011) and reduce algal sensitivity to elevated pCO_2 (Padilla-Gamiño et al. 2016). Although fleshy algae are expected to outcompete coralline algae under OA, abundances of fleshy algae may be limited by seasonal regulation, in which summer gains resulting from increases in light, nutrients, and pCO_2 are leveled by winter losses. Besides raising pCO_2, climate change is also expected to increase storm intensities and wave forces (Bromirski et al. 2005, Ruggiero et al. 2010), which could increase algal bed disturbances. Considerations of environmental variability and ecosystem change involving competing environmental stressors complicate predictions about the effects of climate change on biological communities and require further examination. By leveraging historical data, we were able to compare

potential changes in algal interactions over the last three decades in the natural environment, where abiotic conditions vary and interact. While our study may raise more questions than it answers, advancing beyond single species and single stressor climate change experiments is a step toward greater biological relevance.

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Figures

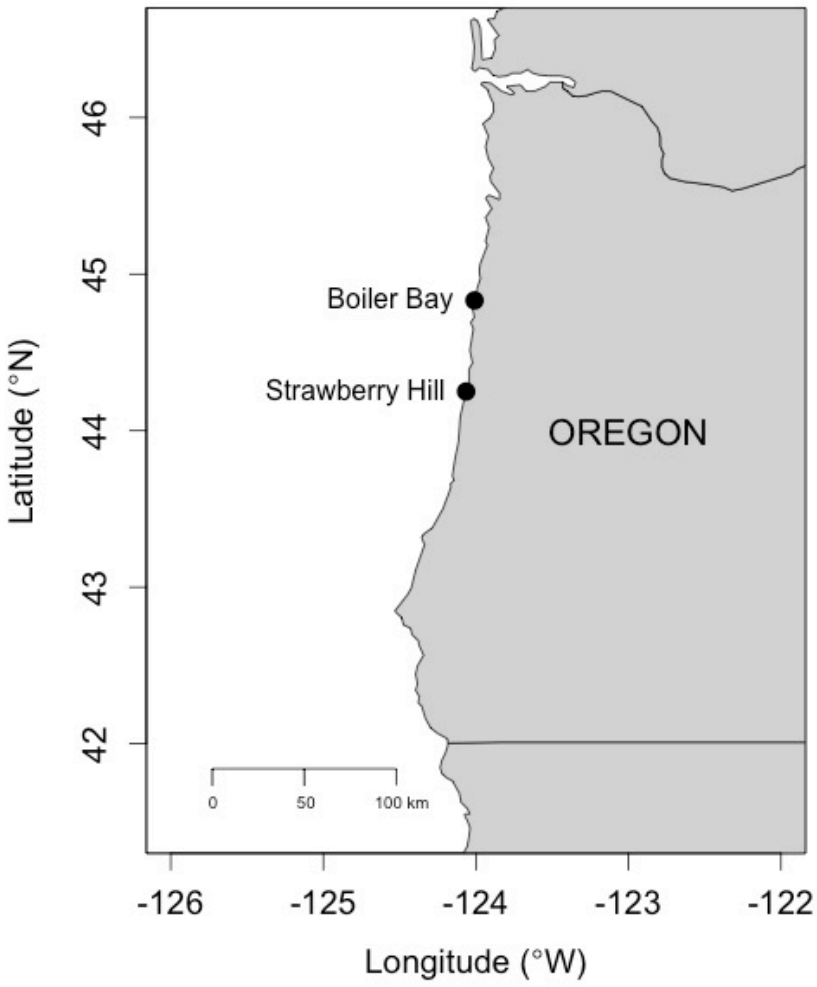

Figure 3.1. Map of study sites in Oregon.

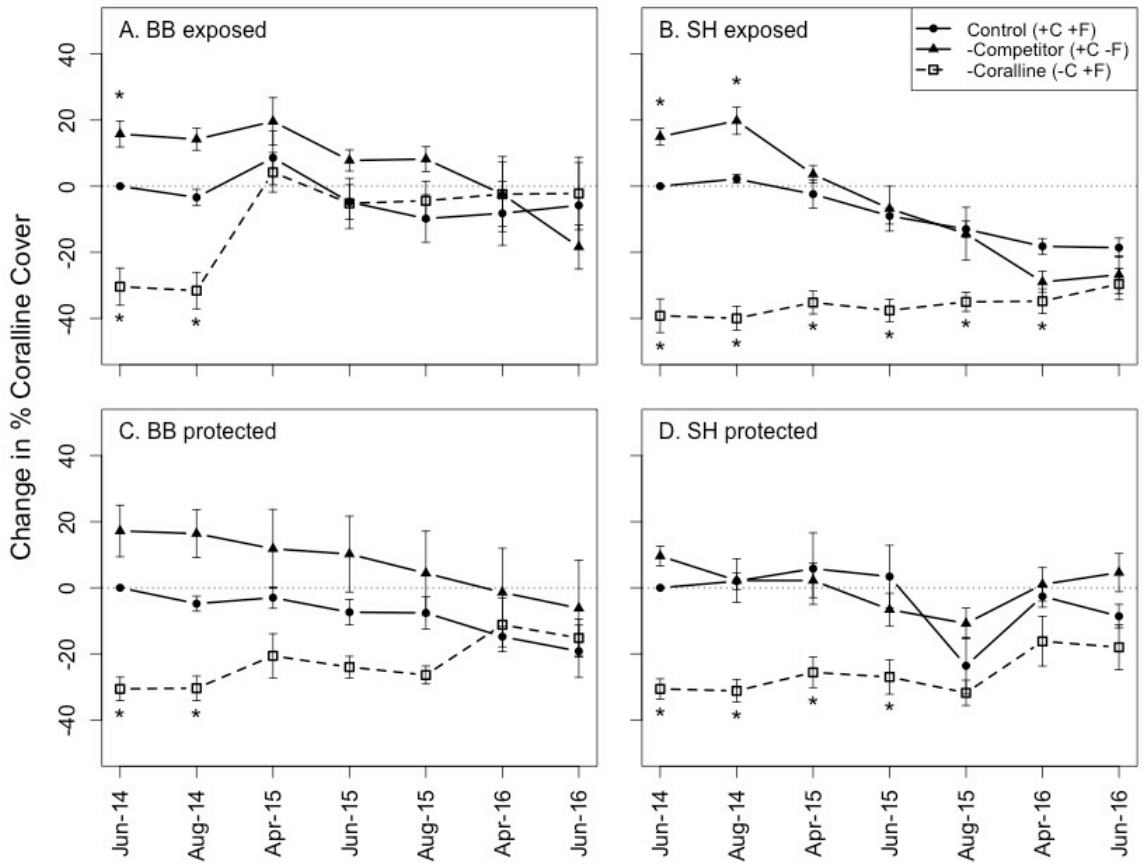

Figure 3.2. The change in percent articulated coralline cover over time in the three treatments, control (+C +F), *Dilsea*/*Neorhodomela* removal (+C -F), and coralline removal (-C +F), at A) BB exposed, B) SH exposed, C) BB protected, and D) SH protected areas. *Dilsea* was removed in exposed areas, while *Neorhodomela* was removed in protected areas. Changes in percent cover were calculated by subtracting coralline cover in June 2014 prior to manipulations from cover on each subsequent date after manipulations. Asterisks indicate a significant difference in percent cover change between the control and the indicated treatment at a given time point.

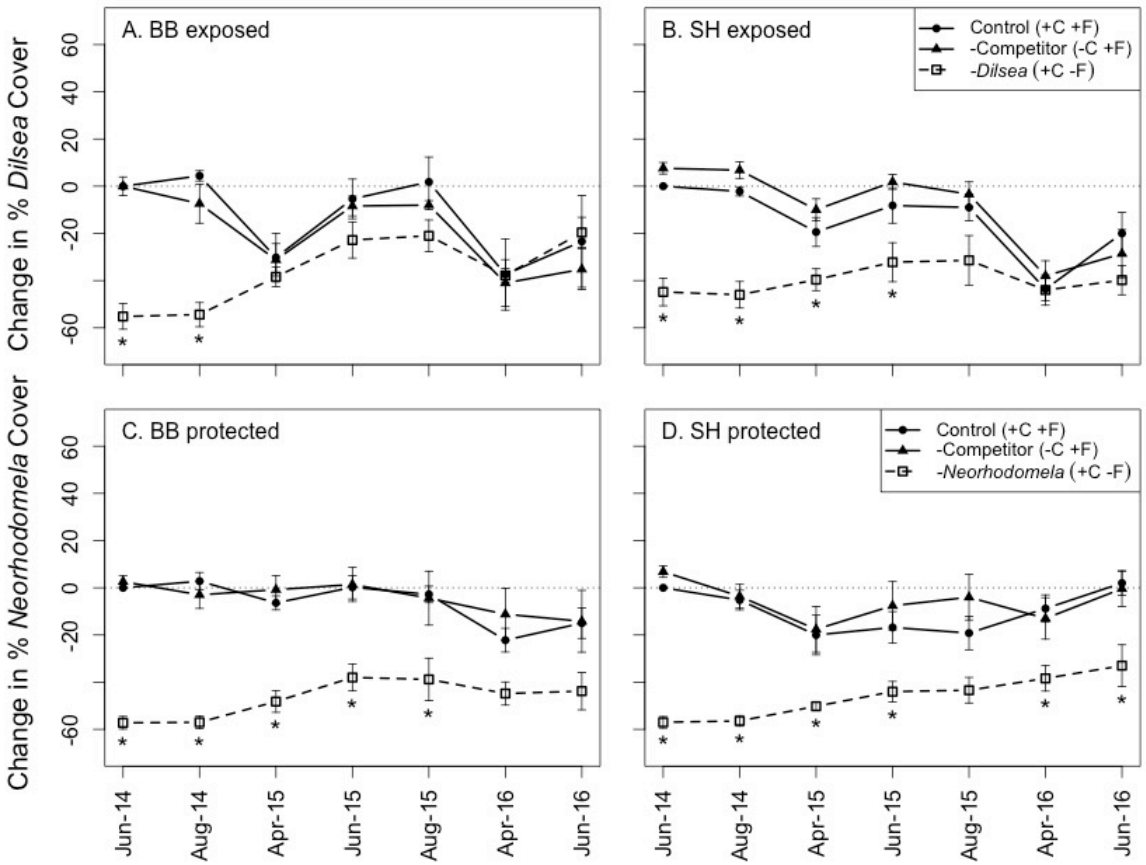

Figure 3.3. The change in percent *Dilsea* and *Neorhodomela* cover over time in the three treatments, control (+C +F), coralline removal (-C +F), and *Dilsea*/*Neorhodomela* removal (+C -F), at A) BB exposed, B) SH exposed, C) BB protected, and D) SH protected areas. Changes in percent cover were calculated by subtracting *Dilsea* or *Neorhodomela* cover in June 2014 prior to manipulations from cover on each subsequent date after manipulations. Asterisks indicate a significant difference in percent cover change between the control and the indicated treatment at a given time point.

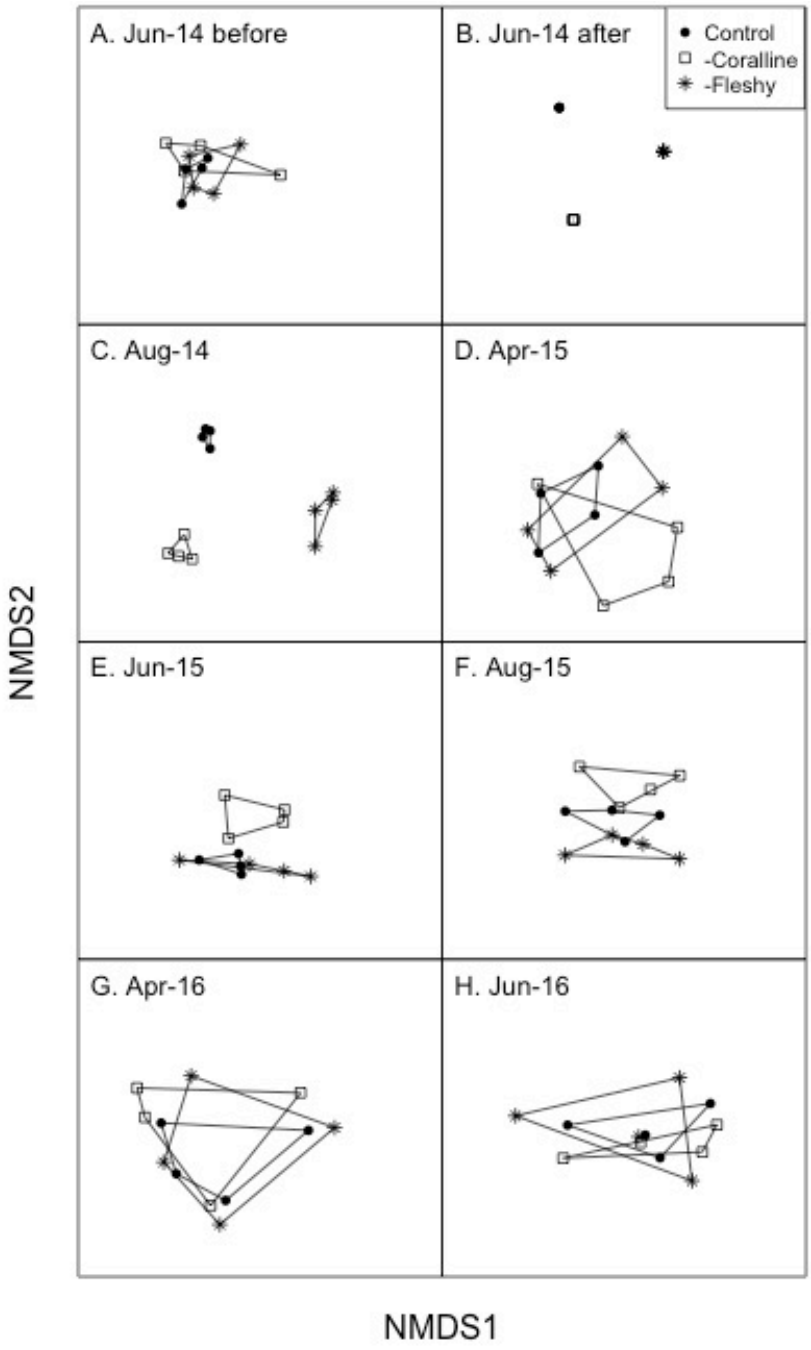

Figure 3.4. Two-dimensional non-metric multidimensional scaling (NMDS) ordinations of experimental plots, averaged across replicates and grouped by treatment, in community space (average stress = 0.09). To simplify plots, community members are not shown. Separate ordinations were plotted for each time point: A) June 2014 before manipulations, B) June 2014 after manipulations, C) August 2014, D) April 2015, E) June 2015, F) August 2015, G) April 2016, and H) June 2016.

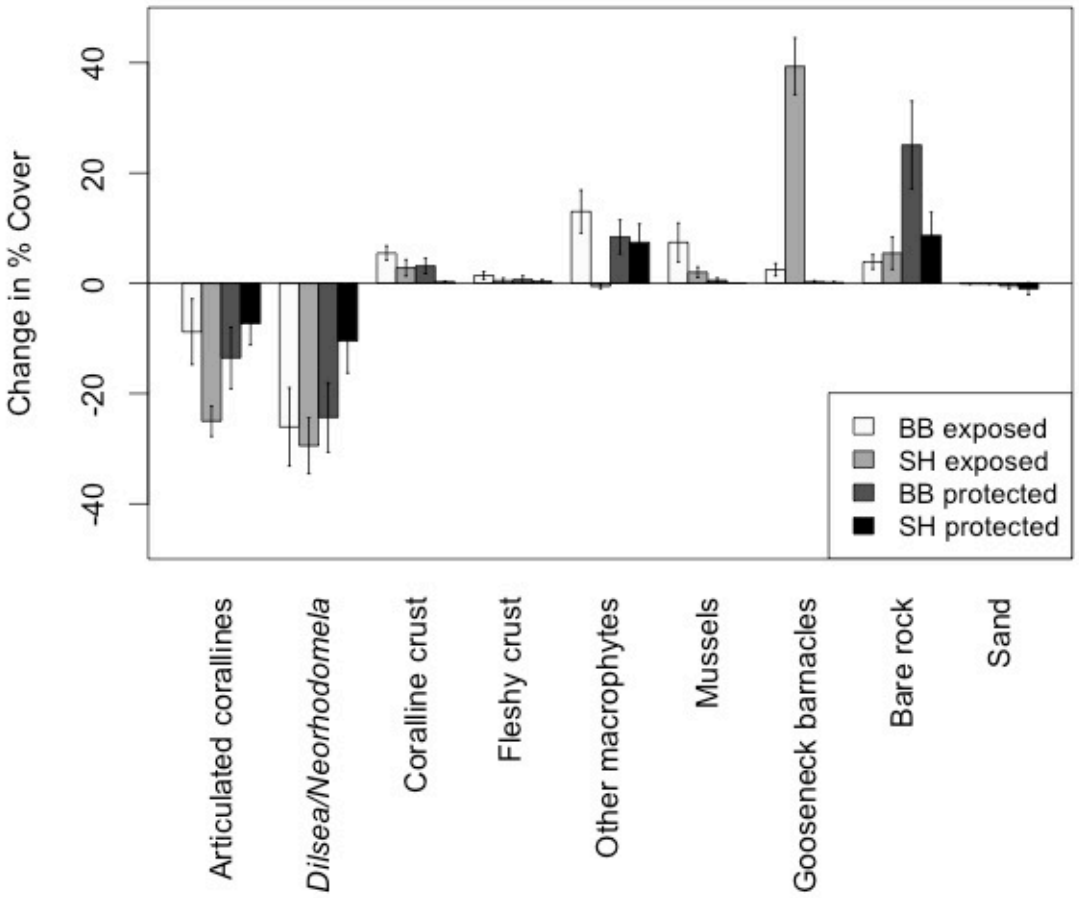

Figure 3.5. The change in percent cover of select space occupiers in all plots between BB and SH exposed and protected areas from the beginning of the experiment, June 2014 prior to manipulations, to the end of the experiment, June 2016.

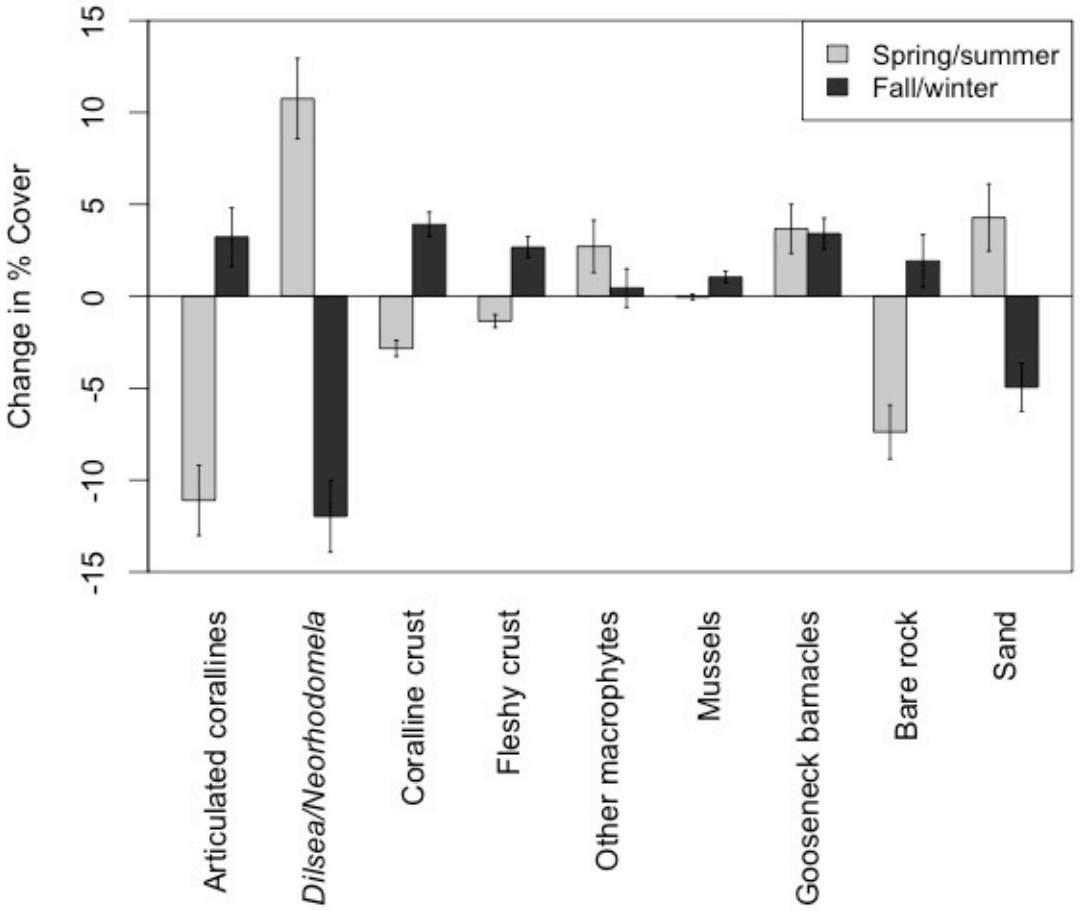

Figure 3.6. The change in percent cover of select space occupiers in all plots between spring/summer and fall/winter. Changes in percent cover over spring/summer were calculated by subtracting cover in April 2015 from cover in August 2015, the only full spring-summer span in the experiment. Changes in percent cover over fall/winter were calculated by subtracting cover in August 2014 and 2015 from cover in April 2015 and 2016, respectively.

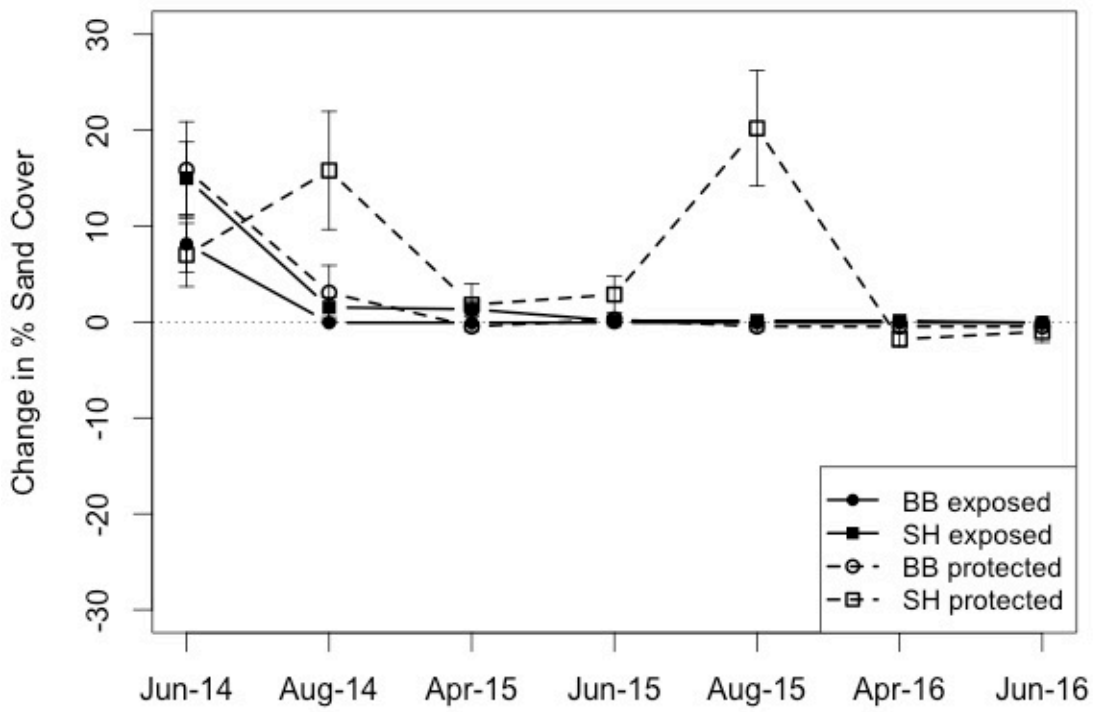

Figure 3.7. The change in percent cover of sand over time at BB and SH exposed and protected areas. Changes in percent cover were calculated by subtracting sand cover in June 2014 prior to manipulations from cover on each subsequent date after manipulations.

Tables

Table 3.1. Multiple linear regression models for overall changes in coralline and fleshy algal cover. Site, exposure, tide height, treatment, and site × exposure were included as independent variables. The reference groups are Site = FC, Exposure = exposed, and Treatment = +C +F (control). Significant *P*-values are shown in boldface.

Coefficients	**Estimate**	**SE**	***t*-value**	***P***
Articulated coralline algae				
Intercept	-0.049	0.068	-0.711	0.480
Site = SH	-0.169	0.068	-2.488	**0.016**
Exposure = protected	-0.017	0.075	-0.225	0.823
Tide height	-0.071	0.076	-0.943	0.350
Treatment = -C +F	-0.032	0.059	-0.551	0.584
Treatment = +C -F	0.012	0.059	0.212	0.833
Site × exposure = SH × protected	0.191	0.102	1.880	0.066
Dilsea/Neorhodomela				
Intercept	-0.117	0.082	-1.430	0.159
Site = SH	-0.047	0.082	-0.573	0.569
Exposure = protected	0.071	0.090	0.790	0.433
Tide height	-0.125	0.091	-1.383	0.173
Treatment = -C +F	-0.055	0.070	-0.789	0.434
Treatment = +C -F	-0.201	0.070	-2.865	**0.006**
Site × exposure = SH × protected	0.115	0.122	0.942	0.351

Table 3.2. Results of PERMANOVAs on Bray-Curtis similarities testing the effects of site, exposure, tide height, treatment, and site × exposure on community composition in experimental plots at each time point examined. Significant *P*-values are shown in boldface.

Source of variation	**df**	**SS**	**MS**	***F***	R^2	***P***
A. June 2014 before manipulation						
Site	1	0.058	0.058	3.246	0.046	**0.018**
Exposure	1	0.066	0.066	3.680	0.052	**0.010**
Tide height	1	0.115	0.115	6.436	0.091	**< 0.001**
Treatment	2	0.038	0.019	1.069	0.030	0.386
Site × exposure	1	0.043	0.043	2.413	0.034	**0.048**
Residuals	53	0.950	0.018		0.747	
Total	59	1.271				
B. June 2014 after manipulation						
Site	1	0.098	0.098	2.812	0.015	**0.046**
Exposure	1	0.133	0.133	3.826	0.020	**0.016**
Tide height	1	0.040	0.040	1.153	0.006	0.334
Treatment	2	4.525	2.263	65.135	0.672	**< 0.001**
Site × exposure	1	0.092	0.092	2.653	0.014	0.053
Residuals	53	1.841	0.035		0.274	
Total	59	6.729				
C. August 2014						
Site	1	0.257	0.257	7.804	0.036	**< 0.001**
Exposure	1	0.396	0.396	12.015	0.055	**< 0.001**
Tide height	1	0.205	0.205	6.228	0.029	**0.001**
Treatment	2	4.539	2.270	68.911	0.632	**< 0.001**
Site × exposure	1	0.034	0.034	1.046	0.005	0.379
Residuals	53	1.746	0.033		0.243	
Total	59	7.177				
D. April 2015						
Site	1	0.495	0.495	8.766	0.088	**< 0.001**
Exposure	1	0.726	0.726	12.849	0.129	**< 0.001**
Tide height	1	0.175	0.175	3.102	0.031	**0.006**
Treatment	2	1.175	0.587	10.399	0.208	**< 0.001**
Site × exposure	1	0.077	0.077	1.354	0.014	0.229
Residuals	53	2.994	0.056		0.531	
Total	59	5.642				

Table 3.2. (Continued)

Source of variation	df	SS	MS	*F*	R^2	*P*
E. June 2015						
Site	1	0.406	0.406	6.774	0.071	**< 0.001**
Exposure	1	0.646	0.646	10.777	0.113	**< 0.001**
Tide height	1	0.195	0.195	3.249	0.034	**0.003**
Treatment	2	1.100	0.550	9.179	0.193	**< 0.001**
Site × exposure	1	0.181	0.181	3.013	0.032	**0.010**
Residuals	53	3.176	0.060		0.557	
Total	59	5.704				
F. August 2015						
Site	1	0.949	0.949	13.646	0.124	**< 0.001**
Exposure	1	1.082	1.082	15.548	0.141	**< 0.001**
Tide height	1	0.413	0.413	5.935	0.054	**< 0.001**
Treatment	2	1.049	0.524	7.537	0.137	**< 0.001**
Site × exposure	1	0.481	0.480	6.907	0.063	**< 0.001**
Residuals	53	3.687	0.070		0.481	
Total	59	7.659				
G. April 2016						
Site	1	0.652	0.652	9.188	0.093	**< 0.001**
Exposure	1	1.344	1.344	18.923	0.191	**< 0.001**
Tide height	1	0.334	0.334	4.706	0.048	**0.001**
Treatment	2	0.409	0.205	2.882	0.058	**0.003**
Site × exposure	1	0.522	0.522	7.357	0.074	**< 0.001**
Residuals	53	3.764	0.071		0.536	
Total	59	7.026				
H. June 2016						
Site	1	0.800	0.800	10.443	0.106	**< 0.001**
Exposure	1	1.091	1.091	14.238	0.145	**< 0.001**
Tide height	1	0.772	0.772	10.080	0.102	**< 0.001**
Treatment	2	0.353	0.176	2.302	0.047	**0.018**
Site × exposure	1	0.460	0.460	6.008	0.061	**< 0.001**
Residuals	53	4.061	0.077		0.539	
Total	59	7.537				

CHAPTER 4 – EFFECTS OF LOCAL- AND REGIONAL-SCALE FORCES ON EPIFAUNAL ASSEMBLAGES ASSOCIATED WITH CALCIFYING AND NON-CALCIFYING ECOSYSTEM ENGINEERS

Abstract

Many marine ecosystem engineers are calcifiers that are threatened by ocean acidification. Included in this group are coralline algae, which provide habitat to diverse epifaunal assemblages. However, unlike most ecosystem engineers, coralline algae have non-calcifying analogs, fleshy algae, that are less vulnerable to ocean acidification. Although it is unclear whether these two habitat types support distinct epifaunal communities, examining the effects of ecosystem engineers can help scale up our understanding of ocean acidification impacts from organismal physiology to whole-community interactions. To this end, we surveyed coralline and fleshy algal habitats at four capes along the Oregon-California coast. We also experimentally reduced the density and height of algal habitats in the field, as these underlying habitat attributes may at least partially account for epifaunal distributions among algae. We found that, on average, epifauna were smaller and more abundant in coralline algae than in fleshy algae, likely due to the observation that thallus density of coralline algae was higher than fleshy algae. While epifauna abundance and richness were positively correlated with thallus density, no relationships were found with algal height. Additionally, neither epifauna richness nor community composition differed between habitat types, while differences in epifauna occurred primarily between capes. These results suggest that the potential degradation of coralline habitats due to ocean acidification can decrease epifauna

abundances, but epifaunal communities are primarily shaped by regional-scale coastal oceanography and biogeography.

Introduction

Ecosystem engineers modify the environment to create habitats and favorable living conditions for other species (Jones et al. 1994). Important marine ecosystem engineers include reef-building organisms like corals and oysters. As calcifiers, however, they are also among the most vulnerable organisms to ocean acidification (Hoegh-Guldberg et al. 2007, Barton et al. 2012, Gazeau et al. 2013, Kroeker et al. 2013a, Wittmann and Pörtner 2013). Any direct negative effects of ocean acidification on calcifying ecosystem engineers will likely have widespread indirect effects on the diverse communities that depend on calcified biogenic habitats. Thus, the ecosystem engineering concept can be applied to help elaborate on the broader effects of ocean acidification on ecological communities.

Although, by definition, ecosystem engineers facilitate other species, the effect of ecosystem engineers on species richness is scale-dependent. Ecosystem engineers almost always increase species richness at the regional scale (Jones et al. 1997). The effect of an engineer on species richness is positive if it both creates unique conditions and supports unique species that are not present elsewhere in the region (Wright et al. 2002). At the local scale, however, the effect of an ecosystem engineer on species richness is not always positive (Jones et al. 1997, Daleo et al. 2006). While habitat modification may benefit some species, it may also displace others. In addition, since ecosystem engineers

are ubiquitous (Wright and Jones 2006), they may compete for space, so the presence of one ecosystem engineer could exclude one or more others. Given the possibility of alternative ecosystem states (Beisner et al. 2003), it can be difficult to determine the effect of an ecosystem engineer on local species richness.

Both calcifying coralline algae and non-calcifying fleshy algae act as ecosystem engineers in rocky intertidal and subtidal environments, often co-occurring. Under ocean acidification, many studies have suggested a decline in coralline algae and a simultaneous increase in fleshy algae (Russell et al. 2009, Hepburn et al. 2011, Porzio et al. 2011, Hofmann et al. 2012a, Koch et al. 2013, Kroeker et al. 2013b, Johnson et al. 2014). Water enriched with CO_2 has a lower carbonate saturation state, hindering calcification in coralline algae (Kuffner et al. 2007, Anthony et al. 2008, Jokiel et al. 2008, Martin and Gattuso 2009, Gao and Zheng 2010, Hofmann et al. 2012b, Ragazzola et al. 2012, Noisette et al. 2013b). Without the need to calcify, fleshy algae are either unaffected or supplemented by increased CO_2 availability for photosynthesis, depending on their carbon concentration mechanism (Kübler et al. 1999, Hepburn et al. 2011, Cornwall et al. 2012b, Connell et al. 2013, Koch et al. 2013). Ocean acidification may ultimately shift the macroalgae composition in rocky shore environments from a mixed coralline-fleshy community to a fleshy-dominated community.

The effect of this loss of calcareous habitat on associated epifauna richness and abundance is unknown. Compared to the difference between coral- and algae-dominated ecosystem states (Done 1992, McManus and Polsenberg 2004), the difference between coralline and fleshy algae is more subtle. Species of both coralline and fleshy algae have

densely growing thalli, with small interstitial spaces that harbor high abundances of epifauna. Results from epifaunal surveys in algal habitat including both coralline and fleshy algae vary in which harbors more epifauna. Davenport et al. (Davenport et al. 1999) discovered more abundant and diverse epifauna in *Corallina* sp. than two species of fleshy algae, *Enteromorpha* sp. and *Hormosira banksii*, sampled from the low intertidal zone in Australia. They suspected that the superior water-retention properties of *Corallina* sp. created a more favorable environment for epifauna during low tide. In contrast, high-zone surveys by Bracken et al. (Bracken et al. 2007) in California tidepools indicate higher epifauna abundance in the fleshy alga, *Cladophora columbiana*, than in any other common tidepool algae, including other fleshy species and the coralline, *Corallina vancouveriensis*. Similarly, research by Hacker and Steneck (Hacker and Steneck 1990) in low-zone tidepools in Maine showed that amphipod abundance in the coralline alga, *Corallina officinalis*, was comparable to that in five fleshy algae sampled. Reconciling these studies, Bates (Bates 2009) surveyed 32 algal species in British Columbia's rocky intertidal and classified algae into different morphological groups, including coralline and various fleshy morphologies. Results revealing substantial variation in epifauna abundance and richness within morphological groups suggested that algae-epifauna associations were host species-specific. However, with respect to making generalizations about algal habitat use by epifauna, Bates (Bates 2009) proposed examining traits shared across a broad spectrum of algal morphologies that could be measured on a continuous scale.

One habitat trait frequently associated with species diversity is the density (complexity) or variation (heterogeneity) of structural components (Tews et al. 2004, Kovalenko et al. 2011). This positive relationship between species diversity and habitat complexity is widespread in nature and has been observed in birds (MacArthur and MacArthur 1961, Roth 1976, Terborgh 1977), rodents (Rosenzweig and Winakur 1969), lizards (Pianka 1967), insects (Murdoch et al. 1972, Lawton 1983), spiders (Uetz 1979), marine and freshwater fish (Gorman and Karr 1978, Luckhurst and Luckhurst 1978), marine invertebrates (Kohn 1967, Heck Jr and Wetstone 1977), and sessile marine organisms (Menge et al. 1985). Notably, the physical habitat traits selected as a measure of habitat complexity or heterogeneity varied between studies and study systems. In marine and freshwater vegetated habitats, epifauna richness and abundance have been associated with habitat traits such as vegetation biomass or density (Heck Jr and Wetstone 1977, Dean and Connell 1987a, Attrill et al. 2000, Kelaher and Castilla 2005), percent cover of vegetation (Levin and Hay 1996, Best et al. 2014), thallus height (Levin & Hay 1996), thallus width (Taylor and Cole 1994), thallus volume (Hacker and Steneck 1990), interstitial volume (Hacker and Steneck 1990, Warfe et al. 2008), fractal dimensions (Gee and Warwick 1994, Warfe et al. 2008, Thomaz et al. 2008), and sediment accumulation (Kelaher and Castilla 2005, Huff and Jarett 2007). Despite the lack of standardization in metrics of habitat complexity, many studies have demonstrated a link between algal habitat complexity and associated epifauna. Some proposed mechanisms underlying this link include greater resource availability, heightened protection from predation and environmental stress, and increased likelihood of

colonization provided by complex habitats (Heck Jr and Wetstone 1977, Lawton 1983, Dean and Connell 1987b).

Still, the effects of algal habitat structure on associated epifauna may be limited. Other variables, like site (Liuzzi and Gappa 2008, Moulton and Hacker 2011, Torres et al. 2015) and shore height/depth (Kelaher et al. 2003, Cacabelos et al. 2010, Berthelsen et al. 2015), may be equally if not more important than algal habitat characteristics in determining epifauna distributions.

While coralline and fleshy algae are ecosystem engineers that will likely respond differently to ocean acidification, there is a lack of knowledge on their respective influence on associated epifauna. Thus the community response to ocean acidification, mediated by changes in algal habitat, is unclear. To better predict the response of epifauna communities to anticipated habitat shifts, we asked 3 questions: (1) how does epifauna abundance, richness, and community composition differ between coralline and fleshy algae? (2) Can simple habitat complexity metrics, i.e. algal density and height, help to explain algae-epifauna associations? And (3) what is the influence of large-scale environmental variation on the distribution of epifauna in algal habitats?

Methods

Study sites

This study was conducted at four rocky intertidal sites spanning about 600 km along the Oregon-northern California coast: Fogarty Creek (FC), Strawberry Hill (SH), Cape Blanco (CB), and Cape Mendocino (CM) (Table 4.1; Figure 4.1). Each site is

located at a different cape (FC at Cape Foulweather, SH at Cape Perpetua) and varies in both environmental factors and community structure (Menge et al. 2015). Consequently, the composition of macroalgae differs at each site, resulting in a different mix of algal species sampled at each site (Table 4.1). Although coralline algae were abundant at all sites and consisted of both *Corallina vancouveriensis* and *Bossiella plumosa*, which frequently intermingle, their relative abundances differed between sites. The fleshy species sampled at each site was selected based on abundance and proximity to coralline algae. Five sampling areas were established in the low zone within each site to systematically disperse survey efforts and experimental plots.

Epifauna surveys

We conducted epifauna surveys monthly during minus tides May-July 2012 and May-August 2013. In most cases, samples were collected from all four sites within a one-week tide series. At each site, one 10 x 10 cm^2 patch of both coralline and fleshy algae was sampled from each of five areas, resulting in 280 samples total (5 replicates x 2 types of algae x 4 sites x 7 visits). After measuring the lengths of five thalli in each patch to estimate average thallus height of the patch, algae and any sediment in the patch were scraped from the substrate using a small pry bar. Samples were placed into plastic Ziploc bags and frozen for later processing.

Algal habitat manipulations

In conjunction with the epifauna survey, we conducted an experiment manipulating the density and height of coralline and fleshy algae to better understand the effect of these general habitat traits on associated epifauna. The experiment took place

June-August 2013 at the same four sites (FC, SH, CB, CM) and five sampling areas within a site used by the epifauna survey, targeting the same algal species. In each sampling area, two 10 x 10 cm^2 plots of both coralline and fleshy algae were established and marked with marine epoxy putty (A-788 Splash Zone compound, Pettit Marine Paint, Rockaway, New Jersey, USA) at four corners, resulting in 80 plots total (5 replicates x 2 types of algae x 2 treatments x 4 sites). In June 2013, two habitat treatments were applied to each set of plots: algae density reduction (D) and algae height reduction (H). In treatments D and H, algae were thinned by manually removing thalli from holdfasts and trimmed with garden shears, respectively. Before and after algal habitat manipulations, the lengths of five thalli were measured in each plot to estimate average algae height. Manipulated algae grew over time, requiring additional thinning and trimming in July 2013 to maintain the treatments. All algae removed from plots during manipulations in June and July 2013 were collected in Ziploc bags and frozen for later processing. In August 2013, experimental plots were destructively sampled following the same methods as in the epifauna surveys, allowing two months for epifauna to emigrate from or immigrate to manipulated habitats. The August 2013 epifauna survey functioned as the experimental control.

To estimate the immediate change in algal density resulting from the manipulations, a separate set of 48 10 x 10 cm^2 plots (3 replicates x 2 types of algae x 2 treatments x 4 sites) was manipulated and destructively sampled directly after manipulation. In addition, the lengths of five algal thalli were measured in each plot before and after manipulations. Both algae removed from the plots during manipulation

and algae remaining in the plots after manipulation were collected and placed in separate Ziploc bags to be frozen for later processing.

Sample processing

In the laboratory, each sample was processed on an 850 μm sieve resting in a large glass bowl. Frozen samples were thawed with artificial seawater before separating into three main components: algae, epifauna, and sediment. Algae were identified and sorted into two coralline algae species, *Corallina vancouveriensis* and *Bossiella plumosa*, and two fleshy algae groups, target species and non-target species, according to the site from which samples were collected (Table 4.1). Epifauna retained on the sieve (> 850 μm) were sorted to the lowest taxon possible and counted. Except for the gooseneck barnacle, *Pollicipes polymerus,* most barnacles were crushed during collection and were thus omitted from processing, although they did not make up a large component of the epifauna sampled. Sediment consisted of all fine matter (< 850 μm) collected in the glass bowl after thorough rinsing of any remaining material on the sieve. Once divided into individual ceramic or aluminum containers, all sample components were dried to constant mass at 70°C and weighed.

In this study, we define algal density as thallus volume divided by canopy volume (Hacker and Steneck 1990). Although density typically has units of mass/volume, we used thallus volume instead of dry biomass in order to standardize measurements between coralline and fleshy algae and capture an algal structural trait that is more relevant to epifauna. Thallus volume for each type of alga was measured by water displacement in a 100 mL graduated cylinder. Empirical measurements of thallus volume

were taken for all samples collected in the July and August 2013 epifauna surveys and all samples from the algal habitat manipulation experiment. For epifauna survey samples collected before July 2013, thallus volumes were estimated using standard curves between dry biomass and volume for each type of algae. Canopy volume was calculated as the product of the plot area (100 cm^2) and the average thallus length in the plot.

Data analysis

Univariate epifauna responses were compared between coralline and fleshy algal habitats in the epifauna survey and between D and H treatments in the habitat manipulations using two-sample *t* tests. Analyses of variance (ANOVAs) were used to evaluate epifauna abundance and richness in response to multiple explanatory variables, including site, month, year, habitat type, and site × habitat type in the epifauna survey, and treatment and habitat type in the algal manipulations. ANOVAs were also used to determine differences in algal density and height between sites and habitat types in the epifauna survey and between treatments and habitat types in the algal manipulations. Data were checked for normality using the Shapiro-Wilk test. To satisfy normality assumptions, most responses were $\log_{10}$ transformed. Homogeneity of variance was checked with plots of residuals against predicted values. In post-hoc interaction contrasts, Bonferroni corrections were applied to address multiple comparisons. All analyses were performed in R version 3.2.3 (R Core Team 2015), with post-hoc contrasts implemented in the phia package (De Rosario-Martinez 2015).

In examining algae-epifauna associations in the habitat manipulations, generalized linear models (GLMs) were used to supplement ANOVAs. While algal

density and height were represented by categorical treatment groups in the ANOVAs, habitat characteristics were treated as continuous measurements in the GLMs. Epifauna richness was modeled using a Poisson distribution and log link, but abundance required a negative binomial regression model, implemented in the MASS package (Venables and Ripley 2002), because data were overdispersed.

Factors affecting epifaunal community composition in both the survey and the experiment were assessed using permutation-based multivariate analyses of variance (PERMANOVAs) (Anderson 2001), with 5000 permutations each. Additionally, differences in community composition between habitat types and sites in the epifauna survey were visualized with a non-metric multidimensional scaling (NMDS) ordination. To reduce the NMDS to two dimensions while maintaining stress below 0.20 (Clarke 1993), heterogeneity was minimized in the data by averaging values across replicates, collapsing the number of sample units from 280 to 56. Data in all multivariate analyses were square-root transformed to compress high abundance values and expand low abundance values, reducing the influence of dominant taxa (McCune et al. 2002). Multivariate analyses were performed on Bray-Curtis similarities in the vegan package (Oksanen et al. 2016).

In the algal habitat manipulations, only data on mobile epifauna were analyzed because they are more capable of responding to changes in habitat. Sessile and burrowing epifauna (17 taxa) were omitted because their post-disturbance recruitment and immigration following manipulations may not be adequately captured within the time frame of the experiment.

Results

Epifauna surveys

In the epifauna surveys (N = 280), we counted 52,240 individual epifauna belonging to 85 identifiable taxa (Table 4.2, Table A1). Five taxa were particularly dominant (*Lacuna marmorata*, *Mytilus* spp., *Synidotea ritteri*, gammaridean amphipods, and *Barleeia haliotiphila*), comprising 80% of the organisms surveyed, while most other taxa were rare (Figure 4.2; Table A1).

Overall, epifauna were more abundant in coralline algae samples than in fleshy algae samples (mean ± SE: coralline 221 ± 14, fleshy 152 ± 11 individuals; two-sample *t* test, $t_{277.56} = 3.59$, $P < 0.001$). This pattern was largely driven by two gastropod mesograzers, *Lacuna marmorata* and *Barleeia haliotiphila*, together accounting for 36% of the organisms surveyed. *L. marmorata* and *B. haliotiphila* abundances were 2.5 and 1.9 times higher in coralline algae than in fleshy algae, respectively. Discounting both gastropods, epifauna abundances were not different between the two algal habitats (mean ± SE: coralline 127 ± 10, fleshy 111 ± 7 individuals; two-sample *t* test, $t_{277.14} = 0.71$, $P =$ 0. 480).

Similarly, after removing from all samples two high-biomass outlier species, *Katharina tunicata* and *Strongylocentrotus purpuratus*, epifauna biomass was not different between the two algal habitats (mean ± SE: coralline 1.3626 ± 0.1103 g, fleshy 1.4721 ± 0.1202 g dry weight; two-sample *t* test, $t_{266.73} = 0.44$, $P =$ 0. 657). With comparable epifauna biomass in the two algal groups and higher abundances in coralline algae, average epifauna body size was smaller in coralline algae than in fleshy algae

(mean ± SE: coralline 0.0091 ± 0.0008 g, fleshy 0.0135 ± 0.0017 g dry weight; two-sample *t* test, $t_{276.95} = -2.93$, $P = 0.004$). This is consistent with the small sizes of *L. marmorata* and *B. haliotiphila* and their high abundances in coralline algae.

Although some epifauna taxa were more strongly associated with either coralline or fleshy habitats, almost no taxa with sufficient sample size were exclusive to one habitat type (Table A1). Epifauna taxon richness did not differ (mean ± SE: coralline 12.9 ± 0.3, fleshy 13.0 ± 0.4; two-sample *t* test, $t_{272.66} = -0.34$, $P = 0.731$), and community structure differed only slightly between the two algal habitats (Table 4.4, Figure 4.4A).

When accounting for other variables in addition to habitat, site and month explained most of the variation in epifauna abundance and richness, with interactions between site and habitat type (Table 4.3, Figure 4.3). Site was also the primary factor structuring the composition of epifauna communities (Table 4.4, Figure 4.4B).

Habitat features differed between coralline and fleshy algae as well as between sites. Coralline algae tended to grow more densely than fleshy algae, especially at FC and CM (Figure 4.5A). Most algae surveyed were turf algae, growing to about 3cm, except *Odonthalia*, the fleshy algae sampled at CM, which was about twice as tall (Figure 4.5B). There was also some variation in relative algal heights between sites. While *Cryptopleura/Hymenena* complex was slightly taller than coralline algae at FC, it was slightly shorter than coralline algae at CB.

Since both habitat density and epifauna abundance were higher in coralline algae than in fleshy algae, epifauna abundance increased with habitat density. After standardizing the response by habitat density, no difference in epifauna abundance

between coralline and fleshy algae remained (mean ± SE: coralline 1784 ± 137, fleshy 1582 ± 103; two-sample *t* test, $t_{271.74} = 0.34$, $P = 0.737$).

Algal habitat manipulations

In the algal habitat manipulations, experimental plots were thinned and trimmed in June and July 2013. More algae were removed in June, when treatments were initiated, than in July, when treatments were maintained. More algal volume was removed in the D manipulations than in the H manipulations (mean ± SE: D removal 16 ± 1 mL, H removal 14 ± 1 mL; two-sample *t* test, $t_{151.17} = 2.15$, $P = 0.033$). In the density reduction treatment (D), algal thalli were removed haphazardly throughout the plot, while in the height reduction treatment (H), the upper portions of thalli were systematically removed. After the initial June manipulations, algal height decreased 48% ± 3% (mean ± SE) in the H treatment and 11% ± 2% in the D treatment. Although immediate changes in algal density resulting from the manipulations could not be measured in the experimental plots, they could be inferred from the separate set of plots that were manipulated and sampled at once. In this set of plots, algal density decreased 30% ± 3% (mean ± SE) in the D treatment and increased 39% ± 8% in the H treatment. Since algal density was defined as thallus volume divided by canopy volume, the H treatment inadvertently increased algal density via a disproportionate reduction in canopy volume as thinner thallus tips were removed. Measurements of the immediate effects of manipulation, however, do not accurately reflect habitat conditions throughout the experiment since algae continued to grow until plots were sampled in August.

At the end of the experiment, the effectiveness of the two treatments was mixed. The D treatment was only effective in fleshy algae, resulting in lower algal density without changing algal height relative to control plots (Figure 4.6A-B). In coralline algae, neither algal density nor height differed between the D treatment and control plots. The H treatment was also more effective in fleshy algae. H manipulations reduced algal height in both coralline and fleshy habitats, but it also reduced density in coralline algae. No differences in epifauna abundance or richness were observed between treatments in either coralline or fleshy algae, except epifauna richness was lower in D treatment fleshy algae than in the control (Figure 4.6C-D). Overall, patterns in epifauna abundance and richness corresponded more closely with differences in algal density than differences in algal height.

While few differences in epifauna abundance and richness were found between treatments, examining habitat variables on a continuous scale provided more insight. For example, algal density was positively related to epifauna abundance (negative binomial GLM, $\chi^2 = 26.10$, $P < 0.001$) (Figure 4.7A) and richness (Poisson GLM, $\chi^2 = 12.22$, $P < 0.001$) (Figure 4.7C). In contrast, algal height was unrelated to epifauna abundance (negative binomial GLM, $\chi^2 = 0.12$, $P = 0.733$) (Figure 4.7B) or richness (Poisson GLM, $\chi^2 = 0.02$, $P = 0.898$) (Figure 4.7D). Habitat type had little influence on epifauna abundance (negative binomial GLM, $\chi^2 = 1.17$, $P = 0.279$) or richness (Poisson GLM, $\chi^2 = 3.42$, $P = 0.064$). Both algal density and site (negative binomial GLM, $\chi^2 = 18.93$, $P < 0.001$) were important predictors of epifauna abundance, but only algal density predicted epifauna richness.

Although the D and H habitat treatments themselves were not particularly instructive, examination of the epifauna removed along with algae in the D and H manipulations support the positive relationship observed between epifauna and habitat density. While only slightly more algae were removed in the D manipulations than in the H manipulations, epifauna abundance (mean ± SE: D removal, 76 ± 9; H removal, 50 ± 6 individuals; two-sample *t* test, $t_{110.94} = 2.57$, $P = 0.011$) and richness (mean ± SE: D removal 5.7 ± 0.3, H removal 3.9 ± 0.3 taxa; two-sample *t* test, $t_{88.02} = 2.99$, $P = 0.004$) were greater in samples of whole algal thalli removed during manipulations (D treatment) compared to tips of algal thalli that were removed (H treatment). This indicates that epifauna were concentrated at the base of algal thalli or in the sediment, rather than at the tips, thereby helping to explain the lack of relationship found between epifauna and algal height. Bivalves and polychaetes, which may be sediment-dwellers or attach to benthic surfaces, were particularly abundant in algae removed in the D treatment relative to the H treatment (Table 4.5). However, algal density and height accounted for little variation in epifaunal community composition in the habitat manipulation experiment (Table 4.4; algal density $R^2 = 0.02$, algal height $R^2 = 0.01$). Similar to the results in the epifauna survey, variation in epifaunal communities was relatively insensitive to all measured variables except site, which interacted with algal habitat, i.e., coralline vs. fleshy algae.

Discussion

Ecosystem engineers such as marine macroalgae play a particularly important ecological role as habitat providers. By understanding how ecosystem engineers respond

to global change, we can gain insight on whole-community changes if we know how they affect the presence and distribution of other organisms. In this study, we focused on coralline and fleshy algae because they are ecosystem engineers whose responses to ocean acidification are relatively well-studied, yet their effects on associated epifauna are unresolved. While we found differences in epifauna abundance between habitat types, rather than an inherent effect of algal identity, our study suggests that algal density was the underlying trait accounting for much of the difference in epifauna abundance. Furthermore, by conducting the study at an extended spatiotemporal scale, we found that site and month also had a large influence on epifauna abundance and richness, and the community composition of animals in algal habitats was primarily determined by site.

Epifauna were more abundant in coralline algae than in fleshy algae, which may be explained by the observation that thallus density of coralline algae was typically higher than that of fleshy algae. These results are consistent with the positive habitat complexity-species diversity relationship often observed in nature (Tews et al. 2004, Kovalenko et al. 2011). In addition, algae-epifauna associations may be dependent on body size. In our field survey, two small gastropod mesograzers, i.e., *Lacuna* and *Barleeia*, were particularly abundant in coralline algae and heavily influenced our findings because they constituted over one-third of epifauna sampled. Denser algal habitats may be characterized by smaller interstitial spaces, limiting the size of residents and providing better protection for smaller organisms, such as these two gastropods. Smaller epifauna can also occupy space more densely than larger epifauna, and since it is more difficult for them to move across algal landscapes, they may demonstrate more

habitat fidelity. In past studies, negative relationships have been found between coralline density and epifauna abundance and richness (Kelaher et al. 2001, Kelaher 2003, Kelaher and Castilla 2005), but it is possible that small-bodied organisms were less abundant in those study areas. One experiment showed that while the abundance of larger epifauna did not differ between four algae species that were studied, the abundance of smaller epifauna was highest in the most densely branched algae (Best et al. 2014). Higher algal density seems to beget higher epifauna density, as long as small epifauna are abundant.

Despite differences in epifauna abundance, we did not find differences in taxon richness or community composition between habitat types. Compared to abundance, richness and community composition retain species information but lose some or all of the fine signals carried by those species in the form of count data. Thus, richness equalizes the relative importance of common and rare species, and community composition trends toward the same outcome. The nuance overlooked in richness and community metrics is exemplified by the two gastropod mesograzers that controlled patterns of epifauna abundance. Although particularly abundant in coralline algae, these gastropods were also common in fleshy algae, so they contributed little to differences in richness and community composition between the two habitat types. Epifauna may have subtle, or even not so subtle, habitat preferences but fail to form exclusive habitat associations. Since the intertidal algae in the study area grow in a patchy, diverse mosaic pattern (Dethier 1984, Menge et al. 1993b, 2005), shifts between algal boundaries would only require mobility on the scale of centimeters. As a result, many epifauna species are habitat generalists. Rather than demonstrating a habitat preference for a particular species

of alga, epifauna may select habitats randomly or based on certain traits and associate with multiple species of algae. An early laboratory experiment demonstrated that algal habitat selection by mobile epifauna was determined by algae biomass, or habitat size (Dean and Connell 1987b). Epifauna preferred large clumps of algae to small clumps, regardless of algal identity. More recent field surveys validate the idea that epifauna occupy broad niches in turf algae (Thrush et al. 2011, Berthelsen et al. 2015).

Although positive relationships were revealed between epifauna abundance and richness vs. algal density, no relationships were found with algal height. More epifauna occupied the base of algal thalli than the tips, perhaps due to less exposure to environmental stressors. Thalli tips are more exposed to desiccating factors like heat, light, and wind when emersed at low tide and more subject to wave action when submersed at high tide. However, if epiphytes concentrate toward the tips of algal thalli, mesograzers may follow. Still, sediment and detritus accumulate around holdfasts at the base of algal thalli, providing food and an additional habitat dimension. The results of this study are consistent with previous studies that also failed to detect a relationship between epifauna and algal height (Kelaher 2003, Kelaher and Castilla 2005). However, algal height may be an influential factor for organisms, such as fish, that inhabit more of the water column (Levin and Hay 1996).

At the local scale, algae-epifauna associations can be influenced by algal density, but the main drivers of epifaunal distributions may lie at larger spatial or temporal scales. Periodic wind-driven upwelling during the spring through fall seasons is a dominant feature of the northern region of the California Current System (CCS), where this study

occurred (Huyer 1983, Snyder et al. 2003). Upwelling brings up cold, saline, nutrient-rich water from depth, which boosts primary productivity and lays the foundation for a diverse coastal marine food web (Bakun 1990, Barth et al. 2007). Our study region is characterized by upwelling that is more intermittent north of Cape Blanco, with longer intervals between upwelling and its relaxation or reversal, and less intermittent south of Cape Blanco (Connolly and Roughgarden 1998, Connolly et al. 2001, Menge et al. 2004, Menge and Menge 2013). Spatiotemporal patterns in upwelling along the CCS have been linked to processes structuring intertidal communities, both directly through recruitment or indirectly through temperature and concentrations of nutrients and phytoplankton (Wing et al. 1995, Menge et al. 1997a, 1997b, 2004, Connolly and Roughgarden 1998, Menge 2000, Connolly et al. 2001, Nielsen and Navarrete 2004, Barth et al. 2007, Broitman et al. 2008, Kavanaugh et al. 2009, Dudas et al. 2009, Menge and Menge 2013). Differences in continental shelf width can also influence the retention of larvae and phytoplankton (Menge et al. 1997a, 1997b, 2015, Kavanaugh et al. 2009, Moulton and Hacker 2011), impacting intertidal community structure at the site scale. Furthermore, at Cape Blanco, a coastal upwelling jet separates from the continental shelf and becomes a meandering equatorward jet (Barth et al. 2000). Thus, Cape Blanco serves as a biogeographic boundary, which could lead to differences in regional species pools that constrain epifauna community composition at our sites.

Our finding that epifauna abundance and richness varied monthly is not surprising given the intra-seasonal variation in upwelling and the probable short life cycles of many epifauna. Monthly differences in epifauna abundance and richness could be explained by

short-term fluctuations in large-scale environmental conditions and ecological subsidies. We also found that epifauna abundances tended to increase from late spring to late summer (Figure 4.3B), perhaps reflecting a lag in recovery after the spring transition from the turbulent winter storm season to the productive upwelling season (Largier et al. 1993). However, since the epifauna survey was only conducted from May to July/August over two years, we were unable to capture seasonal variations in epifauna over a full annual cycle. While we attempted a fall survey in 2012, field conditions become too unreliable after August to sample all four sites in a one-week tide series or in some cases, at all. A more extensive investigation of seasonal changes in epifauna would require either fewer sites, closer sites, or sampling at higher tide heights that can be reliably accessed throughout the year.

Spatial variation in upwelling at the cape-scale may help account for the large impact of site on epifauna abundance, richness, and community composition. However, for logistical reasons, our sites were unreplicated within capes, so we are unable to partition variation between regional-scale differences, like upwelling, and site-scale differences. Many site-scale factors could contribute to landscape heterogeneity affecting epifauna distributions, including coastal geomorphology, wave exposure, the tide height surveyed, habitat availability, etc. To disentangle site- and cape-scale sources of spatial variation, a nested sampling design would be necessary. For example, previous research in our study system employing a nested sampling design found that 52% of the variation in community structure was due to regional (cape) scale factors, while 27% was due to local scale factors (Menge et al. 2015). In another study, analysis of epifauna surveys in

surfgrass habitats at nine sites nested within three of our four capes (Foulweather, Perpetua, and Blanco) focused only on spatial variation at the cape scale, implying that the relative contribution of site-scale variation was minor (Moulton and Hacker 2011).

Compared to oceanic conditions and algal habitat density, algal identity was not a critical determinant for epifaunal communities, suggesting high functional redundancy between coralline and fleshy habitats. While ocean acidification is predicted to weaken coralline algae, fleshy algae seem likely to be more resistant to its effects. If deterioration of coralline algae habitats is balanced by enhancement of fleshy algae habitats, epifaunal communities may remain relatively stable. Thus, functional redundancy may help to buffer ecological communities against environmental change and potential biodiversity loss. However, the existence of functional redundancy in a strict sense is debated because it conflicts with stable coexistence, although weaker redundancy is possible (Loreau 2004). Oftentimes, redundancy is assessed based on a single functional attribute and may be overestimated in consideration of the multi-dimensional functional space that species occupy (Rosenfeld 2002).

In addition to direct habitat provision, coralline algae also provide habitat indirectly through the facilitation of two other intertidal macrophytes. Both the kelp *Saccharina sessilis* (Barner et al. 2016) and the surfgrass *Phyllospadix scouleri* (Turner 1983) preferentially recruit to articulated coralline surfaces. More than 90% of *Saccharina* recruits were found on coralline turfs (Barner et al. 2016), and coralline turfs are the predominant substrate for *Phyllospadix* seeds (Turner 1983). Hence, although these non-calcifying macrophytes are considered to be tolerant of ocean acidification

(Koch et al. 2013), their propagation, especially that of *Saccharina*, is dependent on coralline algae. From a more general perspective, coralline algae play an important role in successional dynamics by promoting the diversity of later colonists (Asnaghi et al. 2015). Just as tropical crustose coralline algae release chemical cues that are important for coral settlement (Morse et al. 1988, Heyward and Negri 1999, Harrington et al. 2004, Webster et al. 2004, Doropoulos et al. 2012), temperate articulated coralline algae may provide unique settlement cues for both algae and invertebrates. Hence, functional redundancy between coralline and fleshy algae seems limited. Although declines in coralline algae anticipated as a result of ocean acidification will not necessarily reduce turf habitat for turf-associated epifauna, the role of coralline algae in recruitment facilitation could influence epifaunal communities via effects on kelp and surfgrass habitats.

The effects of ecosystem engineers are both context- and scale-dependent (Jones et al. 1994, Wright and Jones 2006). Intertidal turf-forming algae may be important ecosystem engineers in the context of a whole site encompassing various habitats, including mussel beds, kelp, surfgrass, sand, gravel, etc. However, by restricting our context within a relevant framework for ocean acidification, we found that coralline and fleshy turf algae subcategories were not distinct enough from each other to host unique epifaunal communities, despite differences in epifauna abundances. On the other hand, conducting our investigations on a broad scale revealed that site/cape had the biggest overall impact on epifauna relative to the other variables considered, suggesting large-scale oceanic processes are the primary driver of epifaunal community structure. A

productive direction for future research may be to assess the persistence of biogeographic patterns in community structure as climate forcing impacts on upwelling systems intensify (Bakun 1990, Schwing and Mendelssohn 1997, Snyder et al. 2003, King et al. 2011, Iles et al. 2012).

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Figures

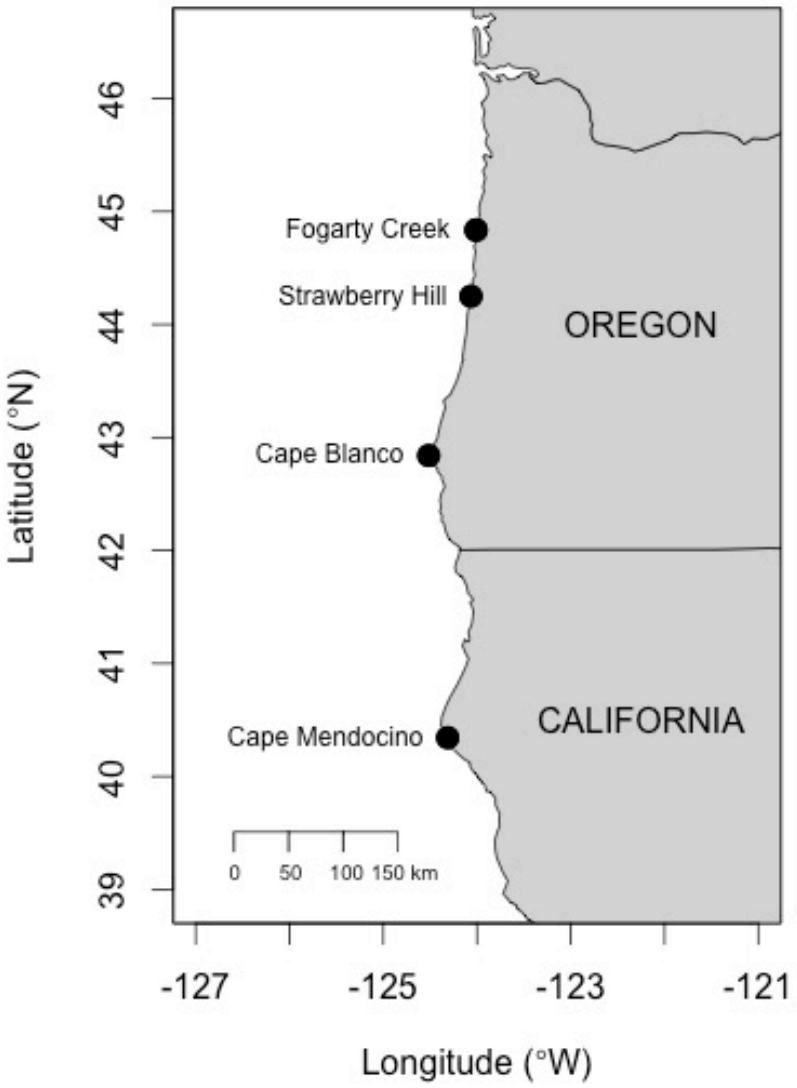

Figure 4.1. Map of study sites along the Oregon-California coast.

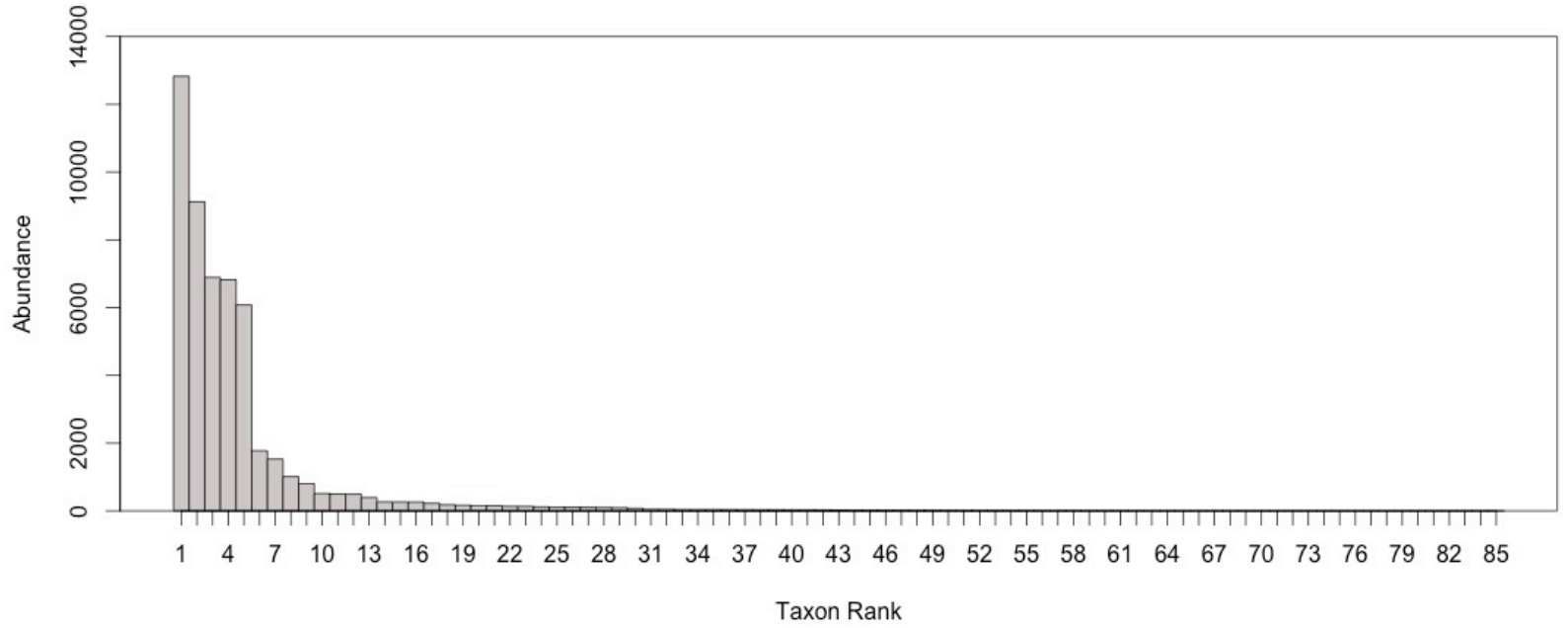

Figure 4.2. Rank abundance curve of epifauna surveyed, representing 52,240 organisms belonging to 85 taxa. The top five ranked taxa (*Lacuna marmorata*, *Mytilus* spp., *Synidotea ritteri*, gammaridean amphipods, and *Barleeia haliotiphila*, respectively) comprise 80% of the organisms surveyed.

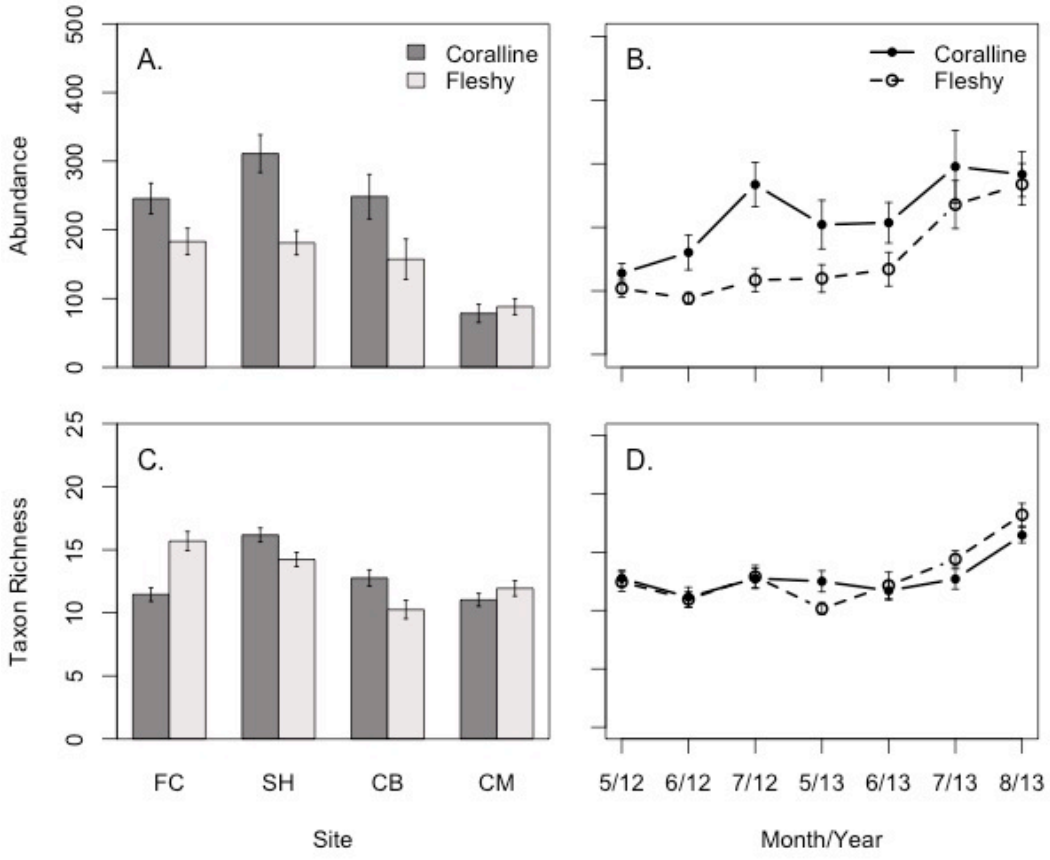

Figure 4.3. Mean abundance and richness (± SE) of epifauna surveyed in coralline and fleshy algal habitats between sites (A, C) and over time (B, D).

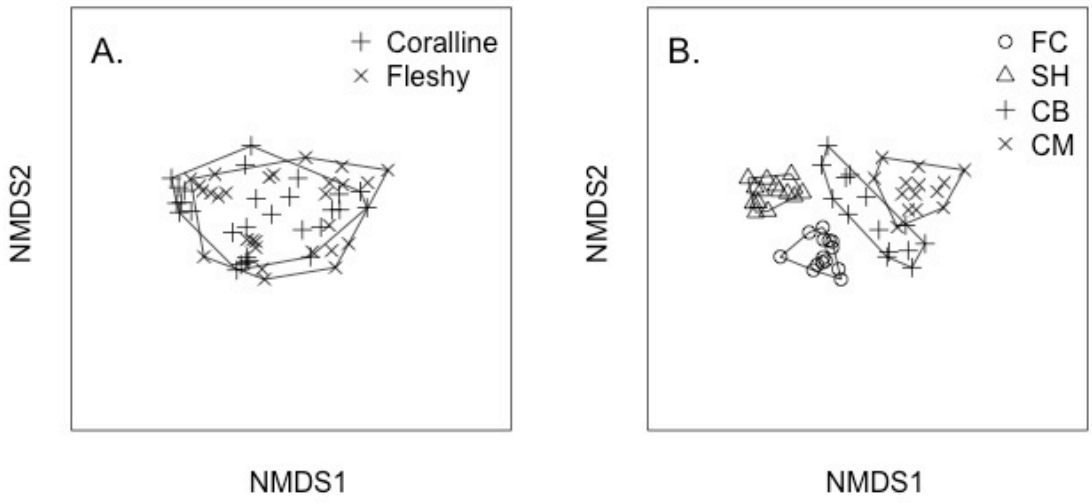

Figure 4.4. Two-dimensional non-metric multidimensional scaling (NMDS) ordination of survey samples, averaged across replicates, in epifauna taxon space (stress = 0.15). To simplify plots, epifauna taxa are not shown. The two plots are the same ordination, grouped by A) habitat type and B) site. More separation between samples grouped by site than by habitat type indicates that epifauna communities are more distinct between sites than between habitat types.

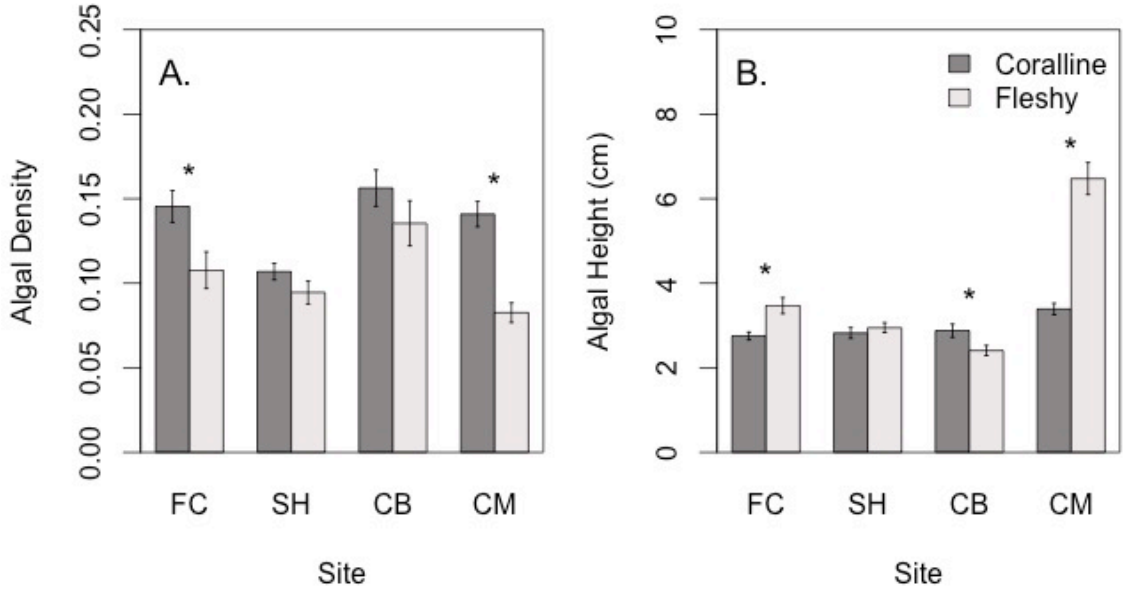

Figure 4.5. Comparison of coralline and fleshy algal habitats sampled at different sites in the epifauna survey, including mean (± SE) A) density and B) height. Asterisks denote significant differences between habitat types within a site.

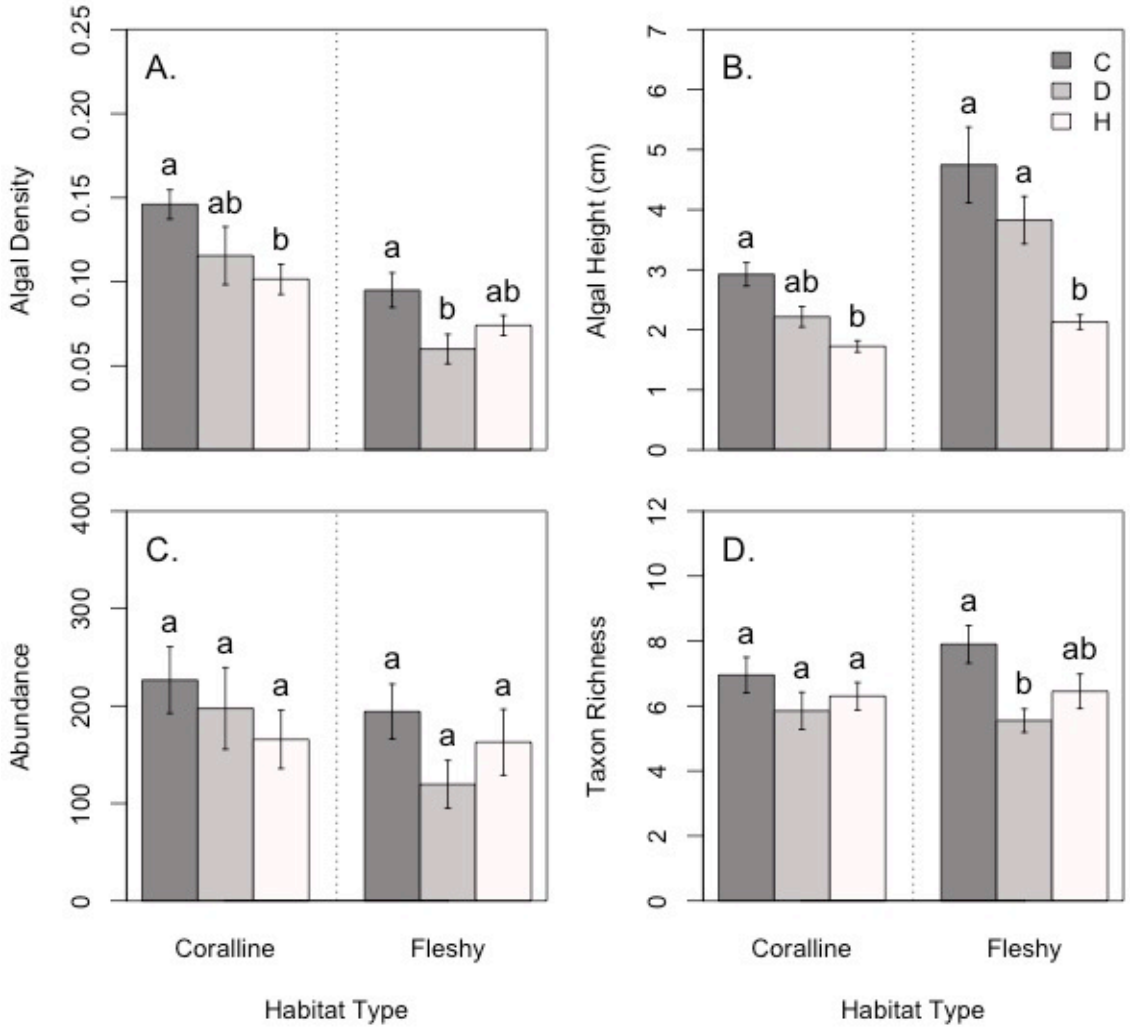

Figure 4.6. Comparison of treatments between habitat types in the algal habitat manipulations, including mean (± SE) A) algal density and B) height, and C) epifauna abundance and D) richness. Letters indicate significant differences between treatments within a habitat type. C = control, D = density manipulation, and H = height manipulation.

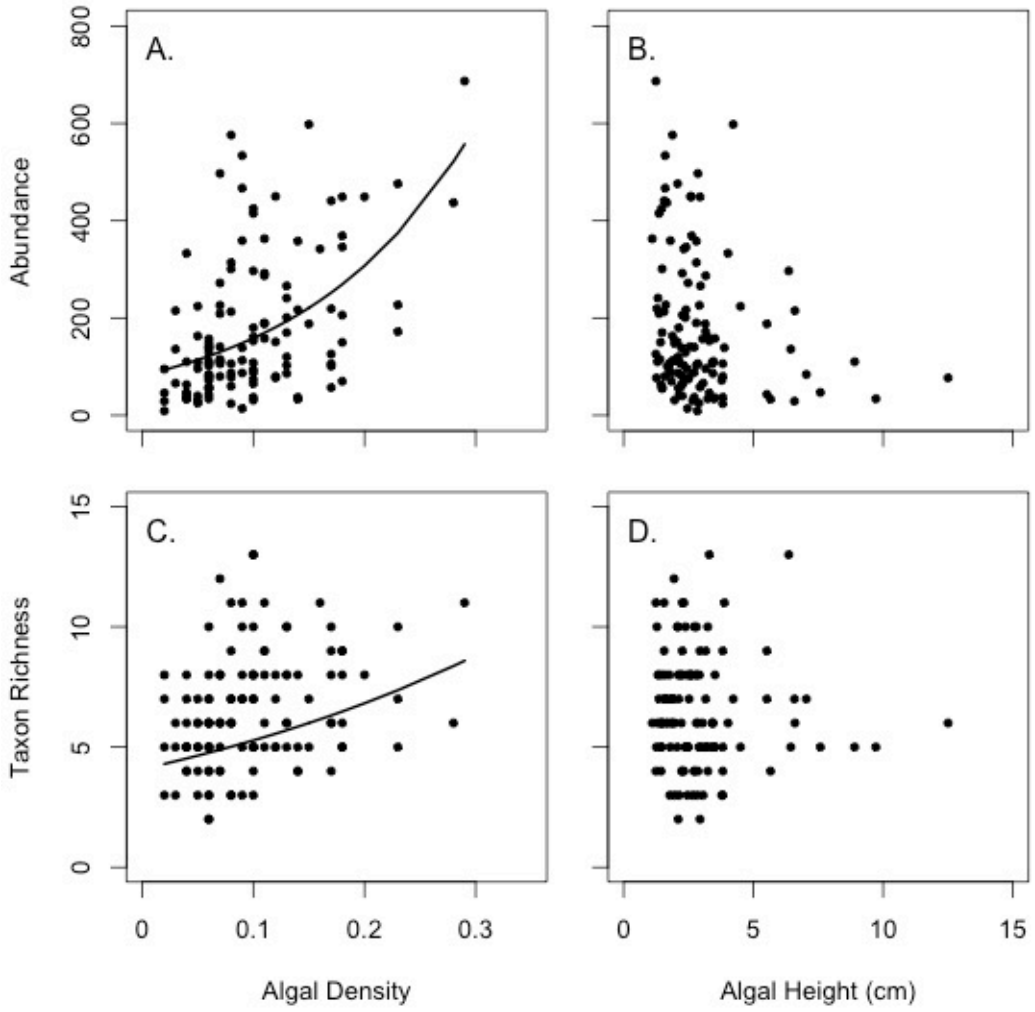

Figure 4.7. Relationships between epifauna and algal habitats, including epifauna abundance and A) algal density and B) height, as well as epifauna richness and C) algal density and D) height. Fitted lines from GLMs demonstrate significant positive relationships of epifauna abundance and richness with algal density, while no relationships are drawn with algal height.

Tables

Table 4.1. Site locations and the algae sampled at each site. Proportions of coralline species were calculated based on total dry biomass of each species collected in coralline samples at each site.

Site	**Location**	**Coralline algae**	**Fleshy algae**
Fogarty Creek (FC)	44.84ºN, 124.06 ºW	*Corallina vancouveriensis* (33%) *Bossiella plumosa* (67%)	*Cryptopleura*/ *Hymenena* complex
Strawberry Hill (SH)	44.25ºN, 124.11 ºW	*Corallina vancouveriensis* (54%) *Bossiella plumosa* (46%)	*Dilsea californica*
Cape Blanco (CB)	42.84ºN, 124.57 ºW	*Corallina vancouveriensis* (51%) *Bossiella plumosa* (49%)	*Cryptopleura*/ *Hymenena* complex
Cape Mendocino (CM)	40.34ºN, 124.36 ºW	*Corallina vancouveriensis* (95%) *Bossiella plumosa* (5%)	*Odonthalia floccosa* f. *comosa*

Table 4.2. Epifauna abundances in the epifauna survey summarized by taxonomic group. Abundances of all 85 taxa are included in Table A1.

Taxonomic Group	**Number of Taxa**	**Cumulative Abundance**	**Abundance in Coralline Samples**	**Abundance in Fleshy Samples**
gastropod	17	19,461	13,325	6,136
bivalve	5	9,711	4,741	4,970
amphipod	3	8,678	4,411	4,267
isopod	10	8,169	5,224	2,945
polychaete	12	4,272	2,174	2,098
holothurian	2	517	493	24
polyplacophoran	6	365	118	247
nemertean	1	267	71	196
decapod	9	168	65	103
cirriped	1	154	62	92
asteroid	3	146	92	54
oligochaete	1	97	31	66
insect	2	53	24	29
tanaid	1	43	11	32
anthozoan	2	39	31	8
sipunculid	1	33	18	15
ophiuroid	1	19	0	19
echinoid	1	14	9	5
pycnogonid	1	13	7	6
platyhelminth	1	8	0	8
bryozoan	1	6	5	1
fish	2	5	4	1
arachnid	1	1	0	1
hemichordate	1	1	1	0
Total	85	52,240	30,917	21,323

Table 4.3. Results of ANOVAs testing the effects of site, month, year, habitat type, and site × habitat type on epifauna abundance and richness in the epifauna survey. Significant *P*-values are shown in boldface.

Source of variation	**df**	**SS**	**MS**	***F***	***P***
log(Abundance)					
Site	3	10.78	3.59	46.65	**< 0.001**
Month	3	4.81	1.60	20.83	**< 0.001**
Year	1	0.39	0.39	5.11	**0.025**
Habitat type	1	1.75	1.75	22.74	**< 0.001**
Site x habitat type	3	1.22	0.41	5.27	**0.002**
Residuals	268	20.64	0.08		
Total	279	39.59			
Richness					
Site	3	675.80	225.30	22.99	**< 0.001**
Month	3	1030.50	343.50	35.07	**< 0.001**
Year	1	1.00	1.00	0.11	0.744
Habitat type	1	2.10	2.10	0.21	0.647
Site x habitat type	3	502.20	167.40	17.09	**< 0.001**
Residuals	268	2625.50	9.80		
Total	279	4837.10			

Table 4.4. Results of PERMANOVAs on Bray-Curtis similarities comparing epifauna community composition across various factors in both the epifauna survey and the algal habitat manipulations. Significant *P*-values are shown in boldface.

Source of variation	**df**	**SS**	**MS**	***F***	***R²***	***P***
Epifauna community (survey)						
Site	3	17.60	5.87	51.34	0.32	**< 0.001**
Month	3	2.38	0.79	6.93	0.04	**< 0.001**
Year	1	0.89	0.89	7.82	0.02	**< 0.001**
Habitat type	1	1.18	1.18	10.30	0.02	**< 0.001**
Site x habitat type	3	1.82	0.61	5.30	0.03	**< 0.001**
Residuals	268	30.63	0.11		0.56	
Total	279	54.50				
Epifauna community (manipulations)						
Site	3	9.82	3.27	32.10	0.42	**< 0.001**
Habitat type	1	0.53	0.53	5.20	0.02	**< 0.001**
Algal density	1	0.49	0.49	4.81	0.02	**< 0.001**
Algal height	1	0.13	0.13	1.25	0.01	0.253
Site x habitat type	3	1.20	0.40	3.91	0.05	**< 0.001**
Residuals	110	11.22	0.10		0.48	
Total	119	23.39				

Table 4.5. Epifauna abundances in the algae removed during habitat manipulations summarized by taxonomic group.

Taxonomic Group	**Number of Taxa**	**Cumulative Abundance**	**Abundance in D Removals**	**Abundance in H Removals**
gastropod	9	5,921	3,232	2,689
amphipod	2	1,822	1,241	581
isopod	7	1,580	1,059	521
bivalve	5	426	343	83
polychaete	8	177	134	43
oligochaete	1	36	19	17
polyplacophoran	1	26	15	11
nemertean	1	25	19	6
insect	2	10	1	9
decapod	5	7	6	1
ophiuroid	1	4	4	0
asteroid	1	3	3	0
cirriped	1	3	1	2
holothurian	1	1	1	0
platyhelminth	1	1	1	0
pycnogonid	1	1	1	0
sipunculid	1	1	1	0
tanaid	1	1	1	0
Total	49	10,045	6,082	3,963

CHAPTER 5 – GENERAL CONCLUSION

There is valid concern that ocean acidification (OA) will cause widespread changes in biological systems, ultimately threatening biodiversity (Hale et al. 2011). The effects of OA may be compounded by multiple environmental stressors acting synergistically and species perpetuating and amplifying effects through interaction networks. Furthermore, research has revealed additional mechanisms, besides calcification, through which OA can influence organisms, e.g. behavioral changes, expanding impacts to non-calcifying organisms (Rosa and Seibel 2008, Munday et al. 2009). While the threats of OA are extensive, they are also variable (Ries et al. 2009, Kroeker et al. 2010). Some species and ecosystems will be less sensitive to OA than others. My dissertation research demonstrates the potential for ecological communities to remain resistant to the effects of ocean acidification.

In Chapter 2, I found that elevated pCO_2 moderately reduced growth rates in *Corallina vancouveriensis*, with stronger effects in treatments with varying levels of pCO_2. However, elevated pCO_2 did not negatively affect other measured responses, nor did it produce synergistic effects when combined with severe desiccation. Under high pCO_2, coralline bleaching and net calcification rates were not significantly affected, and net photosynthesis rates increased. The negative effects of OA on *C. vancouveriensis* may be somewhat offset by energy gains via photosynthesis. Furthermore, there is evidence that *C. vancouveriensis* in upwelling regions may be adapted to high pCO_2 (Padilla-Gamiño et al. 2016). In general, the effects of severe desiccation on *C. vancouveriensis* were more negative than those of high pCO_2 in our experiment.

Desiccation caused substantial bleaching, reduced net photosynthesis rates, and stemmed growth. However, depending on the severity of bleaching, coralline algae can recover from bleaching by regaining pigments (Littler 1972, Figueiredo et al. 2000), shedding bleached portions and growing new tissue (McCoy and Kamenos 2015), or being replaced by new recruits. Coralline algae are known for their persistence (Stewart 1989, McCoy and Kamenos 2015), and despite negative responses to elevated pCO_2 and desiccation in the laboratory, some populations may be more resistant or resilient to environmental change than anticipated.

One factor threatening the resistance of coralline populations to future change is a potential increase in the competitive abilities of fleshy algae under OA. For example, at a CO_2 vent site, coralline algae recruitment and early growth did not differ between high and low pCO_2 zones, but over time, coralline algae were overgrown by fleshy algae in high pCO_2 conditions (Kroeker et al. 2013b). However, in Chapter 3, I found that the lack of competition between coralline and fleshy algae in Oregon rocky intertidal habitats (van Tamelen 1996) has persisted over the last three decades, during a period of rapid acidification (Doney et al. 2009). In reciprocal removal experiments, neither coralline nor fleshy algae grew into adjacent space created from clearances of potential competitors. Observations over two years revealed seasonal fluctuations in the abundance of fleshy algae, particularly *Dilsea californica* in wave exposed areas, with reductions in fleshy algal cover over winter and increases over summer. Thus, pronounced seasonal environmental shifts may take precedence over gradual changes associated with OA in controlling algal abundances and interactions. As OA continues to intensify, the potential

for changes in species interactions increases, but coralline and fleshy algae in our system have not yet started to compete, demonstrating a certain degree of resistance to environmental change.

In Chapter 4, I explored the implications for turf-associated epifaunal communities if coralline populations declined under OA while fleshy algae thrived. Comparisons of epifaunal assemblages inhabiting coralline and fleshy turfs revealed that coralline habitats supported higher epifaunal abundances. Coralline turfs grew more densely than fleshy turfs, likely containing smaller interstitial spaces, which facilitated occupancy by abundant, small-bodied organisms. However, epifaunal richness and community composition were similar across turf types, only differing between sites, indicating a high degree of redundancy in habitat provision by coralline and fleshy turfs, despite differences in algal morphology. Ecological redundancy acts as an insurance policy against species-specific disturbances, increasing ecosystem resilience (Naeem 1998). Following potential declines in coralline turfs due to OA, epifaunal communities may be resistant to change given the persistence and expansion of fleshy turf habitats. Although susceptible to the direct effects of OA, epifauna will be less vulnerable to indirect effects of OA manifested through habitat changes.

Through the combination of several different approaches to integrate the impacts of climate change from species to communities, my dissertation highlights the capacity of biological systems to resist change. Regarding multiple anthropogenic stressors, my results are consistent with meta-analyses revealing that interactive effects on primary producers tend to be additive or antagonistic rather than synergistic (Crain et al. 2008,

Strain et al. 2014). Since primary producers form the foundation of food webs, their resistance to synergistic impacts of multiple stressors helps to maintain ecosystem stability. Cumulative stressor effects on community level responses also tend to be antagonistic while those on population level responses are more synergistic, suggesting that species interactions dilute the negative effects of abiotic stressors (Crain et al. 2008). Similarly in my work, I found that the absence of negative species interactions and redundancy in positive species interactions ameliorated the negative impacts predicted under climate change. The direct effects of OA on coralline and fleshy algae are expected to be amplified by heightened competition (Hofmann et al. 2012a, Kroeker et al. 2013b). However, I failed to detect increased competition in the field between coralline and fleshy algae over the last 30 years, perhaps because abundances of fleshy algae are strongly regulated by seasonal environmental fluctuations that outweigh gradual changes in seawater carbon chemistry. The direct effects of OA on coralline algae are also expected to influence epifaunal communities inhabiting coralline turfs (Garrard et al. 2014, Fabricius et al. 2014). However, I discovered that the epifaunal communities inhabiting coralline turfs were not distinct from those in fleshy turfs and are thus resistant to potential OA-induced declines in coralline turf abundances.

Moving forward, there is a critical need to predict the effects of climate change on not only ecological communities but also entire ecosystems. While we may quantify the responses of organisms, populations, and communities to environmental change, it is unclear how serious these consequences are for ecosystem functioning and stability. Therefore, a top research priority should be the development and refinement of

ecosystem indicators and identification of critical thresholds that provide a fundamental link between various perturbations and ecosystem health or condition. Since there are too many responses to measure and not all are equally informative, it is important to select the few that provide the most pertinent ecosystem perspectives and help signal movement toward limits. For example, climate scientists have agreed on a global warming threshold of 2° C in order to avoid the most destructive and dangerous effects of climate change (Anderson and Bows 2011). This guideline has gained wide attention, increasing the relevance of climate change by anchoring its consequences to a simple number. By establishing and enhancing the accuracy of ecosystem indicators and thresholds, ecologists will be able to more effectively communicate and collaborate with managers and policymakers to set goals and take action on climate change.

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APPENDIX A – CHAPTER 4 SUPPLEMENTAL TABLE

Table A1. Epifauna abundances in the epifauna survey for all 85 taxa identified.

Taxa	**Cumulative Abundance**	**Abundance in Coralline Samples**	**Abundance in Fleshy Samples**
Lacuna marmorata	12827	9130	3697
Mytilus	9123	4525	4598
Synidotea ritteri	6893	4438	2455
Gammaridea	6823	2690	4133
Barleeia haliotiphila	6079	4016	2063
Corophiidae	1776	1681	95
Syllidae	1530	639	891
Terebellidae	1013	690	323
Sabellida	806	378	428
Cucumaria pseudocurata	516	492	24
Nereididae	500	272	228
Idotea schmitti	496	388	108
Modiolus	397	143	254
Hoplonemertea	267	71	196
Cyanoplax dentiens	265	59	206
Idotea wosnesenskii	263	93	170
Lottia spp.	229	88	141
Phyllodocidae	183	58	125
Dynamenella sheareri	169	114	55
Polynoidae	160	75	85
Pollicipes polymerus	154	62	92
Hiatella arctica	140	52	88
Leptasterias hexactis	138	89	49
Cirolana harfordi	119	59	60
Onchidella borealis	116	27	89
Gnorimosphaeroma oregonense	110	72	38
Nucella canaliculata	108	40	68
Ianiropsis kincaidi	103	57	46
Tubificidae	97	31	66
Caprellidae	79	40	39
Dipteran larva	52	24	28
Oedignathus inermis	51	26	25
Pugettia gracilis	44	14	30

Table A1. (Continued)

Taxa	Cumulative Abundance	Abundance in Coralline Samples	Abundance in Fleshy Samples
Tanaidacea	43	11	32
Tellinidae	43	14	29
Arabella iricolor	41	27	14
Mopalia muscosa	39	16	23
Anthopleura elegantissima	37	29	8
Cerithiopsis berryi	36	10	26
Katharina tunicata	33	25	8
Phasocolosoma agassizii	33	18	15
Dodecaceria fewkesi	31	30	1
Gastropoda sp.	24	2	22
Ophiopholis kennerlyi	19	0	19
Pagurus hirsutiusculus	19	11	8
Glebocarcinus oregonensis	18	3	15
Tonicella lineata	17	10	7
Amphissa columbiana	14	3	11
Strongylocentrotus purpuratus	14	9	5
Pycnogonida	13	7	6
Evalea tenuisculpta	12	6	6
Decapoda megalopa	11	5	6
Pugettia producta	11	4	7
Idotea montereyensis	10	1	9
Mopalia spp.	10	7	3
Lasaea adansoni	8	7	1
Notocomplana acticola	8	0	8
Pachycheles rudis	8	1	7
Pisaster ochraceus	7	3	4
Flustrellidra corniculata	6	5	1
Onoba mighelsii	5	0	5
Petrolisthes eriomerus	4	1	3
Alvania compacta	3	0	3
Exosphaeroma amplicauda	3	1	2
Lirobittium purpureum	3	1	2
Munnidae	3	1	2
Phytichthys chirus	3	2	1

Table A1. (Continued)

Taxa	Cumulative Abundance	Abundance in Coralline Samples	Abundance in Fleshy Samples
Polychaeta sp. 1	3	1	2
Polychaeta sp. 2	3	3	0
Anthopleura xanthogrammica	2	2	0
Oligocottus snyderi	2	2	0
Petrolisthes cinctipes	2	0	2
Cirratulidae	1	1	0
Crepidula sp.	1	0	1
Doris montereyensis	1	0	1
Enteropneusta	1	1	0
Eupentacta quinquesemita	1	1	0
Henricia leviuscula	1	0	1
Leptochiton rugatus	1	1	0
Lirularia	1	0	1
Neomolgus littoralis	1	0	1
Ocinebrina interfossa	1	1	0
Ocinebrina minor	1	1	0
Polychaeta sp. 3	1	0	1
Staphylinidae	1	0	1
Total	52,240	30,917	21,323