AN ABSTRACT OF THE DISSERTATION OF

Margot Hessing-Lewis for the degree of Doctor of Philosophy in Zoology presented on September 16, 2011.

Title: Eelgrass-macroalgae Interactions; Context-dependency in Upwelling-influenced Estuaries;

Abstract approved:

Sally D. Hacker Bruce A. Menge

This dissertation investigates the context-dependency of species interactions between seagrass and macroalgae in upwelling-influenced estuaries. In all coastal systems, nutrient loading is multidirectional, resulting from mostly freshwater and marine inputs. The directionality of nutrient inputs may affect the rate of supply of organic matter to the system. In systems where freshwater nutrient loading dominates, and has increased through time, research shows that blooms of fast-growing macroalgae often result in loss of critical seagrass habitats. In upwelling-influenced systems, marine-based nutrient inputs dominate during the summer, also resulting in blooms of ulvoid macroalgae during these productive months. The dominance of marine nutrients in these estuaries, coupled with additional variation in the physicochemical characteristics of seagrass beds, present novel contexts to study the outcomes of species interactions between the seagrass (*Zostera marina L*. (eelgrass) and ulvoid macroalgae. I studied these interactions at two different spatial scales that both address the relative importance of marine versus terrestrial nutrient sources on interaction

outcome. Regionally, I studied between-estuary, latitudinal patterns in species interactions relative to differences in marine and terrestrial drivers of nutrient loading. Within an estuary, I also compared interactions among zones along an estuarine gradient, where nutrient patterns were reflective of the relative contribution of marine-based nutrients.

At both scales of inquiry I employed both observational and experimental approaches to quantify species interaction dynamics. At the regional scale, I used a 5-year observational dataset from four estuaries along the Oregon and Washington coasts to study the relationship between eelgrass and ulvoid macroalgae (Chapter 2). Across latitudes that span ~220 km, macroalgal production was highest in the southern estuaries, and associated with decreased eelgrass production compared to the northern estuaries. However, through time, no estuarine site, regardless of its macroalgal biomass, was associated with declining eelgrass biomass. Contrary to systems where macroalgal production is driven by terrestrial inputs, I found that blooms in upwelling-influenced systems were associated with both marine and terrestrial drivers of nutrient inputs and production.

In Coos Bay (South Slough), at the within-estuary scale, I also found differences in macroalgal and eelgrass biomass among sites along an estuarine gradient. Here too, based on a 2-year seasonal dataset of producer dynamics, I found no temporal relationship between eelgrass and macroalgae producer dynamics (Chapter 3). I used a comparative-experimental framework to understand the impact of macroalgal manipulations (additions and removals) on interactions with eelgrass along this gradient. In intertidal seagrass beds in the marine and polyhaline zones of the estuary I found that interaction strength was neutral and sometimes positive. However, in the riverine zone, interaction strength was negative, caused by decreased eelgrass density following macroalgal manipulation.

To further examine the mechanisms informing interaction outcomes in the marine zone, a large-scale macroalgal manipulation was conducted, coupled with a mesocosm experiment (Chapter 4). For the mesocosm experiment I manipulated macroalgae and nutrients as in the field, but found dissimilar results. In the mesocosms, where water movement was limited and no tidal action occurred, negative effects of macroalgal addition were found. These were associated with increased light attenuation and decreased sediment oxygen levels. Contrary to these results, I found no macroalgal, or covariate effects in the field experiment. I also manipulated water column nutrients in both experiments, and found limited effects of nutrient enrichment on eelgrass, but not macroalgae, in the mesocosm experiment.

Throughout these studies I demonstrated that the mechanisms determining context-dependency in upwelling-influenced estuaries are informed by physical and biogeochemical conditions, coupled with high ambient marine-derived nutrient concentrations. These findings are important to coastal management because they suggest that the strength, direction and mechanisms of interactions are shaped by local abiotic conditions and long-term nutrient regimes, rather than high nutrient concentrations per se. Given the shifting nature of nutrient concentrations in coastal waters associated with both coastal development and climate change, knowledge of context dependency can also be used to assess and forecast future changes in species interactions.

Eelgrass-macroalgae Interactions; Context-dependency in Upwelling-influenced Estuaries

by
Margot Hessing-Lewis

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented September 16, 2011
Commencement June 2012

Doctor of Philosophy dissertation of Margot Hessing-Lewis presented on September 16, 2011.

APPROVED:

Co-Major Professor, representing Zoology

Co-Major Professor, representing Zoology

Chair of the Department of Zoology

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.

Margot Hessing-Lewis, Author

ACKNOWLEDGEMENTS

My co-advisors, Drs. Sally Hacker and Bruce Menge, as well as the labs they oversee (together, dubbed Hackmenge), have provided me with the intellectual structure, access to resources, funding, and editorial strength with which to pursue and complete this dissertation. Both are ardent field workers, and as such, have been role models to me; supporting the same rigorous, ground-truthed, team-based, empirical approach to research as I have sought. Sally Hacker has been especially important to my work, facilitating and participating with all of my field and lab-work and being an invaluable contributor to my research direction. Through their guidance I have been literally and ecologically schooled.

I am thankful to three major sources of funding for enabling my research. The National Estuarine Research Reserve (NERR) Graduate Research Fellowship supported my research throughout my PhD and especially my field work at the South Slough NERR. There, I thank Dr. Steve Rumrill for helping to facilitate and guide my research. Steve, as well as Alicia Helms, Adam DeMarzo and other NERR staff helped me with fieldwork, and allowed me to use their facilities. I am also indebted to them for the excellent work they do maintaining their longterm ecological datasets which were crucial to my research. The Western Regional Aquaculture Center supported portions of my research, and especially Willapa Bay research with Dr. Hacker. There, we collaborated with Dr. Brett Dumbauld and Lee McCoy (USDA) and Dr. Jennifer Ruesink (University of Washington). I am especially thankful to the field help and access to field sites provided by our collaborators. A Mamie Markam

Scholarship supported my work at the Hatfield Marine Sciences Center. There, I greatly appreciate the help of staff in constructing and maintaining the mesocosms, and the support of the Center in general.

My research would not have been possible without the help of many excellent field assistants which endured muddy conditions, early mornings, and many long hours measuring eelgrass blades, etc. In an effort of reciprocity, they were compensated with many natural history moments, camaraderie, scrumptious treats and lots of coffee. I am especially thankful to John Schaefers, Jeremy Henderson, Sea-oh McConville, Micah Rogers and Hollis Bailey who contributed hard labor, good nature and outstanding intellectual capacity in this regard.

As a member of the “Hackmenge” lab and the Zoology Department at Oregon State University I have been supported and encouraged throughout my dissertation. I am especially thankful to the department for teaching assistantships throughout my tenure, especially my participation in the marine biology field course. I thank all of the lab members, and departmental faculty and staff for making it a fabulous environment to be a part of. I am especially thankful to my office-mates Phoebe Zarnetske and Orissa Moulton; my friends and lab-mates Dafne Eerkes-Medrano, Jeremy Henderson, Alison Iles, Sarah Close, Jeremy Rose (as well as many former and future lab members); statistical and R help from Tarik Gouhier; and many other awesome and kind Zoology grad students.

Extra-departmental friends and family were crucial to the completion of my fieldwork, and their physical prowess was well tested in the field. Sandra Uesugi helped in the field and on her bicycle, and many others were corralled into the labor of my love in the mud. My family helped on numerous occasions and was instrumental in the final stages of my experiments. Melody and I braved hailstorms at Fossil Point. Jay and I measured eelgrass and macroalgae galore. Jeremy dragged fencing through the mud, sang Neil Young lullabies to the estuary, and kept things light with his call of “This is science!” Thank you to all those who “do” science, who inform solutions to the world’s environmental problems, and who encourage the next generation of field workers and natural historians to come. We need them.

CONTRIBUTION OF AUTHORS

Chapter 3: Dr. Steve Rumrill facilitated research at the South Slough National Estuarine Research Reserve and provided input on the writing of the manuscript.

Chapter 4: Jeremy Henderson and Sea-oh McConville assisted with fieldwork and data acquisition and participated with writing the manuscript.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

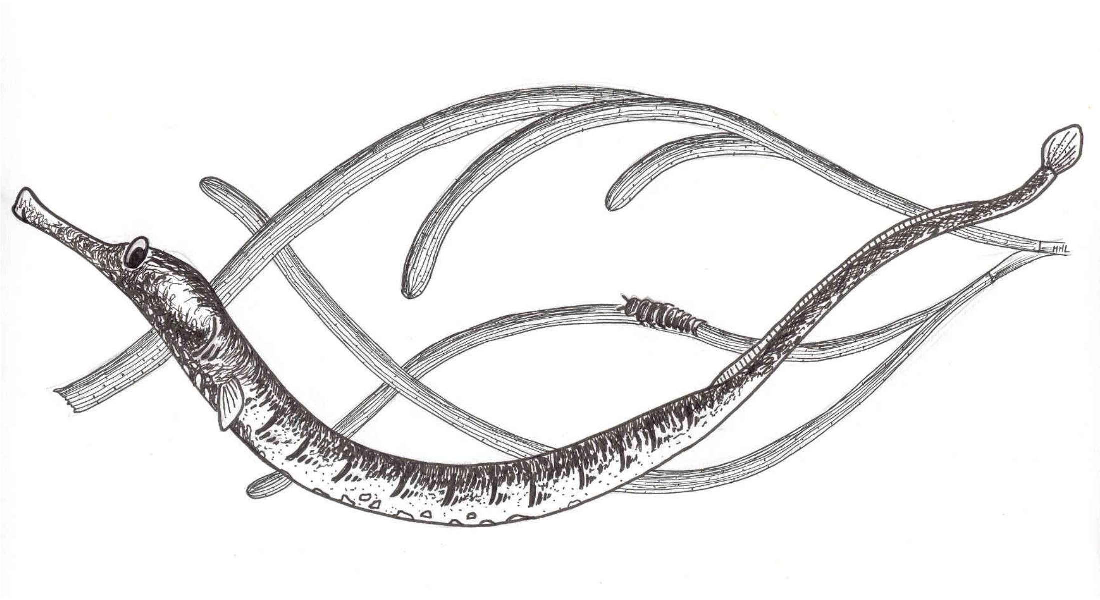
LIST OF APPENDICES

LIST OF APPENDIX FIGURES

LIST OF APPENDIX TABLES

DEDICATION

To the *Zostera marina* of the Pacific Northwest.



Eelgrass-macroalgae Interactions; Context-dependency in Upwelling-influenced Estuaries

1 – General Introduction

Context-dependent Interactions

Understanding how biotic and abiotic context shapes the strength of species interactions is a primary goal in modern ecology (Agrawal et al. 2007). Spatiotemporal variability in species interactions, be they positive, negative or neutral, can often be explained by underlying environmental context (Menge and Sutherland 1987, Hacker and Gaines 1997, Menge 2003, Maestre et al. 2009). For instance, environmental gradients and their associated physicochemical and biological attributes are often used to explain the interplay between common ecological forces that control community dynamics such as competition and facilitation (Hacker and Gaines 1997), top-down and bottom-up control of ecosystem dynamics (Menge 1992, Hacker and Bertness 1995, Burkepile and Hay 2006) and the prevalence of trophic cascades (Shears et al. 2008). These studies are also necessary in order to apply general knowledge of ecological interactions to management of complex natural systems (e.g., Crain and Bertness 2006, Hacker and Dethier 2006, Salomon et al. 2010).

This dissertation illustrates the context-specificity of interactions between ulvoid macroalgae and the seagrass *Zostera marina* L. (commonly known as "eelgrass") in upwelling-influenced Pacific Northwest estuaries. Throughout the dissertation chapters I investigate the physicochemical factors influencing the outcome of species interactions. I show how these factors inform spatial (between and within estuaries) and temporal (inter-annual and seasonal) context dependency, and application of these interactions to coastal management.

Eutrophication and Management

Eutrophication is one of the primary causes of global seagrass decline (Short and Wyllie-Echeverria 1996, Orth et al. 2006, Waycott et al. 2009). Coastal systems affected by eutrophication are increasing globally, primarily in association with land-based delivery of nutrients to coastal water (Doney 2010). Following increased nutrient loading, producer dominance in estuaries often follows a transition from rooted macrophytes (e.g., seagrasses) to macroalgae and phytoplankton (Duarte 1995). The progression in producer response is used by scientists and management agencies to signal the onset of serious change in the marine environment and assess the severity of eutrophication (Bricker et al. 2008). Prevention of seagrass declines are often key to management of coastal systems because of the critical ecosystem functions performed by this habitat, including provision of habitat for economically valuable fish and invertebrate species, nutrient cycling, mitigation of waves and currents, and as a global sink for carbon dioxide (Duarte 2000, Duarte et al. 2010, Antón et al. 2011, Barbier et al. 2011). While land-based eutrophication resulting in macroalgal blooms is a dominant cause of seagrass loss, a growing body of knowledge points to system-specific attributes that may affect response to nutrient enrichment (Cloern 2001). Knowledge of the context-dependency of these responses is therefore necessary for management of seagrass, and important for forecasting the effects of macroalgal blooms in different coastal systems and under changing climate.

Seagrass-macroalgae Interactions

In order to understand the context-dependency of seagrass-macroalgal interactions, it is necessary to consider the mechanisms informing their outcomes. The primary mechanisms by which large accumulations of macroalgae interact with seagrass beds include light limitation and alterations of biogeochemistry within the seagrass meadow water column and sedimentary environment (reviewed by McGlathery 2001, Burkholder et al. 2007, Nelson 2009). These mechanisms result from both the direct and indirect effects of nutrient enrichment, and have the potential to produce self-accelerating feedbacks within coastal systems, interacting with each other as they change the dynamics of the system (Duarte 1995). For instance, water column light attenuation caused by the canopy formation and shading can result in secondary biogeochemical effects. When macroalgae mats are sufficiently large, lack of light penetration within the interior of canopies can cause senescence of the macroalgae itself due to self-shading. As dead macroalgae tissues are remineralized this can cause increased levels of ammonium in the sediment and water column, which can potentially have toxic consequences on seagrass (Krause-Jensen et al. 1996, Hauxwell et al. 2001). Within the sediment, the decomposition of macroalgae material in anoxic sediments can further decrease redox potential and increase the production of sediment sulfides, which can reduce the productivity of seagrass by affecting respiration, nutrient acquisition and other metabolic processes (Pregnall et al. 1984, Burkholder et al. 2007).

While most research has investigated the negative mechanisms responsible for seagrass declines, positive interaction mechanisms have also been documented. At the water-sediment interface, macroalgae accumulations can intercept and sequester the nutrients regenerated in the sediment (e.g., ammonium) or act as a transport vector from the water column to plant biomass (Boyer and Fong 2005), which may be important in nutrient-limited systems. Furthermore, in intertidal seagrass beds, macroalgal accumulations that retain water and provide a cover at low tide may affect seagrass responses to desiccation and temperature stress (Boese et al. 2005). Food web relationships may also mediate the negative effects of macroalgal blooms. Direct grazing on macroalgae and epiphytes by mesograzers is one food web linkage affected by the loss of top predators, as well as the wholesale loss or reduction of large megagrazers from many coastal systems (Heck and Valentine 2006). While these predatory and grazing linkages are not the focus of this dissertation, their effects on species interactions should be kept in mind.

Most likely, the outcome of seagrass-macroalgae interactions, be they positive, negative or neutral, are dependent on the additive and interactive effects of different mechanisms and the feedbacks between these processes. These dependencies are accentuated by the engineering capabilities of macroalgae and seagrasses, and their trophic role as basal producers. While, ultimately, I am interested in understanding the end result of these interactions in terms of their effects on benthic production, knowledge of the relative roles of different mechanisms can help us interpret, predict, and compare interactions between systems.

Upwelling-influenced estuaries

The mechanisms governing seagrass-macroalgae interactions in upwelling-influenced estuaries are not well studied, but may differ from those previously studied due to the physicochemical characteristics of these estuaries, including marine-based nutrient loading regimes and low residence times (high turnover rates of estuarine waters).

I use the term "upwelling-influenced" as a general descriptor of estuaries in the Lower Columbia Bioregion, where land-margin ecosystems are strongly influenced by the California Current; an eastern boundary upwelling system (Simenstad et al. 1997). During the summer months (March-September), northwesterly winds cause upwelling in the nearshore Pacific Ocean of this region. Despite poorly understood circulation patterns within many of these estuaries, research has shown that coastal waters are upwelled and advected into estuaries during the flooding tide (Hickey and Banas 2003, Brown and Ozretich 2009). This has been observed in the South Slough estuary where waters flooding the marine and mesohaline zones of the estuary were similar to adjacent ocean conditions (Roegner and Shanks 2001). This confluence of tidal water and freshwater runoff creates distinct zones in many of these estuaries that can be characterized based on their average salinity regimes (euhaline - marine-dominated zone, polyhaline – mid-estuary mixing zone, oligohaline – upper estuary riverine zone). Estuarine zonation is also characterized by nutrient concentrations, which are correlated with marine-based waters during summer upwelling months (Brown and Ozretich 2009). In South Slough, a characteristic upwelling-influenced estuary, nutrient gradients from marine-dominated to riverine regions indicate that land-based nitrogen loading is not currently excessive and that flooding waters are the primary sources of nitrogen inputs to the estuary (Fry et al. 2003, O'Higgins and Rumrill 2007).

Within the estuary, I examine site differences in interaction outcomes resulting, in part, from the dominance of marine and land-based nutrient delivery between zones of the estuary. I examine scale-dependence of these interactions by scaling-up these investigations to comparisons between estuaries. At this regional scale, latitudinal differences in marine-based forcing, coupled with differences in watershed characteristics, are used to investigate the outcome of species interactions. As a testing ground of novel species interaction dynamics, I also focus this work on intertidal seagrass beds, where environmental variables, and the physical structure of macroalgal canopies, vary from those of shallow subtidal systems where the majority of this research has taken place (McGlathery et al. 2007).

Contexts Explored in Dissertation Chapters

Using a multi-scale approach, I investigate spatial and temporal aspects of context-dependency throughout this dissertation. In Chapter 2, I compare observational trends in eelgrass and macroalgal production between four estuaries in Oregon and Washington, USA, using a 5-year dataset. Latitudinal differences in upwelling are used to examine spatial differences in producer dynamics among estuaries. I found that both macroalgae and eelgrass production varied between estuaries. Most notably, the two southern Oregon estuaries examined (Yaquina and Coos Bay) had much greater macroalgae biomass than the northern estuaries (Willapa and Netarts Bay). I also explored the marine versus terrestrial origin of factors that drive estuarine production, and found that both were important in structuring regional patterns. Across all estuaries, macroalgae biomass was associated with decreased eelgrass biomass, showing the potential for negative interactions between producers. However, I also examined temporal correlative patterns in production within estuaries, and found no evidence for eelgrass declines through time. As a case study from an estuary exemplifying high upwelling-influence, data from Coos Bay was also used to study the drivers of inter-annual variation in macrophyte production. In this southern estuary, nearshore upwelling strength was high compared to the northern sites, and associated with high macroalgal production. Here, I also found no indication of negative interactions, and found that macroalgal production was associated with both land-based and watershed drivers of productivity, as well as local climate.

At a within-estuary scale, Chapter 3 focuses on estuarine location and seasonal patterns in Coos Bay, using observational and experimental data collected over two years. An estuarine gradient, formed by differences in physicochemical conditions is used to explore the context-dependency of eelgrass-macroalgae interactions between sites. Across this gradient, macroalgal biomass was greatest in the marine zone of the estuary, and decreased towards the head of the estuary in the riverine zone. At no sites did I find any negative temporal correlations between producers. I also evaluated the outcomes of species interactions by experimentally manipulating ambient macroalgal conditions (adding or removing macroalgae) between sites. Here again, I found no evidence for negative interactions between producers in the marine zone of the estuary. For macroalgal removal treatments, positive interactions strengths were found at the marine site, and neutral values were found at the polyhaline and riverine sites. I did find that macroalgal addition had negative effects on interaction strength at the riverine site, but neutral effects at both marine and polyhaline sites. These results illustrate the degree of context-dependency found within an estuary, which can be explained by biotic and abiotic patterns across the estuarine gradient.

Another set of macroalgal manipulation experiments (Chapter 4) is used to explore interaction mechanisms at play under the marine zone physicochemical and nutrient contexts that exist in these estuaries. To do so, I compare contextual outcomes from a field experiment conducted in Coos Bay to a similar mesocosm experiment, where no currents or tides occur. In the field, I found no effects of either macroalgal or nutrient addition on eelgrass response. In the mesocosm, however, macroalgal addition had negative consequences on eelgrass, and also increased light attenuation and decreased sediment oxygen conditions; two mechanisms often associated with seagrass declines. Nutrient addition also affected certain eelgrass response metrics in the mesocosm experiment, but no interactions with the macroalgal treatment were observed. In this chapter, venue comparison illustrates the context-dependency of these species interactions. Moreover, across all chapters, I contrast findings from upwelling-influenced estuaries to studies from systems where land-based nutrient delivery dominates as an additional reference point for these context-specific interactions.

2 – Do macroalgal blooms drive patterns of seagrass production in northeast Pacific upwelling-influenced estuaries?

Margot L. Hessing-Lewis and Sally D. Hacker

ABSTRACT

In coastal marine systems, land-based nutrient inputs are often associated with blooms of ulvoid macroalgae, which can negatively affect critical seagrass habitats. To examine the generality of this interaction, we conducted a 5-yr study of macrophyte trends from upwelling-influenced northeastern Pacific estuaries. Across these estuaries, we found that both terrestrial and marine drivers were important in shaping regional patterns in macrophyte production. Macroalgal biomass was ~20 times higher in South coast estuaries (Yaquina Bay and Coos Bay, Oregon) compared to North coast estuaries (Willapa Bay, Washington and Netarts Bay, Oregon), and was negatively correlated ($r_s = -0.70$, $p = 0.00$) with seagrass production. Within-estuary temporal analyses, however, provide no evidence that macroalgal blooms were correlated with inter-annual patterns in eelgrass production. Local trends from Coos Bay showed that eelgrass was associated with climate and marine nutrient loading, rather than negative interactions with macroalgae. This analysis also supports a marine perspective on macroalgal blooms, where local patterns are related to both marine and terrestrial nutrient inputs. As climate change affects both land and ocean-based pathways of marine productivity, the spatial trends observed in this study, coupled with local studies on interaction dynamics, provide new insights on management of coastal eutrophication

2.1 INTRODUCTION

The productivity of coastal estuarine systems is influenced by both land-based nutrient run-off from watersheds and marine-derived nutrient delivery from nearshore environments (Howarth et al. 2011). The directionality (marine versus land) and importance of multiple nutrient input pathways, amongst other factors of production, is system dependent. Studies from estuaries where land-based nutrients dominate demonstrate that nutrient enrichment can shift macrophyte production towards the dominance of fast-growing green macroalgae (e.g., ulvoids), with negative effects on critical seagrass habitats (McGlathery et al. 2001, Burkholder et al. 2007). This research has governed estuarine management, where macroalgae blooms and seagrass declines have become key indicators of human-caused eutrophication (Bricker et al. 1999). However, in some estuaries, such as those along the West Coast of North America, new research suggests that large macroalgal blooms can be associated with nutrient-rich, ocean-derived water transported nearshore during periods of strong coastal upwelling (Brown and Ozretich 2009, Jorgensen et al. 2010). Production of macroalgal blooms on the Oregon Coast can be comparable to East Coast systems (i.e., peak biomass of ~400 g dw m^{-2}, Hog Island Bay, Virginia (Havens et al. 2001) compared to ~320-450 g dw m^{-2}, Coos Bay, Oregon (Hessing-Lewis et al. 2011)) with mean summer biomass surpassing the threshold for negative impacts on seagrass determined from research in Chesapeake Bay, USA (100 g dw m^{-2} (Bricker et al. 2003)). However, macroalgal effects on seagrass communities are understudied on the

Pacific coast of North America (except see Kentula and DeWitt 2003, Jorgensen et al. 2010, Hessing-Lewis et al. 2011). Thus, the classic paradigm that land-based nutrients drive estuarine eutrophication may not hold for systems in this region, which are adjacent to areas of enhanced ocean upwelling.

This marine-terrestrial dynamic is particularly important to understand given the potentially synergistic nature of climate change effects on estuarine systems (Doney 2010). In addition to projected land-based impacts of climate change (i.e., changes in freshwater inflow), changes in marine inputs are also expected (Scavia et al. 2002). Models predict that upwelling may increase with climate change (e.g., Diffenbaugh et al. 2004, Bakun et al. 2010), and could be associated with elevated nearshore nutrient concentrations in this region (Rykaczewski and Dunne 2010). Given the potential role for marine nutrients to affect the severity of land-based estuarine eutrophication (Howarth et al. 2011), marine drivers of climate change could potentially have profound effects on estuarine productivity.

To further understand the role of marine and terrestrial pathways of nutrient enrichment and the effect of macroalgal blooms on productivity of seagrass, we investigated patterns in macroalgae (ulvoids) and eelgrass (*Zostera marina*) variability across multiple upwelling-influenced estuaries on the Pacific Northwest coast (USA). Rocky intertidal communities respond to upwelling conditions along this same coastline (i.e., Menge et al. 2004), but a comparative study of community interactions in protected estuarine systems is lacking. The nearshore northeast Pacific Ocean is influenced by the California Current System (CCS), an Eastern Boundary oceanographic system that delivers tidally advected upwelled waters to estuarine habitats (Roegner and Shanks 2001, Hickey and Banas 2003). At a latitudinal scale, observations of upwelling strength within the CCS show an increase from north to south as alongshore winds strengthen equatorward, however, local features such as river plumes, headlands and continental shelf topography also interact with upwelling to influence nearshore marine productivity (Hickey and Banas 2008). Estuaries along this coastline also encompass a large range of watershed and basin characteristics, including factors that we hypothesized would be related to land-based nutrient loading, such as estuarine watershed catchment area, land use, and human population density (Emmett et al. 2000, Bricker et al. 2008). We used this latitudinal difference in marine-based upwelling-influence, together with variation in terrestrial estuarine influence, to investigate spatiotemporal patterns in production of macroalgae and eelgrass. Our goal was to better understand the potential for macroalgal blooms to affect long-term trends in eelgrass, a critical food and habitat along the coast, and to understand how climate change might influence these systems (Waycott et al. 2009). Spatial and temporal variation in production was examined by comparing four upwelling-influenced estuaries spanning a distance along the coast of ~ 220 km from northern Washington to southern Oregon, USA, for five years (2006-2010). A second analysis was conducted using a temporal dataset from Coos Bay, Oregon, to further explore the interaction of macroalgae and eelgrass as mediated by ocean and land-based inputs. Using this spatiotemporal framework we address three primary questions: 1) Does macroalgae and eelgrass production vary among estuaries and through time? 2) If so, what are the relative roles of marine versus watershed factors in influencing spatiotemporal dynamics of production in these estuaries?, and 3) Does macroalgae negatively affect eelgrass production at regional and/or local scales? Furthermore, we examine the implications of our findings in light of future climate change scenarios involving large-scale regional shifts in oceanographic processes.

2.2 METHODS

2.2.1 Study sites and design

We chose four estuaries along the coast of Oregon and Southern Washington including, from north to south, Willapa Bay, Washington, and Netarts Bay, Yaquina Bay, and Coos Bay, Oregon, that varied in the oceanographic conditions they experienced, their size and catchment areas, and the amount of freshwater input to the system (Fig. 2.1, Appendix 1). To compare differences between estuaries and examine inter-annual variation, we conducted annual (2006-2010) surveys at euhaline, marine-dominated locations within each estuary (exception was Netarts Bay, which was not sampled in 2007, 2009). All survey sites were proximate to the estuary mouth (Willapa Bay ~ 10 km, Netarts Bay ~ 3 km, Yaquina Bay ~ 5 km, Coos Bay ~ 4 km), where high salinities (euhaline: 30-35 ppt) associated with upwelling-influenced marine waters dominate the water column throughout the diel tidal cycle (Brown and

Ozretich 2009). Sampling effort was focused on capturing maximum macroalgal biomass in each estuary by targeting sites with high macroalgal biomass (Appendix 2A) during July, a high biomass month (Appendix 2B).

Surveys consisted of haphazard placement of 0.25 m^2 quadrats within contiguous intertidal eelgrass beds (~ ± 0.1 MLLW). Within quadrats, % cover of eelgrass and macroalgae and eelgrass shoot density were recorded. At low tide, when eelgrass is exposed and when the surveys were conducted, a layer of eelgrass lies flat along the substrate, often covering it completely. Quadrat survey methodology therefore consisted of a) recording percent cover and quantity of macroalgae on top of the eelgrass, b) counting the density of eelgrass shoots, and c) quantifying the percent cover and quantity of macroalgae below the eelgrass layer. We converted our field-based measurements of macroalgae and eelgrass to biomass per area (g dry wt 0.25 m^2) in order to compare production among estuaries. For eelgrass, mean biomass per eelgrass shoot was determined by drying shoots (60°C for 24 hrs) collected haphazardly at each site (N = 20 per site). Average biomass across all years (mean g dry wt) was extrapolated to the quadrat scale (0.25 m^2) by multiplying mean biomass per shoot by mean shoot density per quadrat. For macroalgae, we determined the dry weight (60°C for 24 hrs) removed from quadrats such that biomass (g dry wt 0.25 m^2) could be extrapolated from percent cover (Appendix 2C).

2.2.2 Estuarine physical characteristics

Marine and watershed vectors were tabulated for the four estuaries (see Appendix 1 for data and their sources). Marine parameters included upwelling and coastal current data from nearshore waters adjacent to estuary mouths and the tidal volume per estuary to approximate the quantity of marine water entering the estuary. Watershed parameters included physical characteristics of the estuaries (catchment and estuary area, estuary volume), watershed nutrient loading (nitrogen and phosphorus), watershed land use, population density, freshwater inputs, and local precipitation. For the inter-annual analysis of macrophyte trends in Coos Bay, we focused on marine and watershed nutrient inputs (dissolved inorganic nitrogen and phosphorus) and climate (precipitation and photosynthetically active radiation) using data from the South Slough National Estuarine Research Reserve monitoring program (Appendix 3).

2.2.3 Statistical analyses

We used linear mixed effect models to determine the role of spatial (site) and temporal (yearly) variation on benthic production (eelgrass and macroalgae biomass). For the latitudinal analysis, we modeled biomass as a function of producer-by-site (fixed effect) and sampling year (random effect) using a linear mixed effect (lme) model (Pinheiro et al. 2009) in *R* (R Development Core Team 2009). We accounted for the lack of independence between eelgrass and macroalgal production measured within the same quadrat, and repeated sampling through time by nesting quadrat within site within year in the random model term. The producer-by-site variance structure was also imposed within the model structure using a weighted variance function. For the temporal analysis we modelled biomass as a function of producer-by-year (fixed effect) and quadrat (random effect). Post hoc tests were evaluated using Tukey tests (*R* multcomp package; Hothorn et al. 2008). Interannual relationships between macroalgae and eelgrass were further evaluated at each site using a Spearman correlation (r_s) permutation test (non-normal time series) conducted on ordered, paired mean annual values of macroalgae and eelgrass biomass by site.

The multiple factors related to marine and watershed vectors of nutrient loading and local climate were reduced to one dimension (axis 1 scores) using Principal Component Analysis in *R* (prcomp; R Development Core Team 2009). We then used redundancy analysis (rda vegan package *R*; Oksanen et al. 2010) to determine the linear mapping and direction of the vectors relative to spatial and temporal producer ordinations. Graphical representations based on RDA show the relationship between environmental parameters and groupings of producer biomass for the latitudinal comparison (Fig. 2.2A) and the Coos Bay inter-annual patterns (Fig. 2.2B). Arrows point in the direction of the labeled gradient, and their lengths indicate the strength of their relationship to the producer ordination.

2.3 RESULTS AND DISCUSSION

2.3.1 Regional variation in macrophyte production

Our latitudinal survey of upwelling-influenced estuaries showed striking spatial differences in production along the northeast Pacific coast (mixed effect model: producer-by-site: $F_{7,357} = 131.05$, $p < 0.001$, Appendix 4: macroalgae vs. eelgrass comparisons). Macroalgae varied regionally with mean annual biomass low at the North coast sites (Willapa and Netarts Bay) and high at the South coast sites (Yaquina and Coos Bay) (Fig. 2.1, Appendix 2.4). In contrast, we found less variability in mean annual eelgrass biomass and a reverse north-south pattern, with higher biomass at the North coast sites compared to the South coast sites. The large biomass values in the North coast estuary sites were the result of both high individual shoot biomass and high shoot density (Appendix 1). Latitudinal patterns in production were also evident in the ordination, where North coast sites grouped with eelgrass, and South coast sites grouped with macroalgae (Fig. 2.2A), and both clusters segregated along RDA 1 (accounting for 94% of the producer variance; Appendix 5A). Furthermore, macroalgae biomass and the South coast sites were positively associated with marine and terrestrial vectors of estuarine nutrient delivery, while eelgrass biomass and the North coast sites were not (Fig. 2.2A; Appendix 5B). This analysis shows that both marine and terrestrial factors can play important roles governing macroalgal blooms in upwelling-influenced estuaries. Specifically, high macroalgal biomass was positively associated with a combination of increased upwelling, terrestrial nutrient loading, and other watershed factors influencing estuarine productivity. The role of marine factors governing estuarine production has been understudied due to the predominance of environmental problems associated with land-based eutrophication (Doney 2010). However, in upwelling-influenced estuaries, our regional trends, coupled with detailed local estuarine studies by others (Brown and Ozretich 2009), show that marine drivers are key contributors to estuarine production. For example, in Yaquina Bay, time series analyses show that nutrient concentrations in the lower reaches of the estuary are strongly associated with coastal winds, upwelling and macroalgal blooms (Brown and Ozretich 2009, Kaldy and Brown USA EPA unpublished data). Together, this provides mounting evidence that oceanographic processes can influence estuarine production especially under conditions of strong upwelling.

2.3.2 A negative interaction between macroalgae and eelgrass?

Besides differences in production along the CCS, our results show a negative relationship between macroalgae and eelgrass production across all sites throughout the five years of this study ($r_s = -0.70$, $p = 0.00$). These results suggest that at a large regional scale, where ocean upwelling and watershed nutrients are dominant features of the estuary (i.e., South coast estuaries), macroalgal blooms may negatively impact eelgrass production, resulting in as much as a ~40% reduction in biomass. Support for strong negative interactions between macroalgae and seagrass dominate estuarine ecology, where research from systems with land-based nutrient loading (i.e., Chesapeake, Waquoit Bays) have shown that macroalgal blooms can have negative effects on eelgrass (Valiela et al. 1997, Hauxwell et al. 2003), primarily by decreasing light levels and changing sediment and water column biogeochemistry (McGlathery 2001).

Given this research, and similarly large macroalgal blooms in our system, we considered two lines of evidence for negative interactions in upwelling-influenced systems. First, we analyzed the temporal patterns of production of the two macrophytes. We found no evidence that summer blooms of macroalgae were related to annual changes in eelgrass biomass throughout the five years of this study (Willapa: $r_s = -0.60$, $p = 0.27$; Netarts: $r_s = 0.34$, $p = 0.85$; Yaquina: $r_s = -0.71$, $p = 0.22$; Coos: $r_s = -0.19$, $p = 0.80$). For example, in Yaquina Bay and Coos Bay, years of higher macroalgal production did not result in lower eelgrass production and vice versa. In fact, within-estuary eelgrass variability was quite low and showed no response to high fluctuations in macroalgal production through time (Fig. 2.1A). This suggests that, while macroalgal blooms can potentially have negative effects with eelgrass across large spatiotemporal scales, eelgrass is relatively insensitive or resilient to large fluctuations in macroalgae within particular estuaries. Given that macroalgae had uniformly low biomass in North coast estuaries, we were unable to explore the effect of blooms in these systems.

Alternatively, differences in eelgrass biomass between North and South coast estuaries may be explained by local adaptation to particular estuarine physicochemical conditions (McMillan and Philips 1979) rather than negative interactions with macroalgae. For example, our analyses focused on factors affecting estuarine inputs, but other key parameters known to control eelgrass distribution and morphology, including light levels and other physical, chemical and biological factors (e.g., substrate type, current velocity, grazing pressure) (Koch 2001), may explain why eelgrass production was higher in the north than the south.

The second line of evidence we used to explore the potential negative interaction between macroalgae and eelgrass involved focusing on Coos Bay, where upwelling is strong, macroalgal blooms are high and a long temporal dataset exists of estuarine water properties, nutrient concentrations and local climate (Appendix 3). Similar to the regional spatial analysis, inter-annual differences between producers (mixed effect model: producer-by-year: $F_{9,90} = 34.80$, $p < 0.001$) were found, but inter-annual variability for both producers was lower than latitudinal variability. Based on the ordination (Fig. 2.2B), both high marine and terrestrial nutrient delivery were positively (RDA axis 1; Appendix 5B) associated with high macroalgae production years (2007-2009), whereas low macroalgae production years (2006/2010) (low vs. high macroalgae Tukey test $p > 0.05$) were associated with local climate (increased rain, low light). Previous studies in Coos Bay have also stressed the importance of climatic factors, such as light levels and temperature as regulators of ulvoid biomass (Pregnall and Rudy 1985). As opposed to the regional ordination, macroalgae and eelgrass biomass were not diametrically opposed, and both appeared to respond to changes in marine, terrestrial and climatic vectors, but in different ways. Macroalgae responded to both high marine and terrestrial nutrient inputs, while eelgrass was primarily associated with a combination of marine nutrient loading and local climate (Fig. 2.2B). Temporal variation in eelgrass production within this estuary has been previously documented as a response to regional climate patterns (e.g., El Nino) (Thom et al. 2003). Together with the inter-annual trends, the local evidence from Coos Bay suggests a lack of macroalgal control, where eelgrass biomass responds more strongly to climate and nutrient loading than changes in macroalgae biomass. Furthermore, evidence from a manipulative experiment of macroalgae in intertidal seagrass beds of Coos Bay also demonstrates the potential for neutral and even positive interactions between macroalgae and eelgrass, under conditions of high to moderate macroalgal biomass (Hessing-Lewis et al. 2011). This result is likely due to the impermanence of macroalgal blooms in these systems (summer months only) and a function of intertidal conditions, where macroalgal canopy cover is mediated by tidal action and local currents.

2.3.3 Climate change implications

Within the marine zone of upwelling-influenced estuaries we studied, it is unclear whether macroalgae negatively impacts eelgrass. While regional trends suggest the potential for negative interactions, this evidence runs contrary to temporal interactions and local studies. However, within-estuary studies of interaction dynamics can inform regional-scale effects of climate change, which could alter the dynamics of macroalgal blooms across this region. Contrary to experimental results from the marine zone of Coos Bay, macroalgal manipulations (additions) in the riverine, low macroalgae biomass region of this estuary had negative effects on eelgrass (Hessing-Lewis et al. 2011). These results were attributed to local physicochemical conditions, smaller eelgrass morphology, and lower nutrient concentrations. At the regional scale, these local results may indicate that eelgrass from North coast estuaries (currently with low macroalgal biomass) could respond more severely to future changes in production than those in the South coast. As such, macroalgal blooms in these estuaries may represent the future propensity of these systems to respond to marine-based climate forcing, rather than land-based nutrient delivery alone. This represents a departure from current management thinking which uses macroalgae as an indicator of eutrophication status, and focuses on regulation of land-based vectors of estuarine productivity (Bricker et al. 1999).

Due to the dual roles of marine and terrestrial pathways of nutrient loading (Fig. 2.2), and their potential synergistic effects (Howarth et. al. 2011), climate-related changes to estuarine inputs have potentially profound effects on the future production of northeastern Pacific estuaries (Reum et al. 2011). From a marine perspective, regional upwelling may increase along the CCS, increasing nutrient delivery to these estuaries (Diffenbaugh et al. 2004, Bakun et al. 2010). Increased temperature may play an opposing role as it increases thermal stratification and decreases nutrient transport from depth, but the net effect is still likely to lead to elevated nutrient concentrations in coastal waters (Rykaczewski and Dunne 2010). From a watershed perspective, terrestrial changes include the combined impacts of increased coastal development and climate. While eutrophication assessments in this region do not currently rank these estuaries as susceptible (Bricker et al. 1999), our analysis of forcing factors indicates that macroalgal blooms may be associated with land-based drivers of nutrient loading, including population density. Although coastal population in this region is not growing as fast as other regions of the USA, population growth is apparent across all estuaries in this study (~3% per decade since 1970 based on U.S. Census data). Together with potential climate-related changes in local precipitation, land cover and freshwater inflow (Scavia et al. 2002), land-based alterations of nutrient pathways to estuaries are also expected with climate change.

We suggest that interface habitats such as estuaries, where large shifts in coastal productivity and terrestrial delivery of nutrients are expected, may be the most prone to the effects of climate change. Clearly, additional research documenting the strength, directionality, and synergy of factors acting on the dynamics of estuarine communities is necessary in order to understand the severity of system response to climate change.

ACKNOWLEDGEMENTS

Thanks to the field/lab support associated with this research. At OSU; O. Moulton, P. Zarnetske, J. Henderson, L. Wisehart, D. Eerkes-Medrano, S. McConville, W. Rice-Narusch, H. Bailey, J. Schaefers, M. Rogers, L. McCoy, T. Gouhier. This manuscript was improved by comments from B. Menge. Research in

Willapa Bay was funded by a Western Regional Aquaculture Center (USDA) grant to S. Hacker, B. Dumbauld and J. Ruesink. Research in Coos Bay was funded from an award from the National Estuarine Reserve System (NERR), Estuarine Reserves Division, NOAA. At South Slough NERR, A. Helms, A. DeMarzo, S. Rumrill facilitated data acquisition/processing. Additional support for this research came from OSU Dept. of Zoology, S. Hacker, and B. Menge.

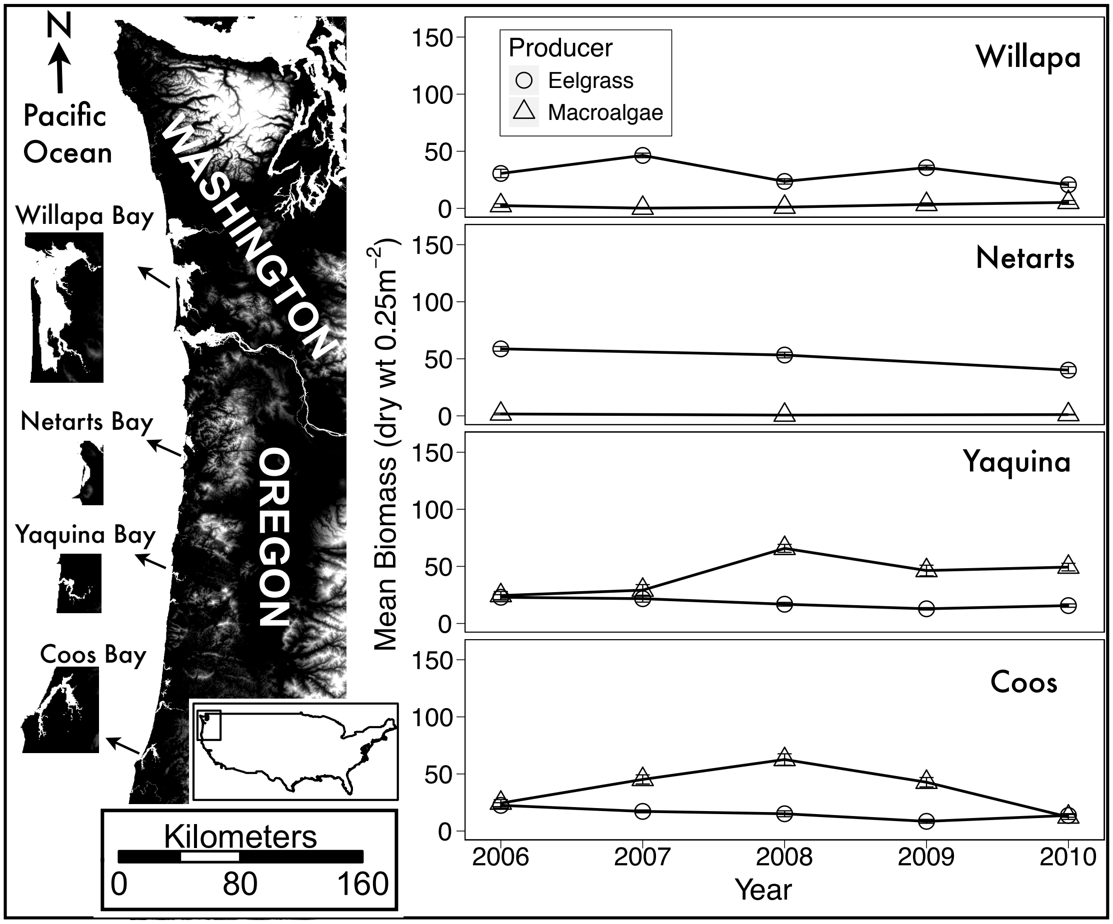


Figure 2.1 Map of study sites and trends in producer biomass (2006-2010: Willapa, Netarts, Yaquina, Coos Bays). Left: Map of surveyed estuaries along the northeastern Pacific coast, USA. Survey sites of all estuaries located within the euhaline, marine zone of each estuary: Willapa Bay (46°40'32 N, 123°55'07 W), Netarts Bay (45°25'03.12 N, 123°56'09 W), Yaquina Bay (44°36'51 N, 124°01'43 W) and Coos Bay (43°20'22 N, 124°19'06 W). Right**:** Mean inter-annual biomass ± SE (dry wt 0.25 m^{-2}) of eelgrass and macroalgae across the surveyed estuaries from 2006-2010.

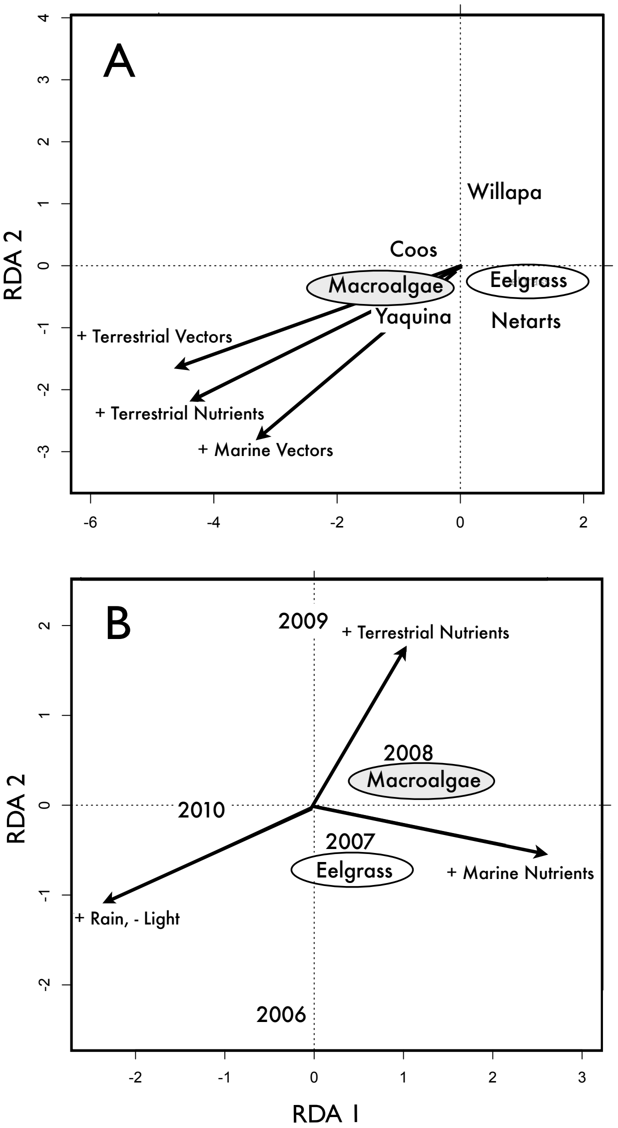


Figure 2.2 Redundancy analysis (RDA) plots showing the ordination of mean annual producer biomass (dry wt 0.25 m-2; macroalgae and eelgrass) by estuarine site, constrained by marine and terrestrial vectors of estuarine production (Appendix 1). Arrows point in the direction of vector change, and arrow length is proportional to the correlation between the ordination and vectors. Appendix 2 provides vector inputs (PCA axis 1 scores), and RDA outputs. A) Ordination of mean producer biomass by estuarine site (2006-2010 mean), constrained by three marine and watershed vectors. B) Ordination of mean annual producer biomass by year (2006-2010) for Coos Bay, constrained by marine, watershed, and climate vectors (Appendix 3).

3 – Context-dependent eelgrass-macroalgae interactions along an estuarine gradient in the Pacific Northwest, USA

Margot Hessing-Lewis, Sally D. Hacker, Bruce A. Menge, Steve Rumrill

Estuaries and Coasts
2011
doi: 10.1007/s12237-011-9412-8

ABSTRACT

Land-based eutrophication is often associated with blooms of green macroalgae, resulting in negative impacts on seagrasses. The generality of this interaction has not been studied in upwelling-influenced estuaries where oceanic nutrients dominate seasonally. We conducted an observational and experimental study with *Zostera marina* L. and ulvoid macroalgae across an estuarine gradient in Coos Bay, Oregon. We found a gradient in mean summer macroalgal biomass from 56.1g dw $0.25m^{-2}$ at the marine site to 0.3g dw $0.25m^{-2}$ at the riverine site. Despite large macroalgal blooms at the marine site, eelgrass biomass exhibited no seasonal or interannual declines. Through experimental manipulations we found that additions of macroalgae biomass (+ 4000 mL) did not affect eelgrass in marine areas but it had negative effects in riverine areas. In upwelling-influenced estuaries, the negative effects of macroalgal blooms are context-dependent, affecting the management of seagrass habitats subject to nutrient inputs from both land and sea.

3.1 INTRODUCTION

Eutrophication of coastal waters worldwide is associated with the loss of seagrass habitat (Orth et al. 2006; Waycott et al. 2009). Among a host of ecosystem perturbations associated with increased anthropogenic nutrient input to coastal waters, land-based nutrient loading and associated macroalgal blooms are of key concern due to their deleterious effects on seagrass meadows (Burkholder et al. 2007) and the associated loss of the many vital ecosystem functions and services associated with these habitats (Barbier et al. 2011; Duarte 2000). Compared to seagrasses, macroalgae can exhibit high productivity and increases in biomass over relatively short periods of time, stemming from its superior ability to sequester nutrients (Hauxwell et al. 2001; Valiela et al. 1997). Blooms of green macroalgae (primarily of the family Ulvaceae; hereafter referred to as “ulvoids”) have been shown to range from roughly 650g dw m^{-2} (Hog Island Bay, Virginia, USA - Havens et al. 2001) to upwards of 1800g dw m^{-2} (Venice Lagoon, Italy - Sfriso et al. 1992). On the Oregon coast, research in Coos Bay has documented maximum summer bloom conditions of ~750g dw m^{-2} (Pregnall and Rudy 1985), almost four times as dense as the threshold for negative impacts on seagrass in Chesapeake Bay, USA (Bricker et al. 2003).

The interactions that result from these productivity differences between seagrass and macroalgae are complex, and their outcomes may depend on both environmental conditions, as well as the ability of seagrass to influence these interactions via feedbacks associated with their strong engineering capabilities (Carr et al. 2010; Havens et al. 2001; Valiela et al. 1997). Light attenuation by macroalgal canopies is one primary cause of seagrass loss under eutrophic conditions (Hauxwell et al. 2001; Krause-Jensen et al. 1996; Valiela et al. 1997) and can be exacerbated by water column nutrient enrichment (Burkholder et al. 1992), oxygen changes due to senescence of the macroalgae (Hauxwell et al. 2001), and other indirect processes, such as increased turbidity (Carr et al. 2010). The various biogeochemical changes associated with macroalgal canopies in seagrass beds have been reviewed by McGlathery (2001) and Burkholder et al. (2007) and include hypoxia in the water column, anoxia in the sediment, as well as the associated microbial activity and production of sulfide-containing compounds associated with low oxygen environments. The additive and interactive effects of the numerous physical and biogeochemical processes produced by macroalgae canopies within seagrass beds suggest that there may be diverse outcomes to these interactions depending on the environmental context in which they occur.

Growing evidence suggests that seagrass response to nutrient enrichment via interactions with macroalgae can result in negative, neutral and potentially positive effects, with changing interaction dynamics through time and space (e.g., Armitage et al. 2005; Eriksson et al. 2007; Jorgensen et al. 2007). This context-dependency of species interactions not only helps inform basic community structure theory (e.g., Bronstein 1994; Hacker and Gaines 1997; Menge and Sutherland 1987) but, along with other ecological research on context-dependency, is necessary to translate broad-scale ecological generalities into management initiatives that address the idiosyncrasies of natural systems, such as the outcomes of biological invasions (Hacker and Dethier 2006, Hacker et al. 2011), human alteration of food webs (Salomon et al. 2010), and ecosystem engineering (Crain and Bertness 2006).

To explore this context-dependency in more detail, we investigated the interaction between seagrass, *Zostera marina* L. (hereafter referred to as “eelgrass”) and ulvoid macroalgae across an estuarine gradient in South Slough, a branch of Coos Bay, located on the southern coast of Oregon, USA (Fig. 3.1). This is a characteristic “upwelling-influenced” estuary in the Pacific Northwest, where the influence of the nearshore California Current System is particularly strong at the estuary’s mouth (Hickey and Banas 2003; Roegner and Shanks 2001). In this system, and other upwelling-influenced estuaries in this region (e.g. Yaquina Bay), marine-derived nutrient inputs dominate over land-based loading during the low precipitation summer months (Brown and Ozretich 2009, Rumrill 2006) and are strongly associated with blooms of green macroalgae (Brown et al. 2007, Kaldy and Brown US EPA unpublished data). Along the relatively undisturbed South Slough estuary, blooms at the mouth of the bay are equivalent in height and biomass to those associated with seagrass declines in other systems (e.g., Hauxwell et al. 2001), but are much greater than macroalgae production in other regions of the estuary. In this study, we were primarily interested in determining how changing macroalgae abundance and gradients in physicochemical conditions along the estuarine gradient affected macroalgae-eelgrass interactions. We used a comparative-experimental approach (e.g., Dayton 1971; Menge et al. 2004), and sites located along the estuarine salinity gradient to address two primary questions: 1) What is the temporal relationship between macroalgae and eelgrass production at different sites within the estuary? 2) How does the direction and magnitude of macroalgae-eelgrass interaction strength change between sites and through time? Based on results from this study, we discuss potential mechanisms influencing interactions along the estuarine gradient, and implications for eutrophication management.

3.2 METHODS

3.2.1 Study System

Coos Bay is located in Southern Oregon (43.35°N, 124.34°W; Fig. 2.1) and lies adjacent to Cape Arago, a region of strong, persistent upwelling within the California Current Large Marine Ecosystem (Rumrill 2006). The mean monthly Upwelling Index (Pacific Fisheries Environmental Laboratory, station 42°N 125°W) for the summer months of this study (June-October) was 51.6 ± 13.97 $m^3s^{-1}100m^{-1}$ coastline (2007) and 81.00 ± 22.95 $m^3s^{-1}100m^{-1}$ coastline (2008) (2000-2010, 10-yr summer mean of 116.05 ±11.9 $m^3s^{-1}100m^{-1}$). Throughout the winter months an average of 142 cm of rainfall enters the estuary, compared to less than 10 cm within the summer months (Rumrill 2006). The research was conducted in the South Slough National Estuarine Research Reserve (SS NERR) located in a southern branch of the larger Coos Bay estuary (Fig. 3.1). The South Slough is approximately 9 km long, with an average width of 600m, a wet surface area of 783 ha, and a volume of 2 million m^3 (Harris et al. 1979). It is composed of 222 ha of intertidal habitat (mudflats, seagrass beds (95 ha), tidal marshes) and 16 ha of subtidal channels. Tidal flushing rates within the South Slough are high, with an estimated tidal prism of 9.34 million m^3 (Harris et al. 1979). Eelgrass beds are not continuous throughout South Slough, but relatively large, contiguous, perennial eelgrass meadows are present throughout its length. We used three sites with large eelgrass meadows for the research, including Barview (marine), Valino (polyhaline), Danger (riverine) (Fig. 3.1). These sites are representative of the overall estuarine gradient in this system and are distinguishable by salinity, temperature and nutrient parameters (Table 3.1).

3.2.2 Eelgrass–Macroalgal Observational Patterns

Eelgrass and macroalgae parameters were measured on a monthly basis during the summer months (June-September) and bi-monthly during the winter months (November, January, April) from June 2007 to April 2009. Eelgrass density through time was monitored along a permanent 100 m transect line haphazardly located in each eelgrass bed perpendicular to the channel edge, at approximately +0.1 MLLW (corresponding to maximum density of eelgrass shoots; Thom et al. 2003). Density of shoots was counted in 0.5 x 0.5m quadrats (0.25 m^2) at 10 m intervals along each transect line (n=10 per site). Thirty eelgrass shoots were haphazardly sampled from the eelgrass bed (adjacent to transect) at each site. Eelgrass was returned to the lab, measured for length and width, scraped of epiphytes, scored for percent blade desiccation and then dried (60°C for 24 hrs) and weighed. The desiccation index developed by Boese et al. (2003) was used to quantify the percentage of blade area with non-pigmented (bleached) necrotic tissue. Biomass at the quadrat scale (0.25 m^{-2}) was extrapolated by multiplying mean biomass per shoot by mean shoot density within the quadrats. Macroalgae biomass was collected from quadrats (0.25 m^{-2})

haphazardly placed within each site (n=5-9 per site) by clipping the macroalgae sheets at the edge of the quadrat, and removing it from around the eelgrass shoots. All ulvoid macroalgae was cleaned with freshwater and scraped to remove epiphytes and sediment, and then dried (60° C for 24 hrs) and weighed.

3.2.3 Eelgrass–Macroalgal Interaction Experiment

We manipulated macroalgal biomass during the summer months of 2007 and 2008 to measure its interaction with eelgrass. Macroalgae treatments, which consisted of the addition and removal of macroalgae, were applied twice (August, September) in 2007 and three times (June, July, August) in 2008 to permanently marked eelgrass plots (0.5 x 0.5 m^2). Treatment plots were blocked haphazardly at each site (n = 9 per treatment) within ± 0.2m of the permanent transect lines. The macroalgae addition treatment involved anchoring sheet-form ulvoids (including the species *Ulva linza* and *Ulva lactuca* (Gabrielson et al. 2006) on the top of the sediment using metal stakes and ropes strung across the plot. Care was taken to ensure that the eelgrass was not blanketed by macroalgae or damaged by the stakes or ropes. The macroalgae removal treatment consisted of carefully removing all macroalgae from the quadrat area, including attached thalli and macroalgae partially embedded in the substrate. A control was established in which macroalgae anchors were applied without manipulating the natural abundance of macroalgae. For the addition treatments, macroalgae was collected from Barview (the marine site) and then added based on volumetric quantification (Robbins and Boese 2002). We added 4000 mL macroalgae to each plot at all the sites which was equivalent to ~160 g dw 0.25 m^{-2} (= ~ 650 g dw m^{-2}), representing a doubling of the average quantity of macroalgae found at the marine site during the peak bloom summer months. Depending on the timing of low tide (when access to the eelgrass beds was possible), ulvoid macroalgae was either gathered for the addition treatments during the same day as the treatment application, or it was held overnight in cold seawater before applying the treatment the following day.

Eelgrass density per replicate plot was counted during each re-visit to the sites. In addition, redox potential was measured three times in different locations within each plot using an Orion Star probe (Thermo electron corporation) fitted with a platinum electrode. At the termination of the experiment (October 2008), all aboveground and belowground material was collected from within the experimental plots. Eelgrass was returned to the lab, measured for length and width, scraped of epiphytes, and then dried (60° C for 24 hrs) and weighed. Sediment samples from the top 3 cm of each plot were also obtained, and later dry sieved to determine the grain size distribution of sand (> 63 *u*m, larger rocks and wood removed) and silt (< 63 *u*m) size.

3.2.4 Statistical Analyses

All statistical analyses were conducted using the *R* platform (R Development Core Team 2009). We analyzed observational eelgrass and macroalgae trends separately using linear mixed effect models (*lme* in *R nlme* package, Pinheiro et al. (2009)) with site, season and site x season, as fixed effects. A random effect term was included in this model structure to incorporate the nestedness of temporal sampling (month within season within year). Macroalgae biomass was log-transformed, and eelgrass biomass was square root transformed in order to improve homoscedasticity and normality assumptions in these models. The analysis of variance techniques used throughout this analysis were robust to inequality of variance and non-normality given the balanced design and large sample size used in these analyses (Underwood 1997). To determine the relationship between macroalgae and eelgrass biomass through time, a correlation permutation test (non-normal time series) was conducted on non-transformed monthly averages.

For the analysis of the experimental data, eelgrass density (square root transformed) was analyzed using a linear mixed effects model (fixed effects: treatment x site x year (2007 vs. 2008), random effects: treatment nested in block). Relative change in density was calculated at the end of the growing seasons in summer 2008 (August 2008) as the change in eelgrass density relative to the initial (August 2007) eelgrass densities. We calculated interaction strength values for both addition and removal manipulations as: treatment - control (based on the raw difference index; Berlow et al. 1999). Comparisons between sites were made using a linear mixed effects model (fixed effect = site, random = experimental block) with square root transformed interaction strengths as the response. Deviations from normality were assessed by employing a generalized linear model (glm) with a Poisson distribution. Results from glm models were compared to linear mixed effect model outcomes but never resulted in differences in treatment effects. Interaction strength by site was also assessed by determination of significant departures from zero (neutral interaction) using Student's t-test with modifications for unequal variance. At the experiment termination (October 2008) aboveground and belowground biomass, shoot length, sediment grain size (sand:silt), and redox potential were analyzed using a linear mixed effect model (fixed effects: site x treatment, random effect: experimental block), with data transformations when appropriate. A priori comparisons were conducted using planned linear contrasts, and were judged significant when $p < 0.05$ (Kuhn et al. 2010). Graphs were produced using the ggplot2 package (Wickham 2009).

3.3 RESULTS

3.3.1 Eelgrass–Macroalgal Observational Patterns

Patterns in macroalgae biomass were highly site and season specific (Table 3.3), with the greatest mean monthly summer biomass at the Barview site, where marine influence is greatest (Fig. 3.2, Table 3.2). Mean summer macroalgal biomass differed among all sites (linear contrasts $p < 0.05$) and was 83 % lower at Valino, in the polyhaline zone, and 99 % lower at Danger, located in the riverine zone (Fig. 3.2, Table 3.2). Among-site differences and rank order in mean macroalgal biomass persisted between seasons (summer vs. winter), except for differences between Barview and Valino, which were not apparent during the winter (linear contrasts $p > 0.05$). Biomass changes between summer and winter were most marked at Barview, followed by Valino (Fig. 3.2, linear contrasts $p < 0.05$). Very little macroalgae biomass was quantified at Danger (Fig. 3.2, Table 3.2), and no seasonal differences were observed (linear contrasts $p > 0.05$).

In contrast, mean eelgrass biomass did not show strong seasonal variation at any of the sites, but differences between sites were found (Fig. 3.2, Table 3.3). Mean eelgrass shoot density and length were similar at Barview and Danger, but density was greater, and shoot length shorter at Danger (Table 3.2, 3.3; linear contrasts $p > 0.05$). Desiccation index was low at all sites (< 2% average blade desiccation), with values of zero for the winter months (Table 3.2). Interannual and seasonal variation in eelgrass biomass showed no relationship to temporal trends in macroalgae biomass at any of the sites (permutation test: Barview (rho = 0.07, $p = 0.76$); Valino (rho = 0.23, $p = 0.39$), Danger (rho = 0.19, $p = 0.45$).

3.3.2 Eelgrass–Macroalgal Interaction Experiment

Eelgrass densities in the eelgrass-macroalgal interaction experiment were affected by both site, treatment and year (Fig. 3.3, Table 3.4). At Barview, treatment effects (both addition and removal) (Fig. 3.3, Table 3.4) were only observed as the experiment progressed through 2008 (Fig. 3.3; linear contrasts, $p < 0.05$). At Valino, eelgrass density did not vary with treatment, but did vary temporally (Fig 3.3; linear contrasts, $p < 0.05$). At Danger, the addition treatment effects appeared in 2007, and endured through 2008, with no effect of removal (Fig. 3.3; linear contrasts, $p < 0.05$). Taking into account relative change through time, and differences from the control treatments, interaction strengths also differed between sites (Fig. 3.4, Table 3.4). For the addition treatment, differences occurred across all sites (Table 3.4; linear contrasts, $p < 0.05$). With addition of macroalgae, interaction strength of macrophytes on eelgrass at Danger was negative, suggesting competition (t-test, $p < 0.001$), but was not different from zero (neutral interaction) at Barview and Valino (Fig. 3.4 addition; t-test, $p < 0.001$). Removal interaction strengths also varied among sites (Fig. 3.4 removal, Table 3.4), with positive (facilitative) interaction strengths at Barview that were different from zero (t-test, $p < 0.001$), and neutral effects (t-test, $p > 0.05$) at both Valino and Danger. The quantity of macroalgae removed was not constant across sites due to the natural differences in macroalgae abundance between sites (Table 3.2). For instance, the average volume macroalgae removed at Danger during August 2007 and 2008 (13.3 mL) was 1% that of Barview (1318 mL) and 4% that of Valino (345 mL). At the termination of the experiment (October 2008), variation among sites persisted, but treatment effects on the eelgrass (Fig. 3.5) and sediment (Table 3.5) parameters measured did not. Site effects were apparent for aboveground biomass (fixed effect: Site, $F_{2,62}=19.6$, $p < 0.001$), belowground biomass (fixed effect: Site, $F_{2,63}=9.8$, $p < 0.001$) and shoot length (fixed effect: Site, $F_{2,59}= 44.0$, $p < 0.001$). Valino had increased aboveground and belowground biomass compared to both Barview and Danger (linear contrasts $p < 0.05$), and average blade length was also greatest at Valino, followed by Barview, then Danger (Fig. 3.5; linear contrasts $p < 0.05$). No full model effects of Treatment or Treatment x Site (fixed effects, $p > 0.05$) were found for any of these parameters. Sand-to-silt ratio and redox potential also varied with site, but not treatment (Table 3.5). Across-treatment sand:silt was greater at Barview and Valino than Danger (Table 3.5; linear contrasts, $p < 0.05$) and redox potential was lower across all treatments at Barview relative to Valino and Danger (Table 3.5; linear contrasts, $p < 0.05$).

3.4 DISCUSSION

3.4.1 Context-dependency of eelgrass-macroalgae interactions

Our results show that interactions between eelgrass and macroalgae, often negative under eutrophic conditions (see reviews: Burkholder et al. 2007; McGlathery et al. 2001), can vary dramatically across sites located along an estuarine gradient within an ocean upwelling-influenced estuary. We found that eelgrass and macroalgae interactions ranged from neutral or slightly positive in the more marine-influenced zones of the estuary, to strongly negative at the riverine, freshwater-influenced site (Fig. 3.4). We also found that while removing macroalgae resulted in either positive or neutral responses of eelgrass depending on the site, additions of macroalgae also had slightly positive or neutral effects on eelgrass, except at the riverine site where macroalgae is naturally sparse and the interactions were uniformly negative (Fig. 3.4, 3.5). These treatment effects became significant during the second year of this experiment (Fig. 3.3), indicating that macroalgae effects only become apparent when blooms persist through time. It is likely that a combination of factors mediate the balance between the positive, as well as the negative, effects of macroalgae on eelgrass, and that this balance is tipped in one direction or the other depending on the physical conditions or site-context within the estuary.

The experimental results are supported by the patterns of eelgrass and macroalgae at the different sites observed through time (Fig. 3.2). Despite large macroalgal blooms at the marine site throughout summer 2007 and 2008, eelgrass biomass was either unchanged or changed only slightly from year to year, with no correlation between trends in macroalgae and eelgrass biomass throughout the annual cycle (Fig. 3.2). Similar results were found at the polyhaline and riverine sites, but these sites also had macroalgal abundances that were lower than that of eelgrass and were a small fraction of those at the marine site (Fig. 3.2). Thus, while other studies have found that increased macroalgae productivity is often correlated with declines in seagrass density and biomass (Burkholder et al. 2007), we found no concurrent seasonal patterns or correlations with seagrass biomass.

3.4.2 Mechanisms responsible for eelgrass-macroalgae interactions

The variability of outcomes between eelgrass and macroalgae at different sites along the estuarine gradient point to some potential mechanisms that might be controlling this interaction. Our findings show that the potential negative effects of macroalgae blooms are magnified in the riverine site of this estuary, and tempered in the marine reaches of the estuary, where other mechanisms may lead to neutral or positive effects of macroalgae on eelgrass.

While we did not measure water column light attenuation during this study, evidence suggests that the riverine reaches may be more light-limited than other zones of this estuary. Turbidity is generally higher (20-30 NTU) with more frequent high pulse events associated with storm events at Danger than at the other sites (Rumrill 2006). Elevated turbidity levels can be related to decreased water column light levels, which may affect eelgrass photosynthetic capabilities (Moore et al. 1997; Thom et al. 2008; Zimmerman et al. 1995). Because eelgrass at this site (Danger) may have pre-existing physiological stress associated with low light levels, additional reduction of light by the macroalgae addition treatment could have had a larger impact on plants. Reduction in light, as well as decreased salinity (Nejrup and Pedersen 2008) may be key factors related to the shorter shoot length found at this site (Table 3.2). Due to this small plant morphology, macroalgae additions held in place at the sediment surface may have covered a greater extent of the shoot's photosynthetic surface than the longer blades able to extend beyond the imposed macroalgae canopy at Barview and Valino (Figure 3.5c, Table 3.2, 3.3).

Other key mechanisms known to negatively affect seagrass are associated directly with the sediment environment and macroalgae degradation. It is currently unclear what factors are limiting macroalgal blooms at the polyhaline and riverine sites, but parameters such as water column nutrients, salinity, temperature, light and grazing are likely key (Table 3.1; Cohen and Fong 2004, Hauxwell et al. 1998; Nelson et al. 2008; Rivers and Peckol 1995). The average biomass of macroalgae was very low at Danger during all months sampled, and likely the result of macroalgal drift into this region of the estuary (Fig. 3.2, Table 3.2). The physicochemical factors limiting the distribution of ulvoids in this region may also have acted to degrade macroalgae additions at a greater rate than elsewhere in the estuary, which potentially could have altered the nutrient conditions in the sediment and contributed to decreased oxygen (redox potential) with macroalgae additions (Table 3.5). Differences in macroalgae degradation rates may have resulted in differences in the duration of the addition treatments across sites. At all sites, we observed that the imposed macroalgae canopy created by the addition treatment remained for the duration of the week during which the low tide series occurred. We do not know how long this treatment endured as subtidal site re-visitations were not conducted. Thus, our repeated summer macroalgae manipulations are to be treated and interpreted as pulse, rather than sustained treatments. Among-site differences in sediment grain size also exist along the estuarine gradient (Table 3.3; Rumrill 2006). The dominance of finer, silty sediments at the riverine site could have exacerbated anoxia and depressed gas exchange with seagrass shoots (Ralph et al. 2006). Furthermore, our experimental manipulation structure itself may have affected sedimentation rates at this site, which may increase shoot burial. While we found significant negative effects of macroalgae addition on both aboveground and belowground eelgrass biomass at this site (Fig. 3.5), we found no conclusive evidence that this was correlated with the factors discussed here (increased silt load or decreased redox potential) (Table 3.5). This is surprising because this experiment was meant to accentuate interactions that occur primarily at the sediment interface, by not allowing macroalgae to drift horizontally or vertically within the eelgrass bed. Further experiments that replicate the natural movement of macroalgae within seagrass beds are thus necessary to fully understand the mechanisms at play in these intertidal environments.

In the middle reaches of the estuary, at Valino, eelgrass was not affected by macroalgae manipulations (Fig. 3.3, 3.4, 3.5). In this zone of the estuary, elevated eelgrass shoot biomass, and longer shoot morphology (Table 3.2, 3.3, Fig. 3.5) may have allowed eelgrass to adapt to the conditions by extending beyond the macroalgae-sediment interface where they are less susceptible to the negative effects of light reduction, decreased oxygen levels and sedimentation. Belowground biomass was also greater at this site, but its role in stress mitigation (Hemminga 1998) in this system has not been well investigated. In other systems, it has been found to allow seagrass to withstand periods of shading by acting as a source of carbohydrates (Peralta et al. 2002). We found no effects of macroalgal removal at this site or at Danger, which is expected given the low macroalgae biomass in this region of the estuary.

Contrary to the riverine site, the marine site showed little evidence that mechanisms associated with light reduction and changes in the sediment associated with ulvoid macroalgae are acting negatively on eelgrass. Redox potential was lower in the marine zone at the end of the experiment than at the other two sites (Table 3.5), and within the low range of reported values for seagrasses (Terrados et al. 1999).

However, these large negative values did not vary by treatment, and are consistent with the hypothesis that *Zostera marina* can adapt to low oxygen sediment conditions that may be associated with macroalgae blooms in this zone of the estuary, depending on the nature of the water column conditions (Terrados et al. 1999). Additionally, it does not appear that the macroalgal canopies occupy a sufficient portion of the water column, or are dense enough to negatively affect eelgrass in these intertidal systems. While snorkeling, we observed that the un-manipulated macroalgal canopy occupied a 5-20 cm layer above the sediment during high tide, and we documented repeated macroalgae accumulations that blanketed many of the seagrass shoots at low tide during our sampling. These values contrast with canopy heights of 20-75 cm found along a gradient of nutrient loading in shallow, subtidal Waquoit Bay, MA, but lie within the critical heights associated with eelgrass declines (9 -12 cm) in experimental macroalgae manipulations conducted in this system (Hauxwell et al. 2001). In our system most large seagrass shoots appear to emerge from macroalgae accumulations, thereby reducing the photosynthetic stress associated with macroalgae canopy formation. In the experimental conditions we mimicked these conditions, allowing eelgrass blades to protrude beyond the artificial canopy attached to the sediment. Given the size of our manipulation plots (0.25 m^{-2}), we expect that some edge effects, including shading from adjacent, non-manipulated macroalgae canopies would be present across all of our experimental treatment plots. Site-specific edge effects caused by differences in baseline algal abundance may be one contributing factor to the observed differences in treatment effects between sites. We did not explicitly measure light attenuation within the macroalgae accumulations at this site, however, and future investigation of light attenuation within intertidal seagrass beds is warranted. Mechanisms associated with light and sediment may be balanced or even overshadowed by the potential positive effects of the canopy structure during times of low tide exposure; such mechanisms have not received sufficient research attention in intertidal seagrass systems. At low tide, intertidal seagrass beds are exposed to both heat and desiccation stress; key factors limiting their upper intertidal distribution (Boese et al. 2005). However, a covering of macroalgae that can retain moisture and protect the seagrass from direct exposure may alleviate these stressors. No major signs of desiccation stress (i.e., bleaching) were observed at any of the study sites (Table 3.2). The values that we recorded were lower (average summer month desiccation values of 0.63-1.6%) than those recorded at a similar tidal height in Yaquina Bay (5%) (Boese et al. 2003) where macroalgae blooms also occur (Kentula and DeWitt 2003). Another potential positive effect of macroalgae layers on seagrass, although not investigated here, is the translocation of dissolved organic carbon (DOC) from macroalgae to seagrass shoots. This has been observed in other seagrass species, and related to increased growth under low light conditions (Brun et al. 2003).

While macroalgae addition had no negative affect on eelgrass in the marine zone, removal of macroalgae had a positive effect, suggesting that while eelgrass co-exists with macroalgae, macroalgal removal may improve the conditions for eelgrass.

Therefore, while we did not find any evidence for negative macroalgae effects under ambient and manipulated (2 x ambient) bloom conditions, the baseline population of eelgrass at this site may already be subject to some degree of sub-lethal stress due to the presence of macroalgae. These results should be interpreted cautiously due to the nature of the removal treatment at this site. Macroalgae removal, while thorough, was only maintained during monthly low tide sampling events, and without a retaining structure, algae was able to repopulate these plots sometime after the manual exclusion. In fact, each time we returned to this site, macroalgal abundance had fully recovered to pre-removal levels. This was the case at all sites, but due to the high baseline macroalgal abundance at the marine site, the potential for macroalgae to re-enter removal plots was likely highest at this site, which may reflect the large positive effects found only at this site (Fig. 3.4).

3.4.3 Management Implications

Key to eutrophication management initiatives is an understanding of the ultimate direction and magnitude of seagrass-macroalgae interactions. Currently, along with nutrient criteria and seagrass metrics as secondary symptoms, macroalgal parameters are considered primary indicators of eutrophication and are used as assessment criteria of nutrient enrichment in the United States (Bricker et al. 2003). The use of macroalgae as a bioindicator is based on studies from land-based, anthropogenic eutrophication, where the macroalgae-seagrass interaction has been primarily documented as negative (Burkholder et al. 2007 review). However, growing evidence from this study and other upwelling-influenced estuaries (Brown and Ozretich 2009; Jorgensen et al. 2009) suggests that this metric of eutrophication status may not be appropriate in regions of estuaries where eelgrass can co-exist with macroalgal blooms.

We caution that adaptive management strategies and continued monitoring of these nearshore benthic habitats and water quality nutrient criteria are necessary in coastal areas with growing human populations, such as the Pacific Northwest. Additional nitrogen loading could potentially increase macroalgal biomass to higher levels than observed or manipulated in this study. Research from the Pacific Northwest and around the world show that nitrogen addition rates rarely saturate ulvoid growth (Kamer et al. 2004; Nelson et al. 2008; Teichberg et al. 2010). While our results indicate that eelgrass may not be negatively affected by small increases in macroalgae biomass in the marine-dominated regions of the estuary, increased production in the riverine reaches of the estuary has potentially negative consequences. The specific factors limiting macroalgal growth in the riverine reaches of this estuary are not known. However, based on trends from eutrophic estuaries (Kamer et al. 2004), and the currently low phosphate concentrations in this region of the estuary (Table 3.1) (similar to other upwelling-influenced Oregon estuaries; Brown et al. 2007), macroalgal production may currently be constrained by low watershed nutrient-loading in this region of the estuary (O'Higgins and Rumrill 2007, Fry et al. 2003). While our research documents the current state of macroalgal-eelgrass interactions of one upwelling-estuary, the high degree of context-dependency found both within this estuary, and in comparison to other eutrophic estuaries, suggests the need for continued monitoring and research on these important ecological interactions in a wider variety of estuarine systems.

ACKNOWLEDGEMENTS

Thanks to all the supportive field and lab help associated with this research. At Oregon State University; O. Moulton, P. Zarnetske, J. Henderson, L. Wisehart, D. Eerkes-Medrano, S. McConville, W. Rice-Narusch, H. Bailey, J. Schaefers, M. Rogers, B. Dumbauld, L. McCoy. SS NERR; A. Helms, A. DeMarzo. *R* advice: T. Gouhier. This manuscript was greatly improved by reviewer comments. Research was funded from an award from the National Estuarine Reserve System, Estuarine Reserves Division, Office of Ocean and Coastal Resource Management, National Ocean Service, NOAA. Additional financial support for this research was provided by Oregon State University Department of Zoology, and the Hacker and Menge Labs.

Table 3.1 Physicochemical differences among the study sites along the South Slough NERR estuarine gradient (Barview, Valino, Danger). Mean temperature and salinity data (± SE) from YSI datasondes (Yellow Springs Instruments Model 6600) located in the water column adjacent to each intertidal site. Data collected at 15-minute intervals and averaged over summer (June-September 2007-09) and winter (October-May 2007-09) seasons (min and max range of values also reported). Nutrient grab samples (nitrate + nitrite, phosphate) collected at flood and ebb tide adjacent to datasondes on a near-monthly basis (SWMP protocol (http://cdmo.baruch.sc.edu/)). Mean high and low tide average values (± SE) reported for 2007-2008. Nutrient data acquired and reported in mg/L and converted to μM using the conversions NO_3^-+ NO_2^- μM / 71.39 and and PO_4^{3-} μM /32.9 . Water column data acquisition protocol and data storage implemented by the NERR SWMP (System Wide Monitoring Protocol) program (http://cdmo.baruch.sc.edu/). All flagged data not meeting SWMP quality check protocol removed from this summary.

	Barview (marine)		**Valino (polyhaline)**		**Danger (riverine)**	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Mean Temperature ± SE (°C)	13 ± 0.01	10 ± 0.01	15 ± 0.01	11 ± 0.01	17 ± 0.01	10 ± 0.01
range (min-max)	(8-20)	(5-23)	(9-21)	(3-21)	(4-25)	(0-22)
Mean Salinity ± SE (psu)	33 ± 0.01	29 ± 0.02	32 ± 0.01	26 ± 0.02	19 ± 0.05	10 ± 0.04
range (min-max)	(0-37)	(0-37)	(0-35)	(0-32)	(0-33)	(0-35)

Continued

	Barview (marine)		**Valino (polyhaline)**		**Danger (riverine)**	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Mean Nitrate ($NO_3^{-)}$ + Nitrite (NO_2^-) ± SE range (min-max) **(top: mg/L, bottom: µM)**	0.15 ± 0.01 (0.02-0.39), 10.72 ± 0.68 (1.18-27.74)	0.16 ± 0.00 (0.00-0.35), 11.25 ± 0.33 (0.29-25.04)	0.11 ± 0.01 (0.01-0.26), 7.95 ± 0.87 (0.61-18.80)	0.15 ± 0.01 (0.01-0.38), 10.71 ± 0.59 (0.36-27.20)	0.06 ± 0.01 (0.00-0.42), 4.12 ± 0.64 (0.37-30.06)	0.28 ± 0.01 (0.06-0.66), 20.02 ± 0.97 (4.00-47.33)
Mean Phosphate (PO_4^{3-}) ± SE range (min-max) **(mg/L, & µM)** **(top: mg/L, bottom: µM)**	0.05 ± 0.00 (0.02-0.07), 1.64 ± 0.04 (0.51-2.43)	0.03 ± 0.00 (0.01-0.09), 1.13 ± 0.02 (0.33-3.09)	0.05 ± 0.00 (0.02-0.09), 1.64 ± 0.07 (0.57-3.06)	0.03 ± 0.00 (0.01-0.06), 1.03 ± 0.03 (0.36-1.88)	0.02 ± 0.01 (0.00-0.05), 0.59 ± 0.07 (0.04-1.68)	0.01 ± 0.00 (0.00-0.12), 0.40 ± 0.04 (0.02-4.05)

Table 3.2 Mean macroalgae and eelgrass parameters measured during observational study at three sites (Barview - marine, Valino -polyhaline, Danger - riverine) along the South Slough NERR, estuarine gradient (June 2007-April 2009). Mean values determined from pooled samples of all summer and winter months sampled. Summer months = June, July, August, September; winter months = November, January, April. Sample sizes: macroalgae biomass area^{-1} = 6 quadrats site^{-1} month^{-1}, eelgrass density area^{-1} =10 quadrats site^{-1} month^{-1}, eelgrass shoot biomass, length and desiccation index = 30 shoots site^{-1} month^{-1}.

	Barview (marine)		**Valino (polyhaline)**		**Danger (riverine)**	
	Summer	**Winter**	**Summer**	**Winter**	**Summer**	**Winter**
Macroalgae Biomass (mean g dry wt 0.25m^{-2})	56.1 ± 5.3	2.2 ± 0.6	9.7 ± 2.3	1.4 ± 0.4	0.3 ± 1.3	0.1 ± 0.2
Eelgrass Biomass (mean g dry wt 0.25m^{-2})	24.8 ± 1.9	18.6 ± 2.3	30.5 ± 2.1	17.1 ± 0.8	14.6 ± 1.0	8.1 ± 0.7
Eelgrass Shoot Density (mean shoot 0.25 m^{-2})	28.5 ± 1.6	22.9 ± 2.6	23.4 ± 1.2	21.4 ± 0.9	33.2 ± 1.9	34.0 ± 2.7
Eelgrass Shoot Length (mean cm)	106.1 ± 9.9	76.6 ± 5.7	108.1 ± 7.1	91.0 ± 6.4	74.4 ± 5.4	45.4 ± 5.7
Desiccation Index (% blade surface)	1.56 ± 0.25	0	0.63 ± 0.18	0	0.76 ± 0.22	0

Table 3.3 Linear mixed effect model results from eelgrass-macroalgae observational study. Effects of season (summer vs. winter) or site on producer biomass (g dry wt $0.25m^{-2}$) at three sites (Barview – marine, Valino – polyhaline, Danger – riverine) along the South Slough NERR estuarine gradient. Differences between sites compared during both summer and winter seasons (fixed effect = site). ns = no significant differences ($p>0.05$); *$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$

Response	**Sample Size (N)**	***Fixed Effect***	**df Fixed effect, df Error**	***F* (p)**
Macroalgae Biomass	258	*Site*	2, 240	0.9 (**)
		Season	1, 1	51.13 (ns)
		Site x Season	2, 240	100.09 (***)
Eelgrass Biomass	420	*Site*	2, 402	46.77 (***)
		Season	1, 1	6.97 (ns)
		Site x Season	2, 402	0.46 (ns)
Eelgrass Density	420	*Site*	2, 402	14.54 (***)
		Season	1, 1	2.29 (ns)
		Site x Season	2, 402	2.92 (*)
Eelgrass Length	1261	*Site*	2, 1243	214.75 (***)
		Season	1, 1	10.17 (ns)
		Site x Season	2, 1243	13.74 (***)

Table 3.4 Linear mixed effect model results from eelgrass-macroalgae manipulation experiment. Effects of treatment and season-year on eelgrass density (shoots $0.25m^{-2}$) at three sites (Barview – marine, Valino – polyhaline, Danger – riverine) along the South Slough NERR estuarine gradient. Interaction strengths (treatment – control) differences for addition and removal also reported. ns = no significant differences ($p>0.05$); $*p < 0.05$; $**p < 0.001$; $***p < 0.0001$

Response	**Sample Size (N)**	***Fixed effect***	**df Fixed effect, df Error**	***F* (p)**
Eelgrass Density	729	*Site*	2, 687	3.82 (*)
		Treatment	2, 16	8.37 (**)
		Year	1, 687	224.01 (***)
		Site x Treatment	4, 687	10.97 (***)
		Site x Year	2, 687	1.36 (ns)
		Treatment x Year	6, 287	4.96 (*)
		Site x Treatment x Year	4, 687	4.06 (*)
Addition Interaction Strength	27	*Site*	2, 16	19.39 (***)
Removal Interaction Strength	27	*Site*	2, 16	7.29 (*)

Table 3.5 Physical sediment parameters (sand:silt, redox potential) measured at the different estuarine gradient sites (Barview - marine, Valino - polyhaline, Danger - riverine) at the termination of the experimental macroalgae manipulation (October 2008). N = 27 plots site^{-1}. Linear mixed effects modeling results reported. ns = no significant differences ($p>0.05$); *$p < 0.05$; **$p < 0.001$; ***$p < 0.000$

		Site			**Mixed effect model results**		
		Barview (marine)	**Valino (polyhaline)**	**Danger (riverine)**	***Fixed effect***	**df Fixed effect, df Error**	***F* (p)**
Sand (g): Silt (g)	**Control**	5.0 ± 1.0	6.2 ± 1.0	3.1 ± 1.6	*Site*	2, 64	10.3 (**)
	Addition	5.5 ± 0.9	5.8 ± 0.6	2.0 ± 0.3	*Treatment*	2, 64	0.39 (ns)
	Removal	5.7 ± 1.2	8.6 ± 2.7	1.7 ± 0.2	*Site x Treatment*	4, 64	0.76 (ns)
Redox Potential (mV)	**Control**	-335.0 ± 15.2	-171.0 ± 41.2	-143.2 ± 8.4	*Site*	2, 64	61.2 (***)
	Addition	-336.3 ± 21.5	-182.6 ± 43.3	-186 ± 19.0	*Treatment*	2, 64	1.2 (ns)
	Removal	-309.2 ± 14.1	-173.4 ± 40.7	-147.7 ± 10.7	*Site x Treatment*	4, 64	0.38 (ns)

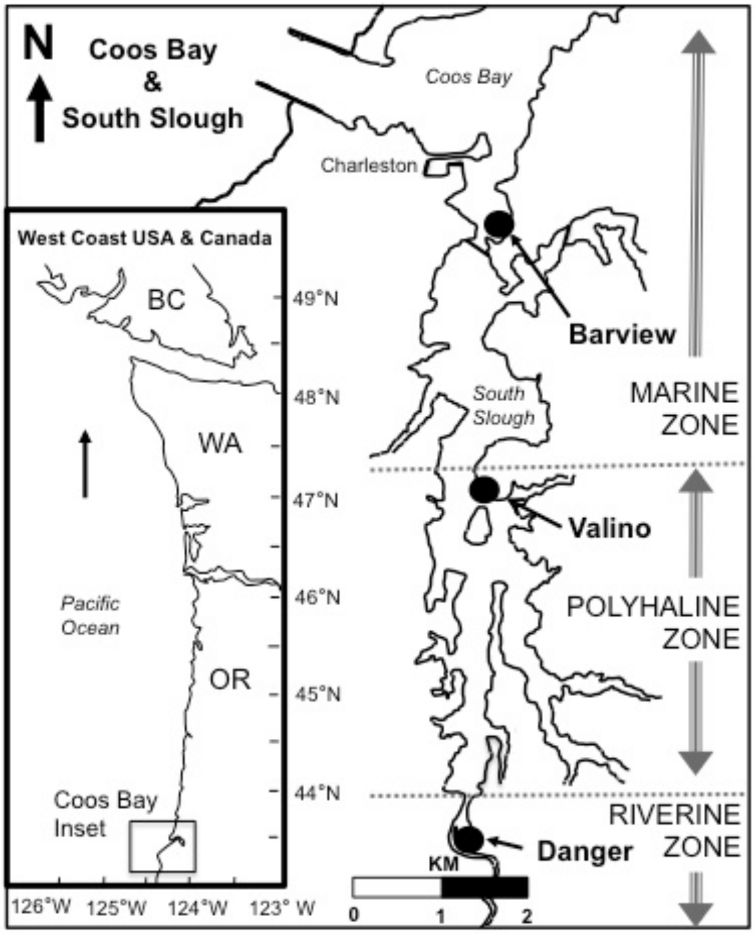


Figure 3.1 Location of study sites along the South Slough National Estuarine Research Reserve estuarine gradient, Coos Bay, Oregon.

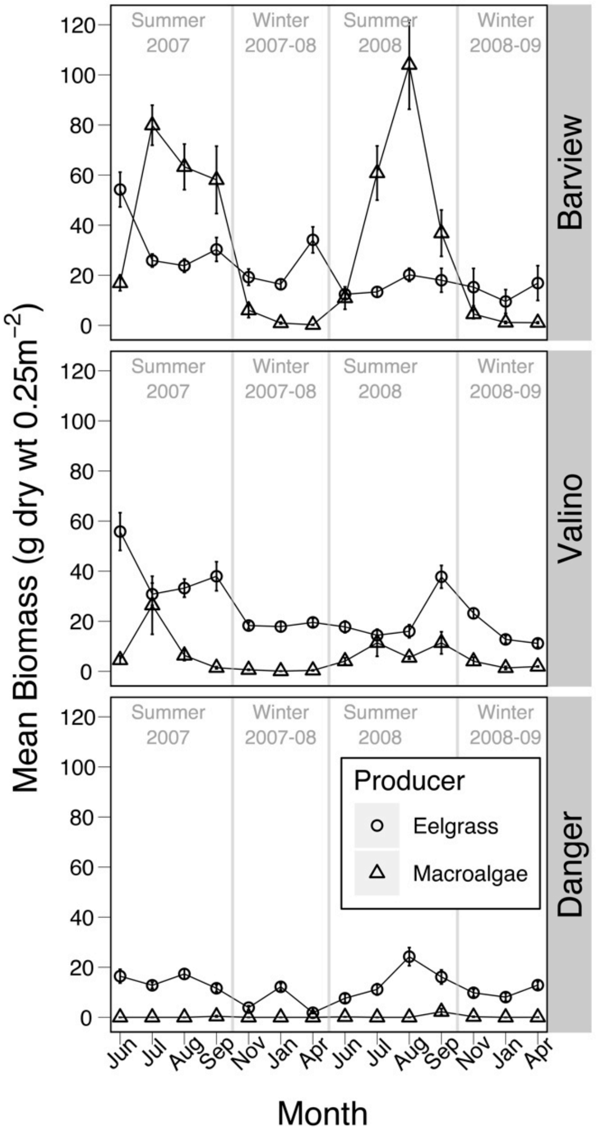


Figure 3.2 Mean (± SE) macroalgae and seagrass biomass at 3 sites located along the estuarine gradient within South Slough, Coos Bay, Oregon, USA from June 2007 to April 2009 (Barview -marine, Valino - polyhaline, Danger - riverine). Macroalgae biomass N = 6 quadrats site^{-1} month^{-1} g dry wt 0.25m^{-2}, 10 quadrats site^{-1}month^{-1}. Eelgrass biomass N = 10 quadrats site^{-1}month^{-1} shoot density 0.25m^{-2} x 30 shoots site^{-1} month^{-1} g dry wt.

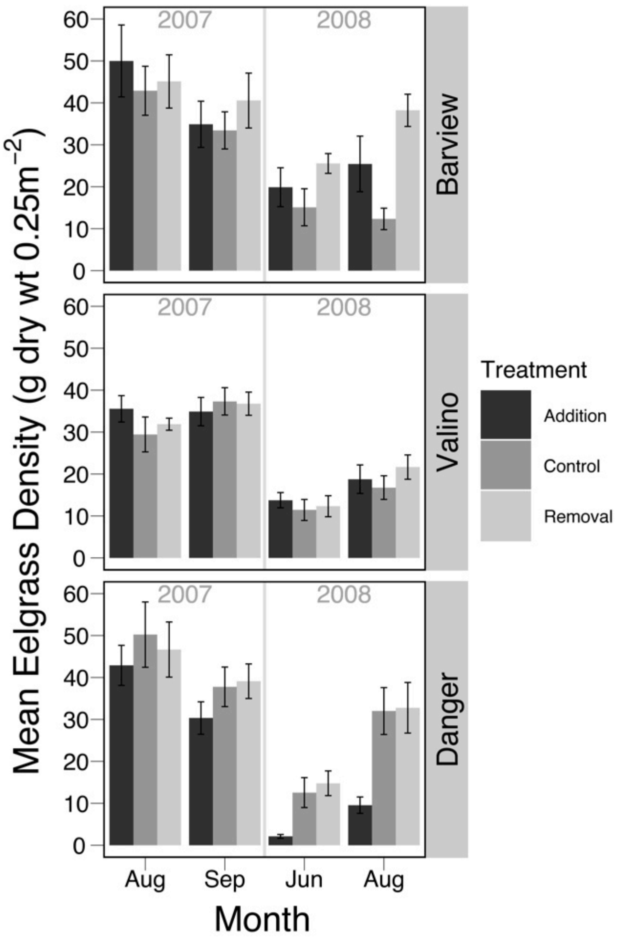


Figure 3.3 Mean (± SE) eelgrass densities (0.25 m^{-2}) in eelgrass-macroalgae manipulation experimental plots at three sites (N = 27 plots site^{-1} month^{-1}) along the South Slough estuarine gradient (Barview - marine, Valino - polyhaline, Danger - riverine). First and last summer month sampled each year (2007 and 2008) is a subset from all months sampled.

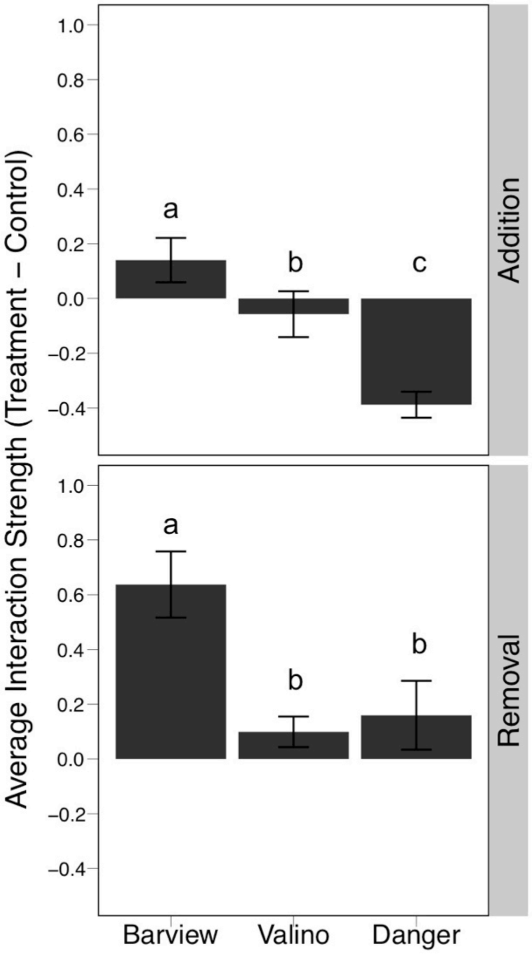


Figure 3.4 Mean (± SE) eelgrass density response to the macroalgae manipulation experiment (N = 27 plots site^{-1} month^{-1}). Addition and removal treatments at three sites along the South Slough estuarine gradient (Barview - marine, Valino - polyhaline, Danger - riverine). Interaction strength calculated as the difference between manipulated and control treatments for the relative change in eelgrass density from initial (August 2007) to final (October 2008) experiment conditions. Significant differences between sites indicated by letter annotations (t-test linear contrast conducted independently for each treatment)

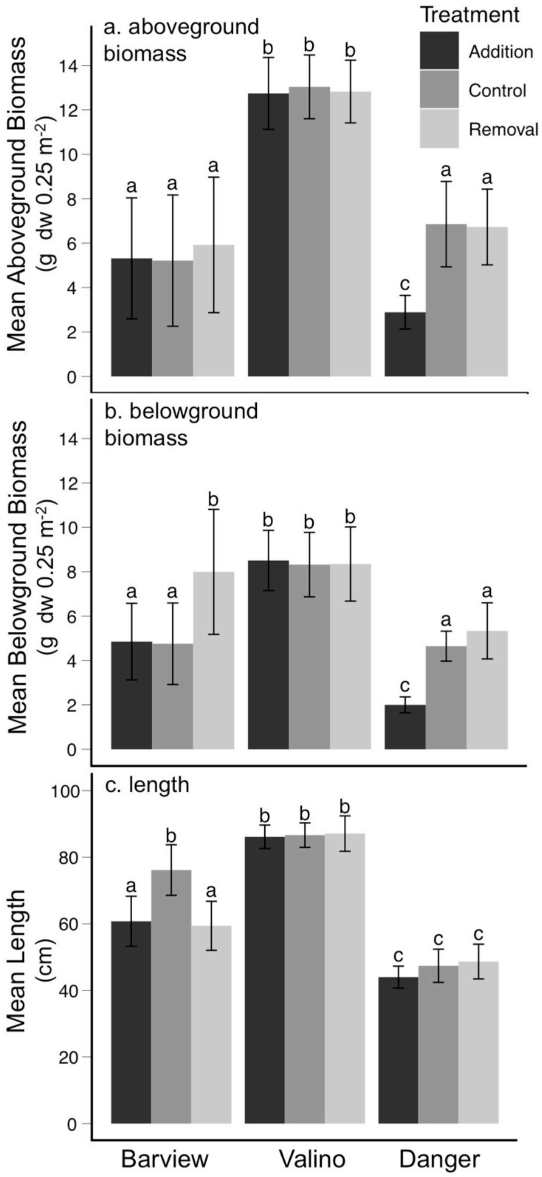


Figure 3.5 Eelgrass parameters measured at the end of the eelgrass-macroalgae experiment (October 2008) at three sites (N = 27 plots site^{-1}) along the South Slough estuarine gradient (Barview - marine, Valino - polyhaline, Danger - riverine): a) Mean (± SE) aboveground eelgrass biomass (mean g dry wt 0.25 m^{-2}). b) Mean (± SE) belowground eelgrass biomass (mean g dry wt 0.25 m^{-2}). c) Mean (± SE) eelgrass length (cm). Significant differences between sites indicated by letter annotations (t-test linear contrast conducted independently for each parameter).

4 – Constrasting outcomes of nutrients and macroalgae on seagrass from upwelling-influenced field and mesocosm experiments

Margot L. Hessing-Lewis, Sally D. Hacker, Bruce A. Menge, Sea-oh McConville,
Jeremy Henderson[1]

ABSTRACT

Increasing nutrient inputs to coastal systems often result in blooms of ulvoid macroalgae, which can have negative consequences on seagrass via shading, as well as other mechanisms. We conducted a field experiment in an upwelling-influenced estuary, where marine-derived nutrients dominate, to understand the direct effects of nutrients and indirect effects of macroalgal production on the seagrass *Zostera marina*. We conducted a complementary experiment manipulating the same factors in mesocosms. In the field, we found no effect of either nutrients or macroalgal treatments on seagrass blade length, density or biomass. In the mesocosms, however, macroalgal treatments had large effects on these same metrics. Addition of macroalgae led to increased light attenuation and decreased sediment oxygen levels. The additive effect of these two factors was associated with larger effects on eelgrass than shading alone, which was evaluated using mimic algae treatments. Nutrient effects on seagrass biomass were also found in the mesocosms, but these were not as pronounced as macroalgal effects, and no interaction between nutrients and macroalgae were found. Contrary results from these parallel experiments are most likely due to high rates of water movement, and the intertidal location of the study site; key physical factors mitigating light reduction and biogeochemical changes *in situ*. Adaptation of ulvoids and eelgrass to high nutrient concentrations may also explain the context-specific results reported here. In upwelling-influenced systems, we find that both physical and nutrient contexts inform novel interaction dynamics.

4.1 INTRODUCTION

Land-based nutrient inputs can have multiple negative effects for estuarine systems, including hypoxia, die-offs of fish and invertebrate species, and harmful phytoplankton and macroalgal blooms (Bricker et al. 2008). Seagrasses have been particularly impacted by the direct and indirect effects of these nutrients (Duarte 1995, Nixon et al. 2001, Cardoso et al. 2004, Burkholder et al. 2007). Although increased nutrient concentrations in coastal waters can affect seagrasses directly, many studies show that the primary pathway governing nutrient effects on seagrass is indirect, via algal blooms (Short et al. 1995, Havens et al. 2001). Of concern are blooms of fast-growing macroalgae, such as ulvoids (*Ulva* spp.), which have often been found to negatively affect seagrass by reducing light levels in the canopy, as well as changing the local biogeochemical environment (McGlathery 2001). Determining the direct and indirect role of nutrients on seagrasses, and the context specificity of these interactions, is important to the management of this critical nearshore habitat, which provides important ecosystem services (Antón et al. 2011, Barbier et al. 2011).

Both seagrass and macroalgal producers can respond positively to increased availability of nutrients, but these responses are primarily observed under low to moderate enrichment in systems where nutrients are the primary limiting factor (Udy and Dennison 1997, Burkholder et al. 2007). Conversely, negative responses, including die-offs, have been documented as a result of physiological responses to high levels of nutrient enrichment (Touchette and Burkholder 2000). Such physiological mechanisms include decreased water-column nitrate inhibition, resulting in carbon limitation, as well as internal ammonium toxicity, which can cause structural weakness in seagrass shoot meristematic tissue (McGlathery 2001). Seagrass responses to nutrient loading show substantial variation however, and similar physiological responses may not hold in particular geographic regions or locations with different historical nutrient input regimes (Kaldy 2009).

While direct physiological response of seagrasses to nutrient enrichment may often contribute to their declines, negative effects of algae, including phytoplankton, epiphytes, and macroalgae, are the most common cause (Burkholder et al. 2007). The mechanisms by which macroalgae have been documented to affect seagrass are many, but changes in the light regime caused by the formation of macroalgal canopies overlaying seagrass beds are most often implicated. Macroalgal blooms can be thick (e.g., 0.25 to > 2m in height - Sfriso et al. 1992, Hauxwell et al. 2001), of high biomass (e.g., 50 to 310 g dry wt m^{-2} - Pregnall and Rudy 1985, McGlathery 2001), and cause such low light levels that negative physiological consequences are common. In addition, low oxygen concentrations caused by increased macroalgal respiration (during non-photosynthetic periods) and degradation of organic matter can increase the sulfide concentrations in the sediment and ammonium in the water-column, which can also affect seagrass (Pregnall et al. 1984, Goodman et al. 1995, Krause-Jensen et al. 1996, van der Heide et al. 2008). Moreover, the structure of macroalgae itself can result in decreased water movement, which reduces advection of oxygen and nutrients within the water-column, and alters nutrient cycling system-wide (McGlathery et al. 2007). Macroalgae accumulations may also have direct mechanical effects on seagrass and their epiphytes as it drifts or accumulates in large aggregations (Irlandi et al. 2004).

Nutrient and macroalgal effects may act on seagrass in concert with other environmental factors and via feedback loops (Duarte 1995). These physiological, structural and physicochemical mechanisms by which nutrients affect eelgrass, either directly or indirectly, are often interactive, and the prominence of specific mechanisms may be context-specific. Nutrient loading to coastal systems is the result of both land-based and marine inputs (Howarth et al. 2011), and in certain coastal systems, such as estuaries adjacent to nutrient-rich coastal waters, marine-based nutrients may even dominate. This is often the case for estuaries adjacent to upwelling zones, where marine-derived nutrients are tidally advected into estuarine waters (Hickey and Banas 2003, Brown and Ozretich 2009). Studies from estuaries adjacent to the California Current upwelling system, along the West Coast of North America, find large blooms of macroalgae in the summer months associated with marine-based nutrients (Brown et al. 2007, Jorgensen et al. 2010, Hessing-Lewis and Hacker in review). These blooms can be as large as those associated with seagrass declines (Hessing-Lewis et al. 2011), but in these systems, declines in seagrass through time have not been found. For example, in a comparison of estuaries along the Oregon coast, the potential for both nutrient and macroalgal effects on seagrass has been documented; upwelling magnitude during summer months was strongly associated with macroalgal blooms, and negatively associated with eelgrass production (Hessing-Lewis and Hacker in review). Surprisingly, experimental evidence (Hessing-Lewis et al. 2011) and observational data (Thom 1990, Kentula and DeWitt 2003) from estuaries exposed to strong upwelling conditions provides little evidence for negative effects of macroalgal blooms on eelgrass. The specific role of nutrients, and how they interact with both seagrass and macroalgae, however, have not been explored in these systems.

To understand how the most common species of seagrass (*Zostera marina* L., hereafter referred to by its common name, eelgrass) in upwelling-influenced estuaries responds to both nutrient inputs and macroalgal canopies, we manipulated these factors in two experiments, one in the field and one in an outdoor lab mesocosm. Our goal was to understand both the direct and indirect effects of nutrients and macroalgae on eelgrass under conditions of high ambient nutrient concentrations. Based on our previous work in upwelling-influenced estuaries where we found limited occurrences of negative interactions, we hypothesized that macroalgal manipulations (either removal or addition of ulvoid macroalgae) would have positive or neutral effects on eelgrass. We also hypothesized that the direct nutrient effects on both eelgrass and macroalgae would be dependent on the extent of producer response to nutrient addition. Macroalgae are often nutrient-limited, and can respond quickly to increased nutrient concentrations (Duarte 1995, Valiela et al. 1997). If this is the case in upwelling-influenced estuaries, we predicted that nutrient addition would augment macroalgal production, and in turn, increase the potential for indirect interactions with eelgrass via shading and other biogeochemical mechanisms. In order to isolate some of these macroalgal mechanisms from nutrient feedback processes, we employed mimic algae, which allowed us to determine the role of light exclusive of macroalgal decomposition and re-mineralization. By comparing results between field and mesocosm venues we aimed to contrast the physiochemical conditions associated with experiment outcomes.

4.2 METHODS

4.2.1 Experiment Location and Design

The field experiment was conducted in Coos Bay, Oregon, at Fossil Point (43°22'3"N, 124°18'11"W). This site is located near (~ 3.5km) the mouth of Coos Bay, along its primary shipping channel. It is exposed to waves entering the estuary from the exposed coast, as well as periodic wakes from boat traffic. From June 2007 to April 2009 we conducted an observational study of macroalgae and eelgrass production to complement experimental results. We used haphazardly placed quadrats to quantify macroalgae production, coupled with field collections of eelgrass biomass and density measurements from a permanent transect line (using the same methodology described in Hessing-Lewis et al. 2011).

The field experiment was carried out in summer 2009. We manipulated macroalgal cover in large plots of eelgrass (2.4 m^2) by adding or removing ulvoid macroalgae. Various species of ulvoid macroalgae grow together in this region. Our manipulations were focused on the dominant sheet forms, including the species *Ulva linza* and *Ulva lactuca*. Macroalgal treatments also included addition of mimic macroalgae (described below), and control plots open to ambient macroalgal conditions. Macroalgae treatments (CONTROL, ADDITION, REMOVAL, MIMIC) were crossed with nutrient addition treatments (AMBIENT, +NUTRIENTS) in a fully crossed design. Plots were delineated with green garden fencing (50 cm high, mesh size of 5 cm) anchored at the corners with PVC pipes used as stakes and reinforced throughout the perimeter with bamboo rods. Treatments were randomly assigned to plots, which were spaced 3-5 m apart. As we document below, this spacing was sufficient to allow for independence of nutrient treatments in this highly diffusive environment. Three blocks of the crossed macroalgae x nutrient design (24 plots total), were located along the main channel, each separated by distances of 30-50 m. All plots were located in the intertidal zone at elevations of -0.1 to +0.1 Mean Lower Low Water (MLLW).

The mesocosm experiment was conducted at Oregon State University's Hatfield Marine Science Center, Newport, Oregon, in 18 flow-through cylindrical tanks (80 cm tall, 90 cm diameter). Bay water was pumped from the adjacent Yaquina Bay at high tide and filtered (50 microns) before circulating through the tanks. Water entered all tanks from spigots near the top of the tanks, and exited via 65 cm tall tubular drains located at the center of each tank. Flow rates were controlled so that all tanks had turnover rates of ~5 L min^{-1}. Yaquina Bay is located 150 km N of Coos Bay and its nutrient conditions are also highly upwelling-influenced during summer months (Brown and Ozretich 2009).

Macroalgal and seagrass collections for the mesocosm experiment were made in Yaquina Bay, at Idaho Point (44°37'1"N, 124° 1'43"W, 4.5 km from mouth of estuary). Mean macroalgae biomass at this site from 2006-2010 averaged 82.2 ± 5.3 g dry wt $0.25m^{-2}$ (N = 20 yr^{-1}), with maximum values of 120.0 ± 12.9 g dry wt (2008; Hessing-Lewis and Hacker in review). Comparably large blooms have also been observed by others in this marine-influenced region of Yaquina Bay (Kentula and DeWitt 2003, Brown et al. 2007). Eelgrass shoots (including 5 cm of rhizome) were transplanted into plastic buckets (23 cm tall) containing sediment (10-15 cm deep) collected from the same site (infauna, primarily polychaetes, were not removed from the substrate). Seven shoots were transplanted in each bucket, and seven buckets were placed at the bottom of each mesocosm tank. Three macroalgae treatments comparable to field treatments (REMOVAL = no macroalgae, ADDITION = macroalgae added and MIMIC = mimic macroalgae added) were again crossed with two nutrient treatments (AMBIENT, +NUTRIENTS) for a total of 6 treatment levels, each with three replicates (N = 18 total). Treatments were randomly assigned to tanks that were arranged in an area open to direct sunlight. Eelgrass was harvested in May 2009 and allowed to acclimate to tank conditions for a period of 1 month prior to treatment application. During this time, dead or unhealthy shoots were replaced, so that initial shoot density and condition was similar across all tanks. Tank surfaces were scrubbed and eelgrass blades were cleaned manually every week in order to reduce epiphytic fouling (primarily diatoms). Fauna, such as juvenile crabs, snails and amphipods were removed manually when possible.

4.2.2 Macroalgae manipulations

To quantify macroalgae in our experiments, we used volume, a surrogate measurement for biomass in the field (Robbins and Boese 2002). Based on a two year dataset relating macroalgal volume (mL $0.25m^{-2}$) to biomass (g dry weight $0.25m^{-2}$) in Coos Bay ($N=199$, $R^2 = 0.82$, $p < 0.001$), our measurements of volume were converted to dry weight as follows: ln macroalgae biomass = 1.08*ln macroalgae volume – 3.49. In the field experiment, ADDITION consisted of the pulse addition of macroalgae (160,000 mL (12.7 kg dry wt) in June, August and September, 140,000 mL (11.0 kg dry wt) in July). This volume represents 5 times the highest ambient macroalgae conditions found in non-addition treatments. Macroalgae was manually removed from both REMOVAL and MIMIC treatments, and CONTROL plots were left with ambient macroalgae quantities. Macroalgae was removed or added at low tide when the plots were exposed. As the tide flooded back into the plots, we kept the added macroalgae from floating out of the plot by manually sinking the macroalgae and displacing air bubbles. In this way, the added macroalgae formed a dense canopy extending up from the sediment surface, approximating macroalgal conditions observed by snorkeling at high tide. Macroalgal additions and removals occurred on a monthly basis throughout the summer. Percent cover and volume of macroalgae were monitored in a permanently marked central quadrat of each plot before monthly macroalgae additions.

In the mesocosm experiment we initially added 8,000 mL (0.5 kg) dry wt of macroalgae per tank in July. Based on volume per substrate area, this was comparable to field plot additions of 60,000 mL (4.4 kg dry wt), approximately 3 times lower than field ADDITION treatments. Smaller volumes were used in the mesocosms as macroalgal loss rates were reduced compared to the open plots in the field. In the tanks we also sunk the macroalgae manually by removing air bubbles so that it formed a canopy close to the sediment surface of the buckets. During weekly tank maintenance we kept macroalgal canopies at average heights of 30-50 cm. At monthly intervals we re-measured macroalgal volume per tank, and added fresh macroalgae to maintain quantities at 6,000 mL (0.4 kg dry wt) in August and 8,000 mL (0.5 kg dry wt) in September. The mesocosm REMOVAL can be considered the control treatment for the mesocosm experiment and approximated field REMOVAL conditions.

We used silicon-impregnated rip-stop nylon to mimic algae for both mesocosm and field MIMIC macroalgae treatments. Sheets of green nylon (approximating the natural color of green ulvoid macroalgae) were cut into rectangles (40 cm wide by 75 cm long). Two sheets were overlaid so that their lengths were perpendicular, and secured around a central bundle of small rocks using a cable tie. The rocks served to anchor the mimic fronds, which were positively buoyant in water. Mimics were added to both mesocosm tanks and field plots at levels that covered the substrate. In each MIMIC algae tank, thirty units of mimic algae were added around the eelgrass shoots, imitating the physical structure of real macroalgae found within the ADDITION treatments.

In the field experiment, approximately 40 units of mimic algae were added to each plot. Units of mimic algae were placed throughout the entire plot, but were focused in the central area, where eelgrass dynamics were closely monitored. The “biomass” of nylon for the mimic canopies was less than the ADDITION canopies, but their light reduction capabilities per surface area were higher. We found that one sheet of nylon attenuated the equivalent amount of light as three average sheets of ulvoid macroalgae. Organisms, such as worms, amphipods, anemones and snails also recruited to the mimics in the field, and sediment accreted in its crevices, in a similar fashion to that observed for large, anchored sheets of ulvoid macroalgae. While the units of mimic algae did fray, they did not decompose as does natural macroalgae.

4.2.3 Nutrient Additions

In the field, we used nutrient diffuser tubes containing Osmocote^{TM} (The Scotts Company LLC) slow-release fertilizer (molar ratio of 19N:6P:12K) to enrich the local water column of the nutrient addition treatments. To focus nutrient addition, 5 nutrient diffuser tubes were arrayed in a horseshoe configuration around the central quadrat area of each plot. Each tube (5.08 cm diameter) was 60 cm long; 30 cm aboveground and 30 cm belowground. 500 g of Osmocote^{TM} was suspended in mesh bags from the top of each tube, and nutrients leached into the water-column via 20 holes (2.4 cm diameter) drilled into the aboveground segment of each pole. A total of 2500 g Osmocote TM was used in each plot. This dosage is similar to that used in other nutrient addition experiments in subtidal seagrass beds (i.e., 500 g m^{-2} N:P = 19:1 - Antón et al. 2011, 1250 g m^{-2} N:P = 16:3 - Heck et al. 2006). Control tubes of the same dimensions, with no holes or nutrients were placed in the AMBIENT treatments in order to control for the physical structure of the tubes. Nutrient treatments were first applied in July. After 45 days, the fertilizer was gathered to measure the amount dissolved, and fresh fertilizer (1500 g per plot) was added for another 45 days.

In the tanks, Osmocote^{TM} was also suspended in thin mesh within small diffuser tubes (20 cm long, with 12 holes) suspended 20 cm above the tank bottom. Two tubes of 150 g each (300 g total per tank) were deployed initially in July (for 39 days), with a re-deployment of fresh fertilizer in August (for 56 days). Nutrient quantities added to the mesocosm experiment corresponded to levels added in the field (g Osmocote^{TM} m^{-2}).

We collected water samples to analyze for evidence of nutrient enrichment by the Osmocote^{TM} additions. Samples were collected though Tygon tubing attached to a 50mL plastic syringe. All water samples were kept on ice in a cooler prior to filtration (Whatman DF/F filters) in the lab. All samples were frozen before analysis of dissolved inorganic nitrogen (DIN) and phosphate (PO_4^{3-}) by the University of Washington Marine Chemistry Lab. In the field, we compared samples collected adjacent to the nutrient diffusers from the CONTROL / AMBIENT and CONTROL / +NUTRIENT treatments as the tide flooded the plots (block 1 only, three reps per treatment). In July we collected samples from both treatments 0-10 cm from the diffuser tubes. In August, we collected three samples at three distances from the nutrient diffuser tubes (0, 5, 20 cm) on three different dates. In the mesocosms, three samples were also collected from each replicate CONTROL / AMBIENT and CONTROL / +NUTRIENT treatment. Tank samples were collected at 0 -10 cm from the diffuser tubes on two dates, in July and August.

4.2.4 Eelgrass Response

We measured shoot density (shoots x area^{-1}), shoot length (first rootlet node to tip), sheath length (a proxy measurement for growth), and final biomass for both field and mesocosm experiments. In the field, eelgrass measurements were focused on a subset of the plot conditions using a permanently marked quadrat (0.25 m^{-2}) located in the center of the plot. Five spot density measurements (shoots 0.0625 m^{-2}) were also taken haphazardly throughout the entire quadrat. Because initial density measurements differed between plots, we calculated change in density from the initial measurements as the density response metric for both field and tank experiments. Sheath length was measured from a subset of 5 shoots within the central quadrat, and 15 shoots haphazardly located throughout the plot. Eelgrass shoots were haphazardly collected from around the nutrient diffuser tubes (with a radius of 0.5 m) to measure change in length through time.

In the mesocosm experiment, density and morphometrics of all shoots per bucket were recorded and averaged by tank (buckets were nested within tanks). Biomass measurements (dry weights) were obtained at the end of the experiment by collecting all shoots from the central quadrat and the buckets, washing and scraping all epiphytes from eelgrass in the lab, drying at 60° C for 24 hrs and weighing. In the mesocosms, we also trimmed the eelgrass to the top of the water line on a weekly basis to prevent self-shading (the blades lie horizontal on the water surface once they reach the height of water). We used trimmed biomass as another measurement to approximate growth. In the mesocosms we also collected material that had sloughed from shoots on a weekly basis, a measurement of production. Most eelgrass response measurements were measured on a monthly basis throughout the experiment (June to October 2009; see Table 4.1 for specific dates).

4.2.5 Response Covariates

Light measurements of photosynthetic photon flux density (PPFD) were measured with a LI-193 Spherical Quantum Sensor. Long-term measurements were recorded using a LI-1400 Datalogger, and spot measurements were recorded using a LI-250A Light Meter (all LI products by LI-COR corporation). In the field, we measured light levels on eight occasions as the tide ebbed or flooded within the intertidal plots (measurements were not logistically possible at the highest tides). Sensors were vertically oriented and attached to a stake placed in the substrate within the plot at a height of 20cm above the substrate. On each logging event we recorded light in three plots simultaneously with the LI-1400 (three channel logger). Throughout the intertidal light logging events (August 18-22, September 17-19) we measured light in all blocks, and all macroalgal treatments (we limited our sampling to AMBIENT nutrient treatments). Light measurements were recorded at 5 min intervals throughout each event (and averaged over 30s logging periods). To analyze differences between treatments, we used average values per logging event. Percent surface irradiance in the mesocosms as a measure of light attenuation was calculated as: mean PPFD readings at 5 cm below the water line (surface) / mean PPFD readings at 30 cm depth. For long-term light measurements in the tanks, the sensors were also placed at 30 cm from the base of the tanks (mid-water).

We measured sediment redox potential in the sediment surface (within the top 5 cm) in both the field and the mesocosms using an Orion Star probe (Thermo electron corporation) fitted with a platinum electrode. We measured temperature, salinity, dissolved oxygen and pH in the water column of the mesocosm tanks with an HI 9828 multiparameter water quality portable meter equipped with a pH/ORP sensor (Hanna Instruments). Spot water column measurements were recorded at a depth of 30cm in the tanks, on 13 dates spread throughout the experiment, during daylight hours (see Table 4.3 for specific dates when response covariate measurements were taken).

We used chalk blocks made of dental chalk as integrated measurements of water movement, wave action and sediment scouring in the field and tank environments (Yund et al. 1991). Pre-weighed chalk was screwed into PVC tubes placed at the same height with reference to the sediment in field plots and mesocosm buckets. We measured dissolution rates as dry weight loss per day throughout deployments (field = 18 days, mesocosms = 19 days).

4.2.6 Statistical analyses

Linear mixed effect models were fit to ulvoid and nutrient response metrics, seagrass response parameters and covariates using the nlme package in R (Pinheiro et al. 2009, R Development Core Team 2009). Nutrient and macroalgae treatment effects (fixed factors) were evaluated with ANOVAs. For the field experiment, block and time were random factors in the statistical model. For the mesocosm experiment, lack of independence due to the spatial nestedness of buckets within tanks was also specified using a random effect term. For both experiments, the relationship between measurements on the same experimental unit through time was incorporated by specifying a correlation structure (CorStruct) within the linear model. We used simple linear models to analyze the effects of nutrient enrichment between treatments on two different dates. Comparisons of significant treatment effects for all models were evaluated using post hoc tests (Tukey test).

4.3 RESULTS

4.3.1 Eelgrass response to nutrients and macroalgae

In the field experiment, although eelgrass density declined through time (Fig. 4.1A), neither macroalgal nor nutrient treatments affected eelgrass density (change though time for both spot and central quadrat measurements) (Fig. 4.1A), final eelgrass biomass (Fig. 4.2A), sheath length, or mean length throughout the experiment (Table 4.1). Since there were clear trends in the means through time (Fig. 4.1A), this lack of effect seems partly due to high variation among blocks. Eelgrass length of the final biomass was greater in the CONTROL treatments (Table 4.1), which reflects initial morphological differences between treatments (June 22).

From June to September 2007/2008, we recorded mean summer ulvoid biomass of 71 ± 13.3 g dry wt 0.25 m^{-2} at this site, with peak blooms of 107.4 ± 33.1 g dry wt 0.25 m^{-2} (August 2007) and 123.0 ± 6.8 g dry wt 0.25 m^{-2} (September 2008) (Fig. 4.3). We found no evidence that eelgrass declined throughout these two years, despite large macroalgal blooms. Rather, mean macroalgae and eelgrass biomass were positively correlated throughout the year ($r_s = 0.73$, $p = 0.01$) (Fig. 4.3).

Contrary to the field, the tank experiment revealed substantial macroalgal treatment effects for all eelgrass response metrics, including change in density (Fig. 4.1B), final biomass (Fig. 4.2B), blade length, sheath length, and trim and slough biomass (Table 4.1). Decrease in eelgrass density was greatest for the ADDITION, followed by the MIMIC, and then the REMOVAL (Table 4.1: Comparisons, Fig. 4.1B). A similar trend was observed for eelgrass blade and sheath lengths, where ADDITION lengths were much lower than REMOVAL lengths, however we did not find any observable differences in eelgrass blade or sheath length between MIMIC and ADDITION (Table 4.1: Comparisons). Trim and slough eelgrass biomass were both greater for REMOVAL than ADDITION, which differed from the MIMIC treatment for trim biomass, but not slough biomass (Table 4.1: Comparisons). We did find nutrient effects for both final eelgrass biomass (Fig. 4.3B) and trim biomass in the mesocosm experiment (Table 4.1). For both final eelgrass biomass and trim biomass, the +NUTRIENT treatment resulted in lower values when crossed with either REMOVAL or MIMIC (but not ADDITION).

4.3.2 Nutrient additions and macroalgal response

Slow-release fertilizer (Osmocote^{TM}) dissolution rates were much greater in the field experiment than the mesocosm experiment. In the field, mean Osmocote^{TM} dissolution was 38.2 g day^{-1} (July 7-Aug. 20) and 41.3 g day^{-1} (Aug. 21-Oct. 5) compared to values of 3.0 g day^{-1} (July 16-Aug. 24) and 1.7 g day^{-1} (Aug. 24-Oct. 19) in the mesocosm. In both mesocosm and field experiments we found no difference in slow-release fertilizer dissolution between macroalgae treatments (Table 4.2).

In both field and tank experiments we found evidence that slow-release fertilizer increased the nutrient concentrations in the local water column. For the field experiment, in July, both DIN and $PO_4^{\ 3-}$ concentrations were higher in the +NUTRIENT treatments compared to the AMBIENT treatments (Table 4.2). For August samples, interactions of nutrient x distance were found for both DIN and $PO_4^{\ 3-}$ (Table 4.2). The highest mean (± SE) nutrient concentrations were found adjacent to the tubes (0 cm distance). For DIN and $PO_4^{\ 3-}$, no nutrient addition effects were found at 5 or 20 cm (Table 4.2: Comparisons). In the mesocosms, mean nutrient concentrations were also higher in the +NUTRIENT treatments (Table 4.2).

While nutrient addition treatments increased local nutrient concentrations, +NUTRIENT treatments did not result in different macroalgal percent cover (field experiment) or volume (field and mesocosm experiments) (Table 4.2, nutrient effects). Evidence that the pulse macroalgae treatments endured from month to month was found. Both macroalgal percent cover and volume were greatest for ADDITION, and were different from both CONTROL, MIMIC and REMOVAL (Table 4.2: Comparisons, except see ADDITION vs. REMOVAL percent cover)

4.3.3 Covariate response to experimental treatments

As with the eelgrass responses in the field experiment, we found no macroalgal or nutrient effects for redox measurements (Table 4.3). We also found no effects of macroalgal treatment on light levels within plots during ebb and flood tide logging periods (Table 4.3).

In the tank environments no nutrient effects were documented, but we found macroalgal treatment effects for many of the covariates measured (Table 4.3). Percent surface light (attenuated PPFD measurements) was greatest for ADDITION, but also reduced for MIMIC (relative to the REMOVAL treatments) (Table 4.3: Comparisons). At 30 cm depth we recorded mean PPFD values that follow these same trends: REMOVAL: 924.7 μmol photon m^{-2} $s^{-1,}$ ADDITION: 490.0 μmol photon m^{-2} $s^{-1,}$ MIMIC: 596.8 μmol photon m^{-2} s^{-1}. We also found marked shifts in light attenuation pre and post macroalgal treatment application (Fig 4.4A). Redox values were lowest for ADDITION, which differed from MIMIC (Fig. 4.4B, Table 4.3: Comparisons).

Water column spot measurements of temperature, dissolved oxygen and pH in the mesocosms also showed macroalgal treatment effects (i.e., Temperature; Table 4.3). No differences were found across treatments for salinity. However, for all significant measurements, mean values for the MIMIC treatment were only slightly lower than REMOVAL and ADDITION treatments (i.e., Temperature: REMOVAL – 12.31°C, ADDITION – 12.26 °C, MIMIC – 12.11 °C).

4.4 DISCUSSION

4.4.1 Contrasting outcomes between venues

Contrary to results found in estuarine systems dominated by land-based eutrophication (Burkholder et al. 2007, Kaldy 2009), we found macroalgal and nutrient manipulations did not affect various metrics of seagrass productivity, except under mesocosm conditions (Table 4.1). As hypothesized, for the field experiment, we found no macroalgal treatment effects whereas in the tank experiments, addition of both real macroalgae and mimic macroalgae led to declines in eelgrass density and biomass (Fig. 4.1, 4.2), as well as all other eelgrass metrics measured (Table 4.1). Observational trends from the field site also support the experimental results; we found large summer macroalgal blooms in years preceding the experiment (summer 2007 and 2008), but observed no declines in eelgrass production throughout this timeframe (Fig. 4.3). Similar trends have been seen at other euhaline sites within Coos Bay (Hessing-Lewis et al. 2011), and large macroalgal blooms have been recorded throughout this zone of the estuary in the past (Pregnall and Rudy 1985b, Thom et al. 2003).

4.4.2 Species interaction mechanisms

Differences between field and mesocosm results allowed us to investigate the mechanisms responsible for variation in eelgrass and macroalgal interactions. First, we explore the mechanisms responsible for macroalgae and nutrient effects in the mesocosm study. We then compare key physical characteristics of these venues, and the potential role that this plays in affecting interaction mechanisms between producers.

In the enclosed mesocosm experiments we found that macroalgal canopies (both ADDITION and MIMIC treatments) decreased light levels, changed sediment conditions and altered the physical conditions of the water column (Table 4.3). Both ADDITION and MIMIC treatments attenuated light levels in the tanks to the same extent, contributing to greater shading of eelgrass in these treatments compared to the REMOVAL tanks where higher percent surface irradiance was recorded (Fig. 4.4A, Table 4.3: Comparisons). Thus we attribute lower values for eelgrass shoot density, biomass and length in treatments with macroalgae, in part, to decreased light availability, as has been observed in many other studies (e.g. Orth and Moore 1983, Hauxwell et al. 2003, 2006). Both ADDITION and MIMIC treatments reduced surface irradiance to less than 34%, which is the limit at which negative effects on eelgrass were observed by Ochieng et al. (2010). However, in the Pacific Northwest, studies have found that eelgrass productivity is maximized at 350-550 μmol photon m^{-2} s^{-1} (Thom et al. 2008), which is within the range of mean light measurements at 30 cm below the water surface found within this experiment. Thom et al. (2008) also found that non-light limited growth required an average of at least 7 mol photon m^{-2} day^{-1} in spring and summer months, which is also met in this experiment if our recorded daylight spot measurements are extrapolated through time. Thus, despite elevated shading by real and mimic macroalgae, additional factors likely contribute to the eelgrass declines observed in the mesocosms.

Both ADDITION and MIMIC treatments were also associated with lower redox potential values than the REMOVAL tanks (Fig. 4.4B). Here, macroalgae (ADDITION) led to lower redox potential values than those found in the MIMIC tanks (Table 4.3: Comparisons). Both ADDITION and MIMIC treatments may have decreased oxygen production in the sediment as a consequence of eelgrass shading (i.e., decreased oxygen transport belowground), but only real macroalgae affected sediment oxygen levels via decomposition of organic matter. While seagrass can often tolerate low sediment oxygen conditions (Terrados et al. 1999), anoxia can affect seagrass by interfering with nitrogen metabolism (Pregnall et al. 1984), and lead to the production of toxic sulfide compounds, which can have negative effects, especially in concert with other stressors such as light reduction (Goodman et al. 1995, Koch and

Erskine 2001). While we found macroalgal effects on sediment oxygen conditions, we did not find water-column changes in oxygen concentrations associated with the ADDITION treatment. Macroalgal effects were found between treatments, but lower values for temperature, dissolved oxygen and pH were associated with the MIMIC treatment, rather than ADDITION. While these differences show that the use of mimic algae had small effects on water column processes, the range of values recorded amongst all tanks was very low, and is not expected to be biologically relevant to patterns in eelgrass declines (Nelson 2009, Table 4.4: ambient temperature range). As with other research on the mechanistic causes of seagrass decline (Short and Wyllie-Echeverria 1996, McGlathery 2001), both light and biogeochemical changes are likely responsible for the observed declines in eelgrass in the mesocosm experiment. Our inclusion of the MIMIC treatment, however, shows that while light may be a key mechanism associated with eelgrass declines in the tank environments, the additive effects caused by reduced sediment oxygen conditions are also important. Although MIMIC treatments decreased light levels to the same extent as ADDITION treatments, decreases in eelgrass density (Fig. 4.1B) and biomass (Fig. 4.1B) were much more pronounced for the ADDITION treatments, and may be related to the additional decrease in sediment oxygen concentrations with this treatment (Table 4.3: Comparisons). These associations show that while light alone may result in declining eelgrass production, both reduced light and changes in local biogeochemical conditions may be necessary for precipitous declines. Thus environmental conditions that mitigate either of these mechanisms may allow eelgrass to withstand macroalgal blooms.

4.4.3 The physical context

In the field, high water exchange throughout the experimental plots, and the estuary as a whole, likely contributed to decreased light and biogeochemical effects of macroalgal treatments compared to the tank environments, where water turnover rates were much lower (~ 0.17 L s^{-1}), with no currents or tides. The flushing time for the entire Coos Bay estuary is high (6-8 tidal cycles or 3 days), due in part to large tidal velocities (peaks of ± 1.1 m s^{-1}, mean of 0.4 m s^{-1}) (Roegner and Shanks 2001, Rumrill 2006). High currents and water motion caused by tides and waves are reflected in the high dissolution rates of chalk blocks (Table 4.4) and Osmocote^{TM} observed in the field. Water movement also leads to a dynamic macroalgal canopy that changes shape and location on a daily basis with the ebb and flood of the tide. Tides in Coos Bay are semi-diurnal, occurring twice daily with a mean amplitude of 2.3 m between daily high and low tides (Rumrill 2006). Together, high flushing rates and movement of macroalgae with the currents may alleviate the concentration of low oxygen levels, and other toxic elements in the sediment and water column. Furthermore, a shifting macroalgae canopy may not result in decreased light levels as seen in the mesocosms. At the field site, macroalgae aggregations developed at the sediment-seagrass interface, often forming accumulations of 5-30 cm at high tide through which seagrass blades penetrated. Our measurements of light conditions in the field experimental plots during ebb and flood tide found no effects of macroalgal manipulations on light levels (Table 4.3) despite measurably different macroalgal conditions between plots (Table 4.2; volume and percent cover). These intertidal field conditions vary from estuaries where persistent canopies form above seagrass beds, and where most negative interactions have been reported.

Contrary to our field site, most studies have focused on shallow estuaries, where seagrass beds are subtidal, flushing rates/residence times are low, and in general, water conditions are more similar to our mesocosms where we did observe negative effects on eelgrass. While we attempted to maintain the structural configuration of macroalgae in the mesocosms similar to that found at our intertidal field sites, the resulting macroalgae canopy heights (30 – 50 cm) were likely higher than the mean canopy heights in the field, and less dynamic, leading to larger shading effects. In both experiments, macroalgal canopies were higher than macroalgal canopies of 9-12 cm associated with subtidal eelgrass declines in Waquoit Bay, MA, USA (Hauxwell et al. 2001), but smaller than accumulations upwards of 50 cm described by McGlathery (2001) and Hauxwell et al. (2001).

4.4.4 The nutrient context

While we did not find any effects of nutrient addition in the field experiment, nutrient effects were found in the mesocosm study for eelgrass final biomass and trim biomass (Table 4.1). In the tank environment, nutrient addition led to lower biomass (Fig. 4.3) of eelgrass for both REMOVAL and MIMIC treatments. While the macroalgal effects were greatest for ADDITION, no nutrient effects were found. These results suggest that the indirect effects of macroalgae have greater inhibitory effects than the direct effects of nutrients. Research has shown that in certain cases, nutrient enrichment can result in sustained nitrate uptake, and without inhibition, this can have negative consequences on seagrass physiology (Touchette and Burkholder 2000, Ralph et al. 2006, Romero et al. 2006). These physiological responses to nutrient enrichment, however, likely evolved for seagrasses adapted to low ambient nutrients, where fast-uptake responses for seagrasses were competitively advantageous (Burkholder et al. 1992, 2007). Such responses may by confounded by other factors (i.e., temperature), and direct nutrient effects have not been well studied across a diversity of geographic conditions, including eelgrass adapted to high nutrient, upwelling-influenced conditions (Kaldy 2009). Nonetheless, our results do show the potential for additive negative effects of indirect macroalgal blooms and direct nutrient enrichment on eelgrass.

While nutrient addition effects on eelgrass were found in the mesocosm study, no interactive effects of nutrient and macroalgal treatments were documented in either experiment. This runs contrary to our expectations that nutrient addition would augment macroalgal volume, leading to interactive effects on eelgrass. In fact, we found no effect of nutrient addition on macroalgal volume in either the mesocosms or in situ (Table 4.2: volume and percent cover). This indicates that either 1) nutrient concentrations were too low, or diffusion rates too high, to stimulate macroalgal response, or 2) macroalgae in this system were not nutrient limited, but rather, uptake rates were saturated. In the mesocosms, nutrient additions led to increases in nitrogen and phosphorus concentrations in the water column (Table 2), i.e., mean DIN = 29.43 μM, PO_4^{3-} = 0.27 μM (Table 4.4). These values were well above the lowest ambient nutrient concentrations in the mesocosms, but within the range of values found in Yaquina Bay and Coos Bay waters during the summer season (Table 4). While water turnover rates in the mesocosms were lower than water movement in the field, water circulation may still have prevented the accumulation of highly enriched waters in the mesocosms and thus limited blooms there. In the field we found that diffusion rates of nutrients were very high, and were only different from AMBIENT nutrient concentrations adjacent to the diffuser tubes. Therefore, while we added large amounts of fertilizer, with the potential to create very high nutrient concentrations (i.e., maximum DIN values of 740 μM adjacent to diffuser tubes), water movement again played a role in diffusing nutrients throughout the larger estuarine system.

Because background nutrient levels are high in upwelling-influenced systems (Table 4.4), macroalgae may be nearing nutrient saturation, and may not respond strongly to nutrient enrichment. In a global comparison of ulvoid macroalgae nutrient limitation, Teichberg et al. (2010) found that macroalgae growth rates increased linearly with annual DIN concentrations up to 100 μM, but addition of NO_3^- primarily increased growth rates in estuaries where ambient DIN was low (~ < 10 μM). Mean estuarine concentrations associated with nutrient-rich ocean waters (Brown and

Ozretich 2009) fall within this high range, as well as the nutrient concentrations observed in our studies (Table 4.4), and here too, we observed no growth response. While DIN may not be limiting under higher nutrient concentrations, PO_4^{3-} may be (Wheeler and Bjornsater 1992, Teichberg et al. 2010). Nutrient limitation and saturation, however, are not well studied in Pacific Northwest estuaries (Kaldy 2009, but see Williams and Ruckelshaus 1993), and potentially, macroalgae could respond to system-wide increases in nutrient concentrations beyond those observed in this study. Additional studies concerning macroalgal physiological adaptations to high nutrient concentrations in these systems are warranted. This nutrient context plays an important role governing macroalgal production in upwelling-influenced estuaries. Another macroalgal manipulation experiment in the marine and polyhaline regions of Coos Bay showed that eelgrass responded positively or neutrally to imposed macroalgal additions (Hessing-Lewis et al. 2011). In riverine regions, however, where macroalgal biomass was low, macroalgal addition had a negative effect. Macroalgal blooms have been persistent throughout this estuary for at least the past 70 years (Rumrill 2006). Thus both eelgrass and macroalgae may have adapted to the local physicochemical conditions associated with marine-based nutrient inputs.

4.4.5 Context-dependency and coastal management

Interpretation of seagrass-macroalgal interactions in a variety of environmental contexts is important as they form key parameters in the assessment and mitigation of land-based eutrophication in the United States (Bricker et al. 2003). Macroalgal blooms occur in diverse coastal systems throughout the world (Teichberg et al. 2010), and their local repercussions may vary. We have found that the physical and nutrient context of Oregon coast estuaries differ from systems where land-based eutrophication is prominent, such as the East Coast of the United States. The physical context of the estuary, including water movement and benthic location, appear to play key roles in curtailing the negative effects of macroalgal aggregations and controlling nutrient uptake. In another macrotidal estuary in Patagonian Argentina, estuarine hydrodynamics also played a key role controlling the potentially negative effects of macroalgal accumulations (Martinetto et al. 2011). In this study, and in another upwelling-influenced estuary in Baja California, Mexico (Jorgensen et al. 2007), nutrient inputs also interact with top-down effects of herbivory, an important element that was not explicitly addressed in our study.

Insight on the role of nutrient context on producer interactions is necessary given the potential for future changes in coastal nutrient concentrations. While upwelling strength and magnitude has increased along the California Current, it has done so gradually over the past 43 years (Iles et al. in revision). Under future climate scenarios nutrient concentrations are also expected to increase in upwelling systems (Bakun et al. 2010). Coupled with the potential for additional land-based nutrient delivery with coastal development in this region, a shifting nutrient context for upwelling-influenced estuaries is likely. Eelgrass-macroalgae interactions in the high biomass estuaries that we studied could become negative and increase in magnitude, but only under large, system-wide increases in nutrient concentrations. In low biomass systems where macroalgae is currently limited, their future effects remain unknown and should be closely monitored. Results from the mesocosm experiment also show the potential for dramatic responses to nutrient enrichment and macroalgal blooms under certain physical conditions, such as subtidal eelgrass meadows with low water movement, which may be found in regions of all estuaries, including those with upwelling-influence. Regionally, results from the mesocosm experiment can also be scaled-up to help explain why estuaries with high macroalgae biomass were associated with lower eelgrass biomass, compared to estuaries where macroalgal production was low (Hessing-Lewis and Hacker, in review). Across these estuaries in Washington and Oregon, no evidence for eelgrass declines through time was found. However, the negative direct nutrient effects observed in the mesocosm experiment may, in part, explain the observed large-scale regional variation in production. Where both ambient macroalgae and nutrients were lowest we found the highest biomass/density of eelgrass.

One of the greatest challenges in modern ecology is determining how context influences the nature and strength of species interactions (Agrawal et al. 2007). Our research from upwelling-influenced estuaries provides a clear example that nutrient regime, coupled with physical characteristics of a system, can dictate the outcome of interactions between benthic producers. We also show that experiment venue influences the mechanisms at play in this interaction (Skelly and Kiesecker 2001). As such, results from stand-alone mesocosm studies may have questionable application to real world situations. In an effort to generalize the outcome and mechanisms of species interactions across different systems, we show that mesocosm studies combined with in situ experimentation can yield complementary results. Only by comparing results between venues did we discover the key factors determining context-specificity, and leading to the novel outcomes observed in these upwelling-influenced estuaries.

ACKNOWLEDGEMENTS

Thanks to the field/lab support associated with this research. At OSU; O. Moulton, P. Zarnetske, J. Henderson, L. Wisehart, D. Eerkes-Medrano, S. McConville, W. Rice-Narusch, H. Bailey, J. Schaefers, M. Rogers, L. McCoy, B. Dumbauld, T. Gouhier. Research in Coos Bay was funded from an award from the National Estuarine Reserve System (NERR), Estuarine Reserves Division, NOAA. At South Slough NERR, A. Helms, A. DeMarzo, S. Rumrill facilitated data acquisition/processing. A Mamie Markham Graduate Scholarship provided funds for the mesocosm experiment at Hatfield Marine Science Center (OSU). Science Center staff greatly facilitated construction and maintenance of this experiment. Additional support for this research came from OSU Dept. of Zoology, S. Hacker, and B. Menge.

Table 4.1 Eelgrass response metrics to macroalgae x nutrient treatments in field and mesocosm experiments. Macroalgae, nutrient and macroalgae x nutrient terms were coded as fixed factors in linear mixed effect models. Block was coded as a random factor (3 blocks for the field experiment). Dates when measurements were taken are indicated; all analyses with multiple dates used a correlation structure in the analysis to incorporate lack of independence between sampling dates. Post hoc comparisons of treatment differences evaluated based on Tukey test z values (Control = CON, Addition = ADD, Removal = REM, Mimic = MIM). Significance codes: ns (no significant differences) $p>0.05$; $*p<0.05$; $**p<0.001$; $***p<0.0001$

Experiment	**Response Metric**	**Dates Sampled**	**Fixed Effect**	**df fixed, df error**	***F* (*p*)**	**Treatment Comparisons (Tukey *p*)**
Field	Eelgrass density (central quadrat, change in density from first sampling date) (mean # shoots 0.25 m^{-2} plot^{-1})	June, September	*Macroalgae*	3, 14	1.42 ns	
			Nutrient	1, 14	0.01 ns	
			Macroalgae x Nutrient	3, 14	0.07 ns	
	Eelgrass density (spot samples, change in density from first sampling date) (mean # shoots 0.06 m^{-2} plot^{-1})	monthly: July, September, October	*Macroalgae*	3, 38	1.20 ns	
			Nutrient	1, 38	2.28 ns	
			Macroalgae x Nutrient	3, 38	3.63 ns	
	Eelgrass biomass (mean g dry wt 0.25 m^{-2} plot^{-1})	October	*Macroalgae*	3, 14	1.04 ns	
			Nutrient	1, 14	0.13 ns	
			Macroalgae x Nutrient	3, 24	0.49 ns	
	Eelgrass length (mean cm 0.25 m^{-2} plot^{-1})	October	*Macroalgae*	3, 14	5.09 *	REM, CON * ADD, CON * MIM, CON * ADD, REM ns MIM, REM ns MIM, ADD ns

Continued

Experiment	**Response Metric**	**Dates Sampled**	**Fixed Effect**	**df fixed, df error**	***F* (*p*)**	**Treatment Comparisons (Tukey *p*)**
Field	Eelgrass length (change in length from first sampling date) (mean cm 0.25 m^{-2} $plot^{-1}$)	Monthly: June, October	*Macroalgae*	3, 14	2.63 ns	
			Nutrient	1, 14	1.98 ns	
			Macroalgae x Nutrient	3, 14	1.00 ns	
	Eelgrass sheath length (change in length from first sampling date) (mean cm $plot^{-1}$)	monthly: July, August, October	*Macroalgae*	3, 38	1.55 ns	
			Nutrient	1, 38	1.77 ns	
			Macroalgae x Nutrient	3, 38	0.64 ns	
Mesocosm	Eelgrass density (change in density from first sampling date) (mean # shoots $bucket^{-1}$ $mesocosm^{-1}$)	monthly: June, July, September, October	*Macroalgae*	2, 28	21.43 *	REM, ADD * ADD, MIM * MIM, REM *
			Nutrient	1, 28	1.12 ns	
			Macroalgae x Nutrient	2, 28	0.44 ns	
	Biomass (mean total g dry wt $bucket^{-1}$ $mesocosm^{-1}$)	October	*Macroalgae*	2, 10	46.39 ***	REM, ADD * ADD, MIM * MIM, REM *
			Nutrient	1, 10	5.03 *	
			Macroalgae x Nutrient	2, 10	1.45 ns	
	Eelgrass length (mean cm $bucket^{-1}$ $mesocosm^{-1}$)	monthly: July, September, October	*Macroalgae*	2, 46	11.70 ***	ADD, REM * MIM, REM ns MIM, ADD ns
			Nutrient	1, 46	0.26 ns	
			Macroalgae x Nutrient	2, 46	0.93 ns	
	Eelgrass sheath length (mean cm $bucket^{-1}$$mesocosm^{-1}$)	monthly July, September, October	*Macroalgae*	2, 46	4.34 *	ADD, REM * MIM, REM ns MIM, ADD ns
			Nutrient	1, 46	1.45 ns	
			Macroalgae x Nutrient	2, 46	0.81 ns	

Continued

Experiment	**Response Metric**	**Dates Sampled**	**Fixed Effect**	**df fixed, df error**	***F* (*p*)**	**Treatment Comparisons (Tukey *p*)**
Mesocosm	Trim eelgrass biomass (mean g dry wt mesocosm^{-1})	weekly: June, July, August September, October	*Macroalgae*	2, 279	16.85 ***	ADD, REM * MIM, REM ns MIM, ADD *
			Nutrient	1, 279	3.97 *	
			Macroalgae x Nutrient	2, 279	2.50 ns	
	Slough eelgrass biomass (mean g dry wt mesocosm^{-1})	weekly: June, July, August September, October	*Macroalgae*	2, 297	8.32 **	ADD, REM * MIM, REM ns MIM, ADD ns
			Nutrient	1, 297	0.10 ns	
			Macroalgae x Nutrient	2, 297	0.62 ns	

Table 4.2 Nutrient and macroalgal responses to macroalgae x nutrient treatments in field and mesocosm experiments. See Table 4.1 heading for further details. For treatment comparisons, macroalge treatments include: Control = CON, Addition = ADD, Removal = REM, Mimic = MIM. Nutrient treatments include: Nutrient addition = + NUT, Ambient conditions = AMB and distance from nutrient diffuser tubes (0 cm, 5 cm, 20 cm).

Experiment	**Response Metric**	**Dates Sampled**	**Fixed Effect**	**df fixed effect, df error**	***F* (*p*)**	**Treatment Comparisons (Tukey *p*)**
Field	Osmocote Dissolution Rate (g day^{-1})	July, August	*Macroalgae*	3, 18	0.61 ns	
	DIN (mg L^{-1})	July	*Nutrient*	1, 4	7.06*	
		August	*Distance*	2, 10	4.85*	0 / + NUT, 0 /AMB ***
			Nutrient	1, 10	4.89*	5 cm / +NUT, 5 cm /AMB ns
			Distance x Nutrient	2, 10	4.84*	20 cm / +NUT, 20 cm / AMB ns
	PO_4^{3-} (mg L^{-1})	July	*Nutrient*	1, 4	11.33*	
		August	*Distance*	2, 10	4.33*	0 cm / + NUT, 0 cm / AMB ***
			Nutrient	1, 10	6.54*	5 cm / +NUT, 5 cm /AMB ns
			Distance x Nutrient	2, 10	4.26*	20 cm / +NUT, 20 cm / AMB ns

Continued

Experiment	**Response Metric**	**Dates Sampled**	**Fixed Effect**	**df fixed effect, df error**	***F* (*p*)**	**Treatment Comparisons (Tukey *p*)**
Field	Macroalgae Percent Cover (% cover 0.25 m^{-2})	June, August September, October	*Macroalgae*	3, 86	12.68 **	REM, CON ns ADD, CON * MIM, CON ns ADD, REM ns MIM, REM ns MIM, ADD *
			Nutrient	1, 86	0.36 ns	
			Macroalgae x Nutrient	3, 86	0.29 ns	
	Macroalgae Volume (mL 0.25 m^{-2})	June, July, August, September, October	*Macroalgae*	3, 123	14.41**	REM, CON ns ADD, CON * MIM, CON ns ADD, REM * MIM, REM ns MIM, ADD *
			Nutrient	1, 123	3.25 ns	
			Macroalgae x Nutrient	3, 123	0.45 ns	
Mesocosm	Osmocote Dissolution Rate (g day^{-1})	July, August	*Macroalgae*	2, 13	0.65 ns	
	DIN (mg L^{-1})	July, August	*Nutrient*	1,17	76.89 **	
	PO$_4^{3-}$ (mg L^{-1})	July, August	*Nutrient*	1, 17	43.07 **	
	Macroalgae Volume (mL mesocosm^{-1})	August, September, October	*Nutrient*	1, 14	0.04 ns	

Table 4.3 Covariate responses to macroalgae x nutrient treatments in field and mesocosm experiments. See Table 4.1 heading for further details.

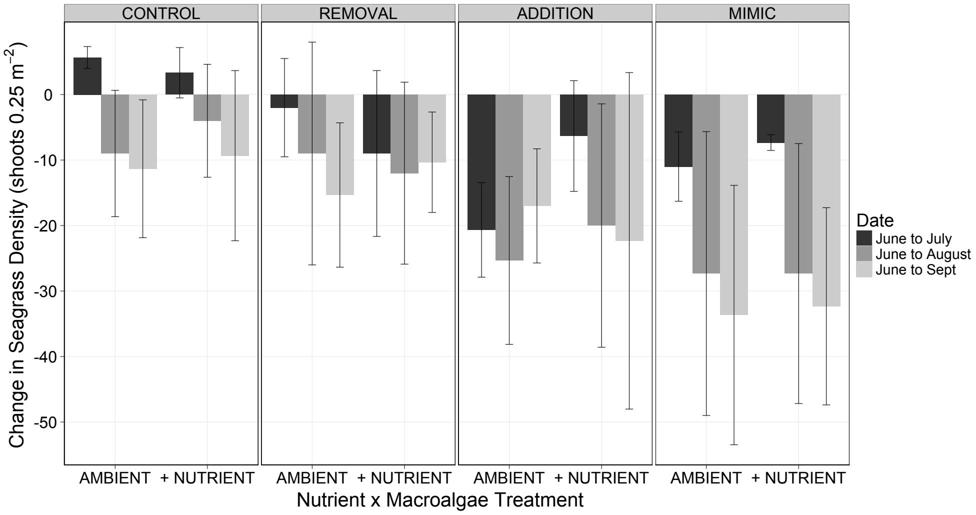
Experiment	**Response Metric**	**Dates Sampled**	**Fixed Effect**	**df fixed effect, df error**	***F*** **(*p*)**	**Treatment Comparisons (Tukey *p*)**
Field	Redox potential (mean mV 0.25 m^{-2} $plot^{-1}$)	monthly: June, August, September, October	*Macroalgae*	3, 86	1.18 ns	
			Nutrient	1, 86	2.02 ns	
			Macroalgae x Nutrient	3, 86	0.51 ns	
	Light (μmol photon m^{-2} s^{-1} $plot^{-1}$)	monthly: August, September	*Macroalgae*	3, 18	1.11 ns	
Mesocosm	Redox potential (mean mV $mesocosm^{-1}$)	monthly: July, September, October	*Macroalgae*	2, 602	12.04 *	ADD, REM *
			Nutrient	1, 602	0.07 ns	MIM, REM ns
			Macroalgae x Nutrient	2, 602	0.45 ns	MIM, ADD **
	Light (attenuated) (mean μmol photon m^{-2} s^{-1})	bi-monthly: July, August, September October	*Macroalgae*	2, 244	12.38 ***	ADD, REM *
			Nutrient	1, 244	0.90 ns	MIM, REM *
			Macroalgae x Nutrient	2, 244	0.37 ns	MIM, ADD ns
	Spot temperature (°C $mesocosm^{-1}$)	bi-monthly: July, August, September, October	*Macroalgae*	2, 244	12.38 *	ADD, REM *
			Nutrient	1, 244	0.90 ns	MIM, REM *
			Macroalgae x Nutrient	2, 244	0.37 ns	MIM, ADD ns

Table 4.4 Physicochemical differences between field and mesocosm experiments. Chalk block dissolution rates are from field (N=12) and mesocosm (N=8) deployments. Field and mesocosm nutrient data are reported in mg/L and converted to µM using the conversions DIN (NO_3^- + NO_2^- + NH_4^+) µM / 71.39 and PO_4^{3-} µM / 32.9 . Mean summer month (June-September 2009) nutrient concentrations for the field were based on nutrient grab samples collected at high tide at the SS NERR (South Slough National Estuarine Research Reserve) system-wide monitoring protocol (SWMP) Boathouse site (SWMP protocol: http://cdmo.baruch.sc.edu/). The Boathouse site is located ~3km from the Fossil Point experiment site. Mean temperature, salinity, DO and pH data (± SE) from YSI Datasondes (Yellow Springs Instruments Model 6600) located in the water column at the Charleston Bridge site (located ~3.5 km from the Fossil Point experiment site). Data were collected continuously at 15 min intervals, and summer means are reported. All flagged data not meeting SWMP quality check protocol were removed from this summary. Mesocosm temperature, salinity, dissolved oxygen and pH data were from spot measurements in tank mesocosms. Mean values reported across all mesocosm tanks and all dates, with range (minimum and maximum mean values).

Experiment	**Field**		**Mesocosm**	
Mean chalk block dissolution rate	4.49 ± 0.19g day^{-1}		0.41± 0.03g day^{-1}	
Mean summer month nitrogen (DIN) concentration mgL^{-1} (& µM)	July mean		July and August mean	
	Ambient: 0.17 ± 0.00 mgL^{-1} (12.18 ± 0.06 µM)	*+ Nutrient:* 0.78 ± 0.23 mgL^{-1} (55.38 ± 16.26 µM)	*Ambient:* 0.30 ± 0.01 mgL^{-1} (21.70 ± 0.77 µM)	*+ Nutrient:* 0.42 ± 0.01 mgL^{-1} (29.93 ± 0.95 µM)
	August (mean: all dates, 0 cm)			
	Ambient: 0.10 ± 0. 00 mgL^{-1} (7.45 ± 0.42 µM)	*+ Nutrient:* 10.37 ± 4.66 mgL^{-1} (740.14 ± 332.54 µM)		

<table>
<tr><th colspan="5">Continued</th></tr>
<tr><th>Experiment</th><th colspan="2">Field</th><th colspan="2">Mesocosm</th></tr>
<tr><td rowspan="2">Mean summer month nitrogen (DIN) concentration
mgL^{-1} (& µM)</td><td colspan="2">Coos Bay data:
South Slough Monitoring Program, 2009
June – September mean flood tide</td><td colspan="2">Yaquina Bay data:
Brown and Ozretich (2009), 2003 & 2003 mean
May - October</td></tr>
<tr><td colspan="2">$NO_3^- + NO_2^- + NH_4^+$
0.19 ± 0.03 mgL^{-1} (13.34 ± 2.33 µM)</td><td colspan="2">$NO_3^- + NO_2^-$ ± SD
11.3 ± 8.8 µM (range: 0.0 to 31.5 µM)</td></tr>
<tr><td rowspan="6">Mean summer month phosphate (PO_4^{3-}) concentration
mgL^{-1} (& µM)</td><td colspan="2">July mean</td><td colspan="2">July and August mean</td></tr>
<tr><td>*Ambient:*
0.006 ± 0.00 mgL^{-1}
(0.19 ± 0.00 µM)</td><td>*+ Nutrient:*
0.01 ± 0.00 mgL^{-1}
(0.32 ± 0.04 µM)</td><td rowspan="3">Ambient:
0.007 ± 0.00 mgL^{-1}
(0.24 ± 0.01 µM)</td><td rowspan="3">+ Nutrient:
0.008 ± 0.00 mgL^{-1}
(0.27 ± 0.01 µM)</td></tr>
<tr><td colspan="2">August (mean: all dates, 0 cm)</td></tr>
<tr><td>*Ambient*
0.006 ± 0. 00 mgL^{-1}
(0.22 ± 0.02 µM)</td><td>*+ Nutrient:*
0.10 ± 0.04 mgL^{-1}
(3.25 ± 1.37 µM)</td></tr>
<tr><td colspan="2">Coos Bay data:
South Slough Monitoring Program, 2009
June – September mean flood tide</td><td colspan="2">Yaquina Bay data:
Brown and Ozretich (2009), 2003 & 2003 mean
May - October</td></tr>
<tr><td colspan="2">0.04 ± 0.00 mgL^{-1} (1.29 ± 0.16 µM)</td><td colspan="2">1.4 ± 0.8 µM (0.0 to 2.9 µM range)</td></tr>
<tr><td>Mean Ambient Temp ± SE
°C (range)</td><td colspan="2">13.53 ± 0.02
(8.4 – 19.0)</td><td colspan="2">12.23 ± 0.06
(11.95 - 12.73)</td></tr>
<tr><td>Mean Ambient Salinity ± SE
ppt (range)</td><td colspan="2">32.93 ± 0.01
(29.6 – 35.8)</td><td colspan="2">33.21 ± 0.06
(33.15 - 33.37)</td></tr>
<tr><td>Mean Ambient DO ± SE
mg L^{-1} (range)</td><td colspan="2">8.47 ± 0.02
(4.5 - 14.3)</td><td colspan="2">9.83 ± 0.10
(8.80 - 10.72)</td></tr>
<tr><td>Mean Ambient pH ± SE
pH (range)</td><td colspan="2">8.25 ± 0.00
(7.5 - 8.8)</td><td colspan="2">8.07 ± 0.02
(7.97 – 8.28)</td></tr>
</table>

A) Field Experiment



B) Mesocosm Experiment

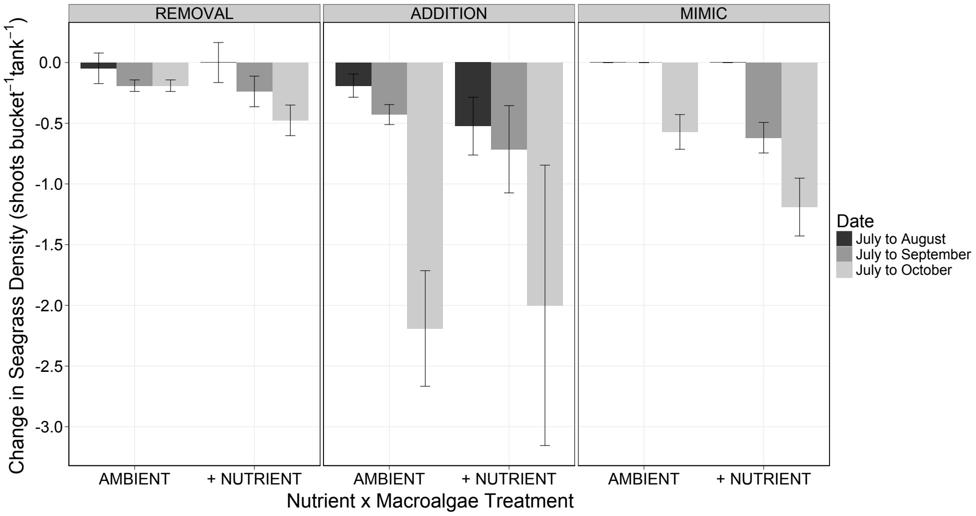
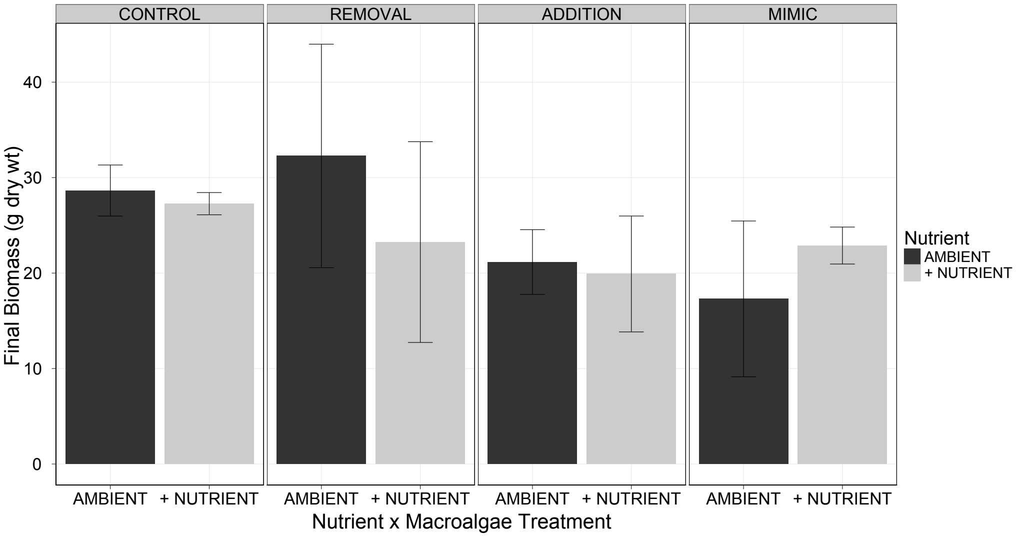


Figure 4.1. Eelgrass density through time in the field and mesocosm experiments by macroalgae and nutrient treatments. A) Mean change in eelgrass density ± SE (shoots 0.25m^{-2} from the central quadrat) from the initial date (June) to subsequent dates (July, August, September), N = 24 date^{-1} B) Mean change in eelgrass density ± SE (mean shoots bucket^{-1} tank^{-1}) from the initial date (July) to subsequent dates (August, September, October), N = 18 date^{-1}.

A) Field Experiment



B) Mesocosm Experiment

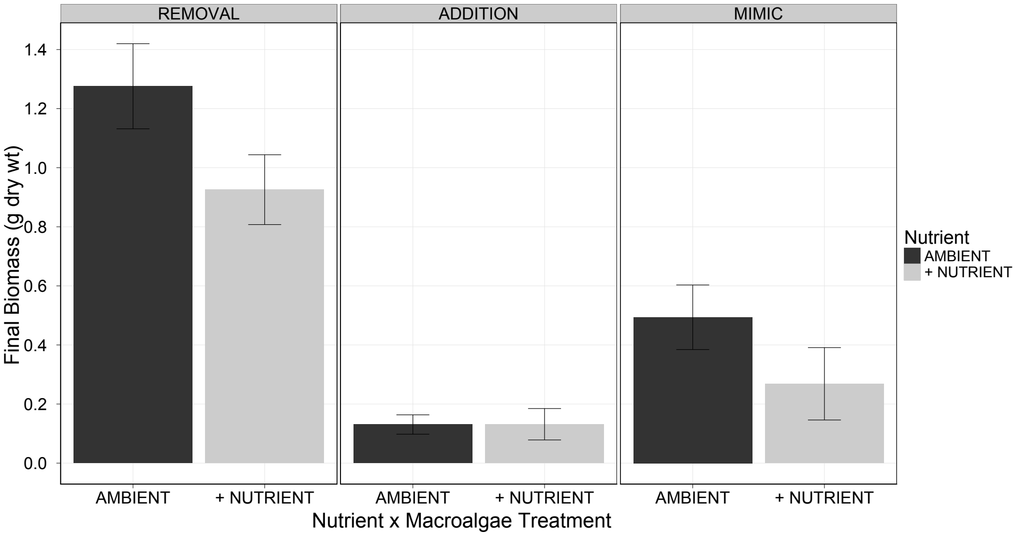


Figure 4.2 Final biomass (g dry wt) from the field and mesocosm experiments by macroalgae and nutrient treatments. A) Mean biomass ± SE per central quadrat (0.25 m^{-2}) of field experiment plots, N = 24. B) Final biomass of shoots (mean biomass ± SE $tank^{-1}$) from the mesocosm experiment, N =18.

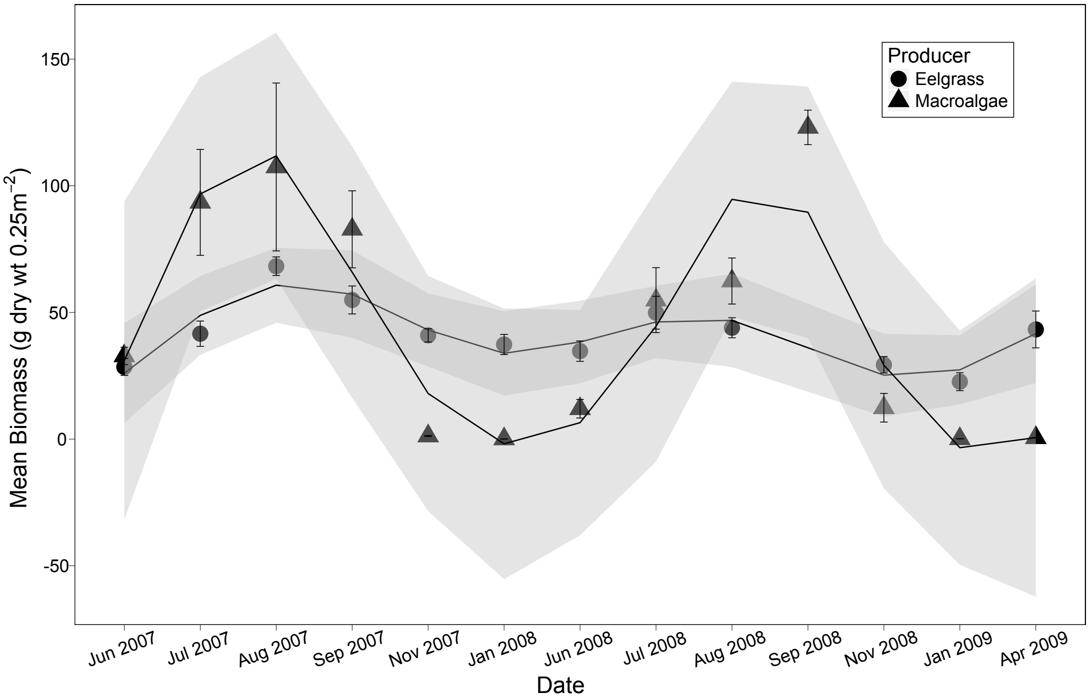


Figure 4.3. Macroalgae and eelgrass biomass mean values ± SE at the Fossil Point experiment site from June 2007 to April 2009. Macroalgae N = 6 , 0.25m^{-2} quadrats month^{-1}. Eelgrass N = 10, 0.25m^{-2} quadrats month^{-1}. Smoother lines fit to this data using natural splines from a linear model (df = 7), grey bands around smoother represent its 95% confidence intervals.

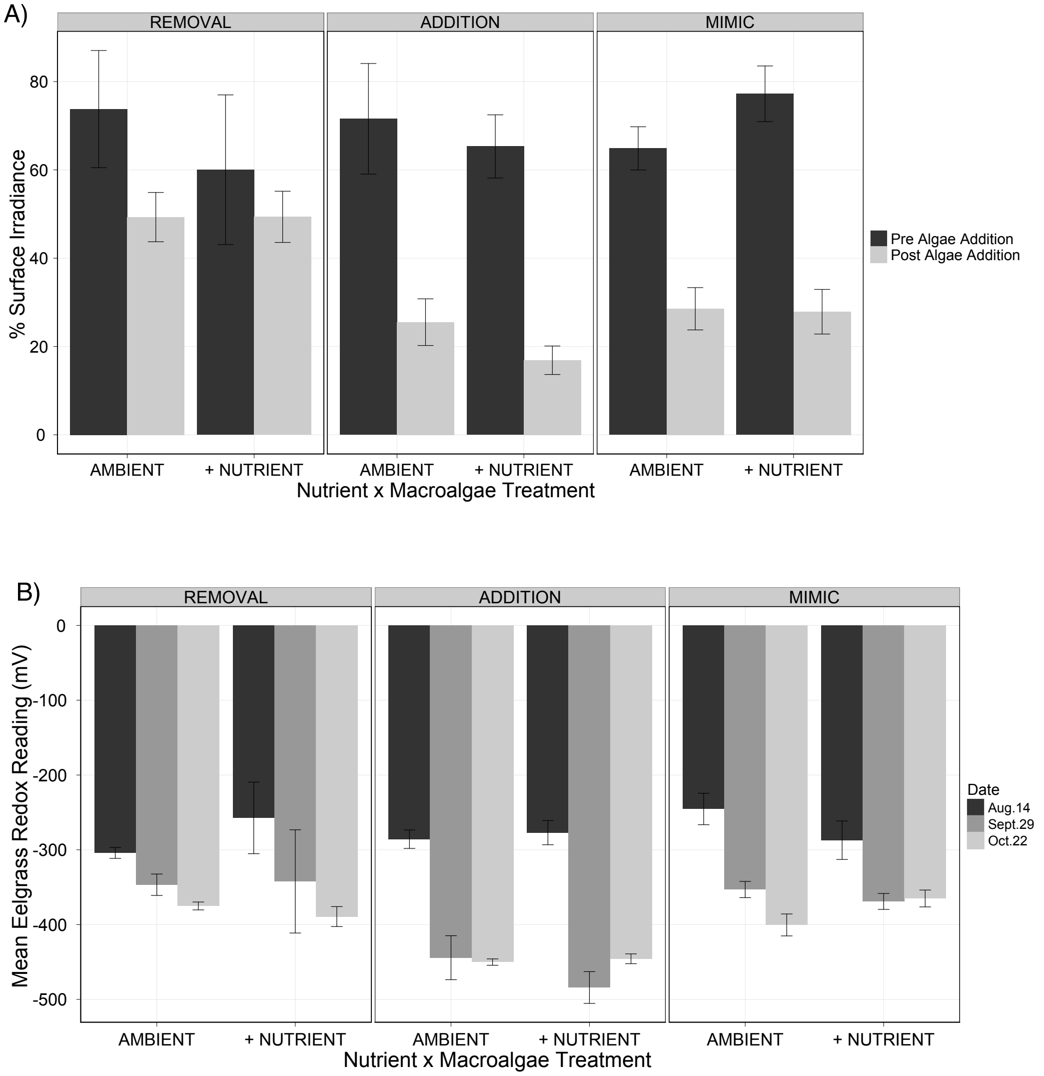


Figure 4.4. Light and sediment oxygen levels in the mesocosm experiment A) Mean % surface irradiance ± SE by mesocosm macroalgae and nutrient treatments. Percent surface irradiance calculated as: mean PPFD at 5 cm below the water line (surface) / mean PPFD at 30 cm depth. PPFD (photosynthetic photon flux density) measured in µmol photon s^{-1} m^{-2}. Pre algae addition dates = mean values July 16 and 17, and post algae addition dates = mean values July 23, 29, 31, August 2, 4, 18, 24, September 26, and October 19. N = 18 date^{-1}. B) Mean redox potential value ± SE (mV) bucket^{-1} tank^{-1} by mesocosm macroalgae and nutrient treatments. N=18 date^{-1}.

6 – Conclusion

Species Interaction Outcomes

Throughout this dissertation upwelling-influenced estuaries were used to examine the context-specificity of seagrass-macroalgae interactions. Contrary to the predominant trends for estuarine eutrophication, I found limited cases where macroalgal blooms led to negative interactions with seagrasses (e.g., eelgrass – *Zostera marina* L.) (McGlathery 2001, Burkholder et al. 2007). Rather, I found neutral, or positive, associations between macroalgal blooms and eelgrass. This was true despite macroalgal production of similar magnitude to studies where blooms have been associated with eelgrass declines (i.e., Hauxwell et al. 2001).

At a regional scale, I found relationships between increased macroalgal production and decreased eelgrass biomass across estuaries, associated with both marine- and land-based nutrient inputs (Chapter 2). However, these broad-scale patterns were not associated with eelgrass declines throughout this 5-year study. Temporal dynamics were examined in all chapters, and throughout, no evidence for strong correlations between macroalgal and eelgrass production was found. Additionally, in my experimental studies (Chapter 3 and 4), no negative interactions in the marine zone of upwelling-influenced estuaries were documented. I found that macroalgal manipulations in the intertidal zone led to neutral and positive interaction strengths at both the marine (euhaline) and polyhaline sites (Chapter 3). In Chapter 4, my large-scale macroalgal manipulations in the marine zone also had no negative effects on eelgrass. Within upwelling-influenced estuaries I did find that certain contexts led to negative interactions, including macroalgal addition at the riverine site (Chapter 3) and macroalgal manipulations in a mesocosm experiment (Chapter 4). Differences in the physicochemical environment between conditions yielding negative versus positive/neutral results can illuminate the factors that I found to be responsible for context-dependency and the mechanisms informing these interactions.

Physical and Nutrient Contexts

In intertidal eelgrass beds, the physical structure of macroalgal accumulations plays an important role influencing species interactions. In these beds, macroalgae canopies form from the sediment to upwards of 30 cm, and eelgrass blades can penetrate though the canopy. Furthermore, at low tide, both eelgrass and macroalgae are exposed to aerial desiccation and heat stress. In many of the estuaries where negative interactions have been found, the eelgrass beds are subtidal, and the canopies form dense layers that blanket the photosynthetic surface of seagrass blades (Hauxwell et al. 2001, McGlathery et al. 2007). These persistent canopy configurations are most often associated with decreased light and changing biogeochemistry in the sediment and water columns; mechanisms most often associated with seagrass declines (McGlathery 2001, Burkholder et al. 2007). In Chapter 3 I focused on macroalgal manipulations on the sediment surface, and found that negative interactions in the riverine zone were associated with decreased oxygen values (redox potential). However, no change in sediment oxygen conditions were found for macroalgal addition in the marine zone, where ambient values were much lower than all other sites, and where I found no evidence for negative interactions. Moreover, no changes in sediment oxygen conditions were found in the large-scale macroalgae manipulation (Chapter 4), where macroalgae addition more accurately reflected the natural structure of macroalgal accumulations. Similar mesocosm experiments, however, showed that both light attenuation and reduced sediment oxygen values were associated with eelgrass declines. Thus, where water movement was limited and no tidal action occurred, the standard mechanisms related to eelgrass loss and macroalgal bloom formation played much larger roles in the outcome of species interactions than they did *in situ*. In sum, the physical context of the macroalgal canopy, its structural configuration and mechanistic role in altering the light and biochemical regime of seagrass beds, leads in large part to the context-dependent interactions that I observed in these estuaries.

This research also suggests that the nutrient context of upwelling-influenced estuaries influences interaction outcome. These estuaries have relatively high nutrient concentrations, and research shows that nutrient loading in the marine zone is dominated by marine-derived nutrients (Kaldy 2009, Brown and Ozretich 2009), although land-based nutrients may also be associated with the regional occurrence of macroalgal blooms (Chapter 2). Contrary to systems with historically low nutrient concentrations, macroalgal blooms in upwelling-influenced estuaries are likely the result of seasonal response to upwelling, rather that increased nutrient loading from land. Eelgrass beds inhabiting the intertidal marine zones of these estuaries appear to be adapted to these seasonally high, bloom-fuelling nutrient concentrations. In both field and mesocosm experiments (Chapter 4) I found no evidence for direct effects of nutrient addition on either eelgrass or macroalgae, implying that both producers may not be nutrient-limited under current ambient conditions. While nitrogen concentrations are seasonally high and may not be limiting, phosphorus concentrations may be, especially in the riverine zone of the estuary, where seasonal values were very low (Chapter 3). In summary, the nutrient context of the estuary is also an important agent in informing producer response to nutrient enrichment. While negative responses to nutrients, both direct and indirect, have been found for seagrasses in historically low-nutrient systems, under high ambient nutrient conditions, bottom-up processes may not limit interactions. In all chapters, I explore this further in regards to future changes in nutrient inputs to these estuaries. For example, under climate change predictions, changes in upwelling, as well as weather patterns, coupled with the dual impact of coastal development, will likely affect nutrient concentrations and inputs. My findings can inform the corollary interactions that result from this shifting nutrient context.

Management Implications

The context-specificity of seagrass-macroalgal interactions is important for managers to consider when assessing the severity of eutrophication. This research shows that both physical and nutrient factors should be taken into account before associating macroalgal blooms with negative repercussions. These trends are also consistent with research from other upwelling-influenced estuaries along the Pacific Eastern Boundary (e.g., Brown et al. 2007, Jorgensen et al. 2007, 2010). Although macroalgae, eelgrass, and nutrient parameters are used in national eutrophication assessments (Bricker et al. 2003), all of these metrics should take local conditions into account. The applicability of these bioindicators as management tools is further discussed in Chapters 3 and 4, and has been considered in detail in work by the United States Environmental Protection Agency (i.e., Brown et al. 2007).

Using the understudied but critical interactions between seagrass-macroalgae, I have demonstrated how context-dependence, and knowledge of the mechanisms associated with this dependence, can be used to inform the generality of this interaction, with application to eutrophication management across a range of different estuarine systems. In general, species interactions are shaped by a multitude of biotic and abiotic factors, and disentangling the key factors responsible for interaction outcome continues to be a primary goal of ecologists. Using a comparative-experimental framework, and studies at multiple scales, I have shown that illuminating these interactions is not only possible, but also a management imperative

Bibliography

Agrawal, A. A., D. D. Ackerly, F. Adler, A. E. Arnold, C. Cáceres, D. F. Doak, E. Post, P. J. Hudson, J. Maron, K. A. Mooney, M. Power, D. Schemske, J. Stachowicz, S. Strauss, M. G. Turner, and E. Werner. 2007. Filling key gaps in population and community ecology. Frontiers in Ecology and the Environment 5:145-152.

Antón, A., J. Cebrian, K. L. Heck, C. M. Duarte, K. L. Sheehan, M.-E. C. Miller, and C. D. Foster. 2011. Decoupled effects (positive to negative) of nutrient enrichment on ecosystem services. Ecological Applications 21:991-1009.

Armitage, A., T. Frankovich, K. Heck and J. Fourqurean. 2005. Experimental nutrient enrichment causes complex changes in seagrass, microalgae, and macroalgae community structure in Florida Bay. Estuaries and Coasts 28(3):422-434.

Bakun, A., D.B. Field, A. Redondo-Rodriguez, and S.J. Weeks. 2010. Greenhouse gas, upwelling-favorable winds, and the future of coastal oceanic upwelling ecosystems. Global Change Biology 16:1213-1228.

Barbier, E.B., S.D. Hacker, C. Kennedy, E.W. Koch, A.D. Stier, and B.R. Silliman. 2011. The value of estuarine and coastal ecosystem services. Ecological Monographs 81:169-193.

Berlow, E.L., S.A. Navarrete, C.J. Briggs, M.E. Power and B.A. Menge. 1999. Quantifying variation in the strengths of species interactions. Ecology 80(7): 2206-2224.

Boese, B.L., K.E. Alayan, E.F. Gooch and B.D. Robbins. 2003. Desiccation index: a measure of damage caused by adverse aerial exposure on intertidal eelgrass (*Zostera marina*) in an Oregon (USA) estuary. Aquatic Botany 76(4):329-337.

Boese, B. L., B. D. Robbins, and G. Thursby. 2005. Desiccation is a limiting factor for eelgrass (Zostera marina L.) distribution in the intertidal zone of a northeastern (USA) estuary. Botanica Marina 48:274-283.

Boese, B.L. and B.D. Robbins. 2008. Effects of erosion and macroalgae on intertidal eelgrass (*Zostera marina*) in a northeastern Pacific estuary (USA). Botanica Marina 51(4):247-257.

Boyer, K. E., and P. Fong. 2005. Macroalgal-mediated transfers of water column nitrogen to intertidal sediments and salt marsh plants. Journal of Experimental Marine Biology and Ecology 321:59-69.

Bricker, S. B., C. G. Clement, D. E. Pirhalla, S. P. Orlando, and D. R. G. Farrow. 1999. National Estuarine Eutrophication Assessment: Effects of Nutrient Enrichment in the Nation's Estuaries. 71 pp. NOAA, National Ocean Service, Special Projects Office and the National Centers for Coastal Ocean Science, Silver Spring, MD.

Bricker, S., J. Ferreira and T. Simas. 2003. An integrated methodology for assessment of estuarine trophic status. Ecological Modelling 169:39-60.

Bricker, S., B. Longstaff, W. Dennison, A. Jones, K. Boicourt, C. Wicks, and J. Woerner. 2008. Effects of nutrient enrichment in the nation's estuaries: A decade of change. Harmful Algae 8:21-32.

Bronstein, J.L. 1994. Conditional outcomes in mutualistic interactions. Trends in Ecology & Evolution 9(6): 214-217.

Brown, C., W.G. Nelson, B.L. Boese, T.H. DeWitt, P.M. Eldridge, J.E. Kaldy, H. Lee, J.H. Power and D.R. Young. 2007. An Approach to Developing Nutrient Criteria for Pacific Northwest Estuaries: A Case Study of Yaquina Estuary, Oregon, USEPA Office of Research and Development, National Health and Environmental Effects Laboratory, Western Ecology Division:146 pp.

Brown, C. and R. Ozretich. 2009. Coupling between the coastal ocean and Yaquina Bay, Oregon: Importance of oceanic inputs relative to other nitrogen sources. Estuaries and Coasts 32(2):219-237.

Brun, F.G., J.J. Vergara, G. Navarro, I. Hernandez and J.L. Perez-Llorens. 2003. Effect of shading by *Ulva rigida* canopies on growth and carbon balance of the seagrass *Zostera noltii*. Marine Ecology Progress Series 265:85-96.

Burkepile, D. E., and M. E. Hay. 2006. Herbivore vs. nutrient control of marine primary producers: Context-dependent effects. Ecology 87:3128-3139.

Burkholder, J. M., K. M. Mason, and H. B. J. Glasgow. 1992. Water-column nitrate enrichment promotes decline of eelgrass *Zostera manna*: evidence from seasonal mesocosm experiments. Marine Ecology Progress Series 81:163-178.

Burkholder, J., D. Tomasko and B. Touchette. 2007. Seagrasses and eutrophication. Journal of Experimental Marine Biology and Ecology 350:46-72.

Cardoso, P. G., M. A. Pardal, A. I. Lillebø, S. M. Ferreira, D. Raffaelli, and J. C. Marques. 2004. Dynamic changes in seagrass assemblages under eutrophication and implications for recovery. Journal of Experimental Marine Biology and Ecology 302:233-248.

Carr, J., P. D'Odorico, K. McGlathery and P. Wiberg. 2010. Stability and bistability of seagrass ecosystems in shallow coastal lagoons: Role of feedbacks with sediment resuspension and light attenuation. Journal of Geophysical Research 115, G03011. doi:10.1029/2009JG001103

Cloern, J. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series 210:223-253.

Cohen, R.A. and P. Fong. 2004. Physiological responses of a bloom-forming green macroalga to short-term change in salinity, nutrients, and light help explain its ecological success. Estuaries 27(2):209-216.

Crain, C.M. and M.D. Bertness. 2006. Ecosystem engineering across environmental gradients: Implications for conservation and management. BioScience 56(3): 211-218.

Dayton, P. K. 1971. Competition, disturbance, and community organization: The provision and subsequent utilization of space in a rocky intertidal community. Ecological Monographs 41:351-389.

Diffenbaugh, N. S., M. A. Snyder, and L. C. Sloan. 2004. Could CO2-induced land-cover feedbacks alter near-shore upwelling regimes? Proceedings of the National Academy of Sciences of the United States of America 101:27-32.

Doney, S. C. 2010. The growing human footprint on coastal and open-ocean biogeochemistry. Science 328:1512-1516.

Duarte, C. M. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. Ophelia 41:87-112.

Duarte, C.M. 2000. Marine biodiversity and ecosystem services: An elusive link. Journal of Experimental Marine Biology and Ecology 250(1-2):117-131.

Duarte, C. M., N. Marbà, E. Gacia, J. W. Fourqurean, J. Beggins, C. Barrón, and E. T. Apostolaki. 2010. Seagrass community metabolism: Assessing the carbon sink capacity of seagrass meadows. Global Biogeochemical Cycles 24, GB4032. doi:10.1029/2010GB003793.

Emmett, R., R. Llanso, J. Newton, R. Thom, M. Hornberger, C. Morgan, C. Levings, A. Copping, P. Fishman. 2000. Geographic signatures of North American West Coast estuaries. Estuaries and Coasts 23(6):765-792.

Eriksson, B.K., A. Rubach, A. and H. Hillebrand. 2007. Dominance by a canopy forming seaweed modifies resource and consumer control of bloom-forming macroalgae. Oikos 116(7):1211-1219.

Fry, B., A. Gace and J.W. McClelland. 2003. Chemical indicators of anthropogenic nutrient loading in four Pacific estuaries. Pacific Science 57(1):77-101.

Gabrielson, P., T. Widdowson and S. Lindstrom. 2006. Keys to the Seaweeds and Seagrasses of Southeast Alaska, British Columbia, Washington and Oregon, Phycological Contribution 7. Hillsborough, NC: PhycoID.

Goodman, J. L., K. A. Moore, and W. C. Dennison. 1995. Photosynthetic responses of eelgrass (Zostera marina L.) to light and sediment sulfide in a shallow barrier island lagoon. Aquatic Botany 50:37-47.

Hacker, S. D., and M. D. Bertness. 1995. A herbivore paradox: shy salt marsh aphids live on poor quality plants. American Naturalist 145:192-210.

Hacker, S. D., and M. D. Bertness. 1999. Experimental evidence for factors maintaining plant species diversity in a New England salt marsh. Ecology 80:2064-2073

Hacker, S.D. and M.N. Dethier. 2006. Community modifications by a grass invader has differing impacts for marine habitats. Oikos 113:279-286.

Hacker, S.D., P. Zarnetske, E. Seabloom, P. Ruggiero, J. Mull, S. Gerrity, and C. Jones. 2011. Subtle differences in two non-native congeneric beach grasses significantly affect their colonization, spread, and impact. Oikos doi: 10.1111/j.1600-0706.2011.18887.x

Harris, D., W.G. McDougal, W.A. Patton and N. Talebbeydokhpi. 1979. Hydrologic study for South Slough Estuarine Sanctuary. Oregon State University, Corvallis: Water Resources Research Institute.

Hauxwell, J., J. McClelland, P.J. Behr and I. Valiela. 1998. Relative importance of grazing and nutrient controls of macroalgal biomass in three temperate shallow estuaries. Estuaries 21(2):347-360.

Hauxwell, J., J. Cebrian, C. Furlong and I. Valiela. 2001. Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. Ecology 82(4):1007-1022.

Hauxwell, J., J. Cebrian, and I. Valiela. 2003. Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. Marine Ecology Progress Series 247:59-73.

Hauxwell, J., J. Cebrian, and I. Valiela. 2006. Light dependence of *Zostera marina* annual growth dynamics in estuaries subject to different degrees of eutrophication. Aquatic Botany 84:17-25.

Havens, K.E., J. Hauxwell, A.C. Tyler, S. Thomas, K.J. McGlathery, J. Cebrian, I. Valiela, A.D. Steinman and S.Hwang. 2001. Complex interactions between autotrophs in shallow marine and freshwater ecosystems: implications for community responses to nutrient stress. Environmental Pollution 113(1):95-107.

Heck, J., and J. F. Valentine. 2006. Plant-herbivore interactions in seagrass meadows. Journal of Experimental Marine Biology and Ecology 330:420-436.

Heck KL, J., J. Valentine, J. Pennock, G. Chaplin, and P. Spitze. 2006. Effects of nutrient enrichment and grazing on shoalgrass *Halodule wrightii* and its epiphytes: results of a field experiment. Marine Ecology Progress Series 326:145-156.

van der Heide, T., A. J. P. Smolders, B. G. A. Rijkens, E. H. van Nes, M. M. van Katwijk, and J. G. M. Roelofs. 2008. Toxicity of reduced nitrogen in eelgrass (*Zostera marina*) is highly dependent on shoot density and pH. Oecologia 158:411-419.

Hemminga, M. 1998. The root/rhizome system of seagrasses: An asset and a burden. Journal of sea research 39:183-196.

Hickey, B.M. and N. Banas. 2003. Oceanography of the U.S. Pacific Northwest coastal ocean and estuaries with application to coastal ecology. Estuaries 26: 1010-1031.

Hickey, B. M., and N. S. Banas. 2008. Why is the northern end of the California Current System so productive? Oceanography 21:90-107.

Hessing-Lewis, M., S. D. Hacker, B. A. Menge, and S. Rumrill. 2011. Context-dependent eelgrass-macroalgae interactions along an estuarine gradient in the Pacific Northwest, USA. Estuaries and Coasts. doi: 10.1007/s12237-011-9412-8

Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric models. Biometrical Journal 50:346-363.

Howarth, R., F. Chan, D. J. Conley, J. Garnier, S. C. Doney, R. Marino, and G. Billen. 2011. Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems. Frontiers in Ecology and the Environment 9:18-26.

Irlandi, E., B. Orlando, and P. Biber. 2004. Drift algae-epiphyte-seagrass interactions in a subtropical *Thalassia testudinum* meadow. Marine Ecology Progress Series 279:81-91.

Jorgensen, P., S.E. Ibarra-Obando and J. D. Carriquiry, J.D. 2010. Management of natural *Ulva* spp. blooms in San Quintin Bay, Baja California: Is it justified? Journal of Applied Phycology 22(5):549-558.

Jorgensen, P., S.E. Ibarra-Obando and J.D. Carriquiry. 2007. Top-down and bottom-up stabilizing mechanisms in eelgrass meadows differentially affected by coastal upwelling. Marine Ecology Progress Series 333:81-93.

Kaldy, J. E. 2009. Water Column and Sediment Nutrients as Limits to Growth of Zostera marina and Thalassia testudinum. Pages 48-65. In Seagrasses and Protective Criteria: A Review and Assessment of Research Status. Office of Research and Development, National Health and Environmental Effects Research Laboratory, EP/600/R-09/-50.

Kamer, K., P. Fong, R. Kennison and K. Schiff. 2004. Nutrient limitation of the macroalga *Enteromorpha intestinalis* collected along a resource gradient in a highly eutrophic estuary. Estuaries 27(2):201-208.

Kentula, M. and T. DeWitt. 2003. Abundance of seagrass (*Zostera marina* L.) and macroalgae in relation to the salinity-temperature gradient in Yaquina Bay, Oregon, USA. Estuaries and Coasts 26(2):1130-1141.

Koch, E.W. 2001. Beyond light: Physical, geological, and geochemical parameters as possible submersed aquatic vegetation habitat requirements. Estuaries 24:1-17.

Koch, M. S., and J. M. Erskine. 2001. Sulfide as a phytotoxin to the tropical seagrass *Thalassia testudinum*: interactions with light, salinity and temperature. Journal of Experimental Marine Biology and Ecology 266:81-95.

Krause-Jensen, D., K. Glathery, S. Rysgaard and P.B. Christensen. 1996. Production within dense mats of the filamentous macroalga *Chaetomorpha linum* in relation to light and nutrient availability. Marine Ecology Progress Series 134: 207-216.

Kuhn, M., S. Weston, J. Wing and J. Forester. 2010. Contrast: A collection of contrast methods. http://CRAN.R-project.org/package=contrast. Accessed 29 Aug 2010.

Maestre, F. T., R. M. Callaway, F. Valladares, and C. J. Lortie. 2009. Refining the stress-gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology 97:199-205.

Martinetto, P., M. Teichberg, I. Valiela, D. Montemayor, and O. Iribarne. 2011. Top-down and bottom-up regulations in a high nutrient-high herbivory coastal ecosystem. Marine Ecology Progress Series. doi:10.3354/meps09173.

McGlathery, K. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters. Journal of Phycology 35:1-4.

McGlathery, K., K. Sundbäck, and I. Anderson. 2007. Eutrophication in shallow coastal bays and lagoons: The role of plants in the coastal filter. Marine Ecology Progress Series 348:1-18.

McMillan, C. and R.C. Philips. 1979. Differentiation in habitat response among populations of New World seagrass. Aquatic Botany 7:185-196.

Menge, B.A. and J.P. Sutherland. 1987. Community regulation: Variation in disturbance, competition, and predation in relation to environmental stress and recruitment. The American Naturalist 130(5):730-757

Menge, B. A. 1992. Community regulation: Under what conditions are bottom-up factors important on rocky shores? Ecology 73:755-765.

Menge, B.A. 2003. The overriding importance of environmental context in determining the consequences of species deletion experiments. In The Importance of Species: Perspectives on Expendibility and Triage, eds S.A. Levin and P. Kareiva, 16-43. Princeton, NJ: Princeton University Press.

Menge, B.A., C. Blanchette, P. Raimondi, T. Freidenburg, S. Gaines, J. Lubchenco, D. Lohse, G. Hudson, M. Foley and J. Pamplin. 2004. Species interaction strength: Testing model predictions along an upwelling gradient. Ecological Monographs 74(4): 663-684.

Moore, K.A., R.L. Wetzel, R.L. and R.J. Orth. 1997. Seasonal pulses of turbidity and their relations to eelgrass (*Zostera marina* L.) survival in an estuary. Journal of Experimental Marine Biology and Ecology 215(1):115-134.

Nelson, W. G. 2009. Seagrasses and Protective Criteria: A Review and Assessment of Research Status. Office of Research and Development, National Health and Environmental Effects Research Laboratory, EP/600/R-09/-50.

Nejrup, L. and M. Pedersen. 2008. Effects of salinity and water temperature on the ecological performance of *Zostera marina*. Aquatic Botany 88:239-246.

Nelson, T.A., K. Haberlin, A.V. Nelson, H. Ribarich, R. Hotcchkiss, K.L. Van Alstyne, L. Buckingham, D.J. Simunds and K. Fredrickson. 2008. Ecological and physiological controls of species composition in green macroalgal blooms. Ecology 89(5):1287-1298.

Nixon, S., B. Buckley, S. Granger, and J. Bintz. 2001. Responses of very shallow marine ecosystems to nutrient enrichment. Human and Ecological Risk Assessment 7(5):1457-1481.

Ochieng, C. A., F. T. Short, and D. I. Walker. 2010. Photosynthetic and morphological responses of eelgrass (*Zostera marina* L.) to a gradient of light conditions. Journal of Experimental Marine Biology and Ecology 382:117-124.

O'Higgins, T. and S. Rumrill. 2007. Tidal and Watershed Forcing of Nutrients and Dissolved Oxygen Stress within four Pacific Coast estuaries: Analysis of time-series data collected by the National Estuarine Research Reserve System-Wide Monitoring Program (2000-2006) within Padilla Bay (WA), South Slough (OR), Elkhorn Slough (CA) and the Tijuana River estuary (CA). Report for the NOAA/UNH Cooperative Institute for Coastal and Estuarine Environmental Technology (CICEET), 58 pp.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2010. vegan: Community Ecology Package. R package version 1.17-4. http:CRAN.R-project.org/ package=vegan. Accessed 20 July 2011.

Orth, R. J., and K. A. Moore. 1983. Chesapeake Bay: An unprecedented decline in submerged aquatic vegetation. Science 222:51-53.

Orth, R.J., T.J.B. Carruthers, W. Dennison, C.M. Duarte, J. Fourqurean, K.L. Heck, A.R. Hughes, G.A. Kendrick, W.J. Kenworthy, S. Olyarnik, F.T. Short, M. Waycott and S.L. Williams. 2006. A global crisis for seagrass ecosystems. BioScience 56(12):987-996.

Pacific Fisheries Environmental Laboratory. 2011. http://www.pfeg.noaa.gov. Accessed 14 Jan 2010.

Parmesan, C., C. Duarte, E. Poloczanska, A. J. Richardson, and M. C. Singer. 2011. Overstretching attribution. Nature Climate Change 1:2-4.

Peralta, G., J.L. Perez-Llorens, I. Hernandez, J.J. Vergara. 2002. Effects of light availability on growth, architecture and nutrient content of the seagrass *Zostera noltii* Hornem. Journal of Experimental Marine Biology and Ecology 269:9-26.

Pinheiro, J., D. Bates, S. DebRoy, D.Sarkar and the R Core Team 2009. nlme: Linear and nonlinear mixed effects models. http://cran.r- project.org/web/packages/ nlme/index.html. Accessed 29 Aug 2010

Pregnall, A. M., R. D. Smith, T. A. Kursar, and R. S. Alberte. 1984. Metabolic adaptation of Zostera marina (eelgrass) to diurnal periods of root anoxia. Marine Biology 83:141-147.

Pregnall, A. and P. Rudy. 1985. Contribution of green macroalgal mats (*Entermorpha* spp.) to seasonal production in an estuary. Marine Ecology Progress Series 24: 167-176.

R Development Core Team. 2009. R: A language and environment for statistical computing. Vienna Austria: R Foundation for Statistical Computing. http://www.R.project.org. Accessed Aug 29 2010.

Ralph, P.J., D. Tomasko, K. Moore, S. Seddon, C.M.O. Macinnis-Ng. 2006. Human impacts on seagrasses: Eutrophication, sedimentation, and contamination. In

Seagrasses: Biology, Ecology and Conservation, eds A.W.D. Larkum, R.J. Orth, C.M. Duarte. Pages 567-593. The Netherlands: Springer.

Reum, J., T. Essington, C. Greene, C. Rice, and K. Fresh. 2011. Multiscale influence of climate on estuarine populations of forage fish: The role of coastal upwelling, freshwater flow and temperature. Marine Ecology Progress Series 425:203-215.

Rivers, J. and P. Peckol. 1995. Summer decline of *Ulva lactuca* (Chlorophyta) in a eutrophic embayment: Interactive effects of temperature and nitrogen availability. Journal of Phycology 31(2):223-228.

Robbins, B.D. and B.L. Boese. 2002. Macroalgal volume: A surrogate for biomass in some green algae. Botanica Marina 45(6):586-588.

Roegner, G.C. and A.L. Shanks. 2001. Import of coastally-derived chlorophyll a to South Slough, Oregon. Estuaries 24(2):244-256.

Romero, J., K.-S. Lee, M. Perez, M. Mateo, and T. Alcoverro. 2006. Nutrient dynamics in seagrass ecosystems. Pages 227-254 Seagrasses: Biology, Ecology and Conservation. Springer, The Netherlands.

Rumrill, S. 2006. The Ecology of the South Slough Estuary: Site profile of the South Slough National Estuarine Research Reserve. Salem, Oregon: NOAA. 238 pp.

Rykaczewski, R. R., and J. P. Dunne. 2010. Enhanced nutrient supply to the California Current Ecosystem with global warming and increased stratification in an earth system model. Geophysical Research Letters 37 (21), L21606.

Salomon, A.K., S.K. Gaichas, N.T. Shears, J.E. Smith, E. Madin and S.D. Gaines. 2010. Key features and context-dependence of fishery-induced trophic cascades. Conservation Biology 24(2):382-394.

Scavia, D., J. C. Field, D. F. Boesch, R. W. Buddemeier, V. Burkett, D. R. Cayan, M. Fogarty, M. A. Harwell, R. W. Howarth, C. Mason, D. J. Reed, T. C. Royer, A. H. Sallenger, and J. G. Titus. 2002. Climate change impacts on U.S. coastal and marine ecosystems. Estuaries 25:149-164.

Sfriso, A., B. Pavoni, A. Marcomini, A. Orio. 1992. Macroalgae, nutrient cycles, and pollutants in the Lagoon of Venice. Estuaries and Coasts 15(4):517-528.

Short, F. T., and S. Wyllie-Echeverria. 1996. Natural and human-induced disturbance of seagrasses. Environmental Conservation 23:17-27.

Short, F. T., D. M. Burdick, and J. E. Kaldy. 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina*. Limnology and Oceanography 40:740-749.

Simenstad, C. A., M. Dethier, C. Levings, and D. Hay. 1997. The terrestrial/marine ecotone. Page 431. The Rainforests of Home: Profile of a North American Bioregion. Island Press, Washington, D.C.

Skelly, D. K., and J. M. Kiesecker. 2001. Venue and outcome in ecological experiments: manipulations of larval anurans. Oikos 94:198-208.

Teichberg, M., S. E. Fox, Y. S. Olsen, I. Valiela, P. Martinetto, O. Iribarne, E. Y. Muto, M. A. V. Petti, T. N. Corbisier, M. Soto-Jimãnez, F. Pãez-Osuna, P. Castro, H. Freitas, A. Zitelli, M. Cardinaletti, and D. Tagliapietra. 2010. Eutrophication and macroalgal blooms in temperate and tropical coastal waters: nutrient enrichment experiments with *Ulva* spp. Global Change Biology 16: 2624-2637.

Terrados, J., C.M. Duarte, L. Kemp-Nielsen, N.S.R. Agawin, E. Gacia, D. Lacap, M.D. Fortes, J. Borum, M. Lubanski and T. Greve. Are seagrass growth and survival constrained by the reducing conditions of the sediment? Aquatic Botany 65:175-197.

Thom, R. M. 1990. Spatial and temporal patterns in plant standing stock and primary production in a temperate seagrass system. Botanica Marina 33:497-510.

Thom, R., A. Borde, S. Rumrill, D. Woodruff, G. Williams, J. Southard and S. Sargeant. 2003. Factors influencing spatial and annual variability in eelgrass (*Zostera marina* L.) meadows in Willapa Bay, Washington, and Coos Bay, Oregon, estuaries. Estuaries and Coasts 26(4):1117-1129.

Thom, R.M., S.L. Southard, A.B. Borde and P. Stoltz. 2008. Light requirements for growth and survival of eelgrass (*Zostera marina* L.) in Pacific Northwest (USA) estuaries. Estuaries and Coasts 31(5):969-980.

Touchette, B. W., and J. M. Burkholder. 2000. Review of nitrogen and phosphorus metabolism in seagrasses. Journal of Experimental Marine Biology and Ecology 250:133-167.

Udy, J. W., and W. C. Dennison. 1997. Physiological responses of seagrasses used to identify anthropogenic nutrient inputs. Marine and Freshwater Research 48: 605-614.

Underwood, A. 1997. Experiments in Ecology; Their Logical Design and Interpretation Using Analysis of Variance. Cambridge, U.K.: Cambridge University Press.

Valiela, I., J. McClelland, J. Hauxwell, P.J. Behr, D. Hersh, K. Foreman. 1997. Macroalgal blooms in shallow estuaries: Controls and ecophysiological ecosystem consequences. Limnology and Oceanography 42(5):1105-1118.

Waycott, M., C.M. Duarte, T.J.B. Carruthers, R.J. Orth, W. Dennison, S. Olyarnik, A. Calladine, J. Fourqurean, K.L. Heck, A.R. Hughes, F.A. Kendrick, W.J. Kenworthy, F.T. Short and S.L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings of the National Academy of Sciences 106(30):12377-12381.

Wickham, H. 2009. ggplot2: Elegant graphics for data analysis. http://had.co.nz/ggplot2/. Accessed Aug 29 2010

Wheeler, P., and B. R. Bjornsater. 1992. Seasonal fluctuations in tissue nitrogen, phosphorus, and N:P for five macroalgal species common to the Pacific Northwest Coast. Journal of Phycology 28:1-6.

Williams, S. L., and M. H. Ruckelshaus. 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. Ecology 74:904-918.

Yund, P.O., S.D. Gaines, M.D Bertness. 1991. Cylindrical tube traps for larval sampling. Limnology and Oceanography 36(6):1167-1177.

Zimmerman, R.C., J.L. Reguzzoni and R.S. Alberte. 1995. Eelgrass (*Zostera marina* L.) transplants in San Francisco Bay: Role of light availability on metabolism, growth and survival. Aquatic Botany 51(1-2):67-86.

APPENDICES

Appendix 1. Macrophyte, marine, watershed, and nutrient characteristics of Willapa Bay, Netarts Bay, Yaquina Bay, and Coos Bay. The macrophyte values come from the surveys described in the Methods. The estuarine characteristics (terrestrial vectors) include catchment area per estuary area, estuary volume, watershed land use (urban, agricultural, forest), population per estuary area, freshwater per estuary area, total suspended solids per estuary area, and daily precipitation per estuary area are compiled from data sources available at NOAA's National Coastal Assessment and Data Synthesis (CA&DS) (http://coastalgeospatial.noaa.gov/) and the National Estuarine Eutrophication Assessment (NEEA) Estuaries Database (http://ian.umces.edu/neea). The physical and hydrological (P&H) characteristics of each estuary include data on estuarine volume and catchment size per estuarine area calculated from NOAA Coastal Assessment Framework (CAF) shapefiles. The freshwater inflow information is obtained from USGS gage stations. The daily precipitation per estuarine area is derived from PRISM shapefiles. The catchment land use is based on USGS Land Use and Land Cover (LULC) for the entire watershed updated with 1990 Census data to enhance the characterization of urban areas. The population numbers are based on catchment areas from 2000 census data. The freshwater data (total suspended solids, terrestrial nutrients: nitrogen and phosphorus per estuary area) are from the National Coastal Pollutant Discharge Inventory (NCPDI). These values represent estimates of pollutant discharges from all point, non-point and riverine sources. The data are approximated from conditions from 1982-1991.

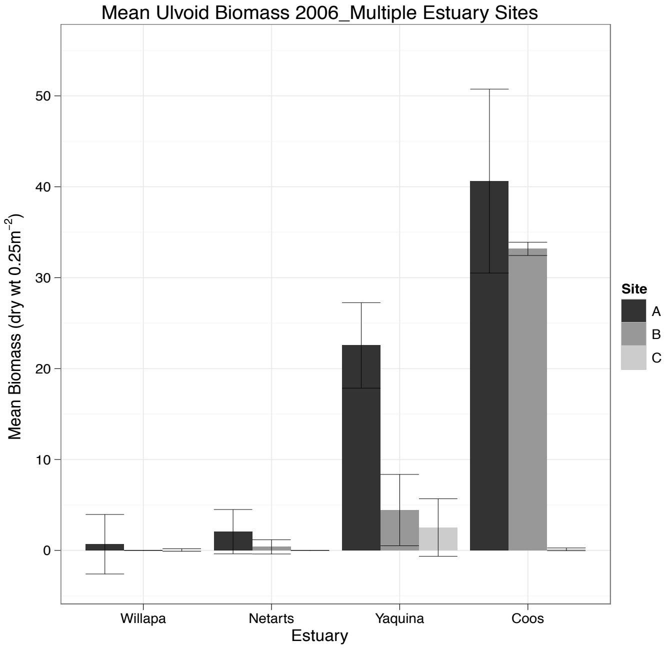
The marine vectors include tidal volume per day, mean upwelling index and neashore and alongshore currents. The tidal volume per day data is from tide station long-term data (NEEA database). For both upwelling and current data, mean monthly values for June and July 2006-2010 were used. Upwelling data was summarized based on upwelling indices computed by the Pacific Fisheries Environmental Laboratory (http://www.pfeg.noaa.gov/) for geographic coordinates closest to the mouths of the surveyed estuaries. The current data (offshore and alongshore) is extracted from CODAR measurements maintained by M. Kosro, College of Oceanic and Atmospheric Science, Oregon State University (http://bragg.coas.oregonstate.edu/). The data are extracted from a 0.5° radius corresponding to geographic coordinates at the mouths of the surveyed estuaries

Appendix 1 continued. Characteristics of Willapa, Netarts, Yaquina and Coos Bays

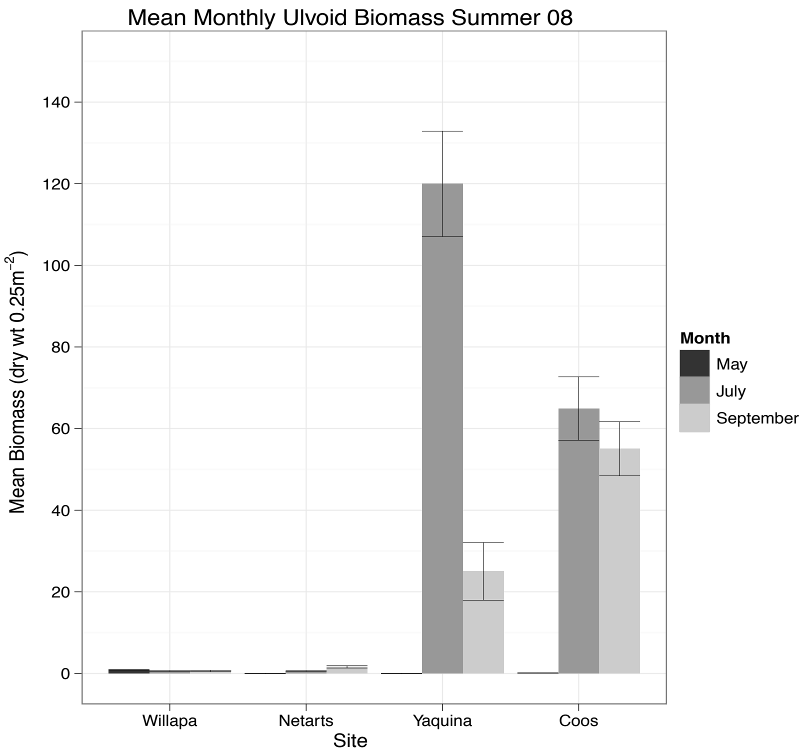
		Willapa Bay	**Netarts Bay**	**Yaquina Bay**	**Coos Bay**
Macrophyte Production	Macroalgae Biomass per Area (g dw 0.25 m^{-2})	5.40 ± 1.16	0.71 ± 0.09	82.17 ± 5.33	42.55 ± 3.99
	Eelgrass Biomass per Area (g dw 0.25 m^{-2})	31.83 ± 1.38	50.71 ± 1.76	15.37 ± 0.98	7.91 ± 0.96
	Eelgrass Density per Area (shoots 0.25 m^{-2})	18.72 ±10.78	89.45 ± 3.19	23.14 ± 1.16	29.52 ±1.76
	Eelgrass Biomass per Shoot (biomass shoot^{-1})	1.68 ± 0.08	0.57 ± 0.01	0.79 ± 0.13	0.52 ± 0.07
Terrestrial Vectors	Catchment (km^2) per Estuary Area (km^2)	6.8	3.1	45.3	33.6
	Volume (m^3)	107.21 x 10^7	1.41 x 10^7	2.98 x 10^7	20.68 x 10^7
	% Catchment Urban	1.14	0.4	2.48	4.26
	% Catchment Agricultural	2.69	0	1.61	1.41
	% Catchment Forest	93.28	79.76	95.08	92.65
	Population per Estuary Area (# km^{-2})	42.24	50.5	586.21	828.14
	Freshwater per Estuary Area (m/d)	20.45	3.56	47.14	14.18
	Total Suspended Solids per Estuary Area (tonne km^{-2}yr^{-1})	797	1270	11357	14750
	Daily Precipitation per Estuary Area (mm d^{-1})	5.6	5.9	4.9	4.5
Terrestrial Nutrients	Total Nitrogen per Estuary Area (tonne km^{-2}yr^{-1})	10016	9338	74600	99272
	Total Phosphorus per Estuary Area (tonne km^{-2}yr^{-1})	140	93	1344	1824
Marine Vectors	Tidal Volume per Day (m^3 d^{-1})	161.89 x10^7	2.96 x10^7	5.14 x10^7	14.03 x10^7
	Mean Upwelling Index (m^{3}s $^{-1}$100m^{-1})	20.16	40.00	50.96	74.94
	Mean Offshore Current (cm s $^{-1}$)	5.59	3.68	7.7	12.47
	Mean Alongshore Current (cm s $^{-1}$)	14.02	15.08	15.33	19.64

Appendix 2

Methodology for determining the sites (A) and the month (B) used in the latitudinal survey of producer biomass, and for extrapolating field-based measurements of macroalgae to biomass area^{-1} (C)



Appendix 2A. Within-estuary site selection. We surveyed three marine sites per estuary (A, B, C) in 2006 to establish the long-term survey site of macroalgae and eelgrass production. All sites were located in the euhaline zone of the estuary, near the estuary's mouth. In all estuaries we chose site A, which had the highest macroalgae biomass to focus our subsequent sampling (geographic coordinates of site A locations described in Figure 2.1). Sites B and C were only sampled in 2006. Biomass values are based on percent cover extrapolations (Appendix 2C).



Appendix 2B. Seasonal surveys. We sampled estuaries for macroalgae and eelgrass biomass in July of each year (2006-2010). In 2008 we sampled all estuaries during three months (May, July and September) and found that peak biomass occurred in July. Biomass was very low in all months in Willapa and Netarts Bays. Biomass values were calculated based on volume extrapolations (Appendix 2C) rather than percent cover because we only quantified macroalgae volume during this seasonal survey.

Appendix 2C. Calculations of macroalgal biomass. To determine biomass of ulvoid macroalgae, we compared two relationships for extrapolating field-based measurements of percent cover and macroalgal volume to dry weight. We quantified macroalgal volume per quadrat (0.25 m^{-2}) from 2008-2010 during our comparative surveys of estuaries, percent cover methodology described in the Methods. Relationships were derived from on an extensive two year dataset (n = 199) from Coos Bay (Hessing-Lewis et al. 2011), which measured macroalgae percent cover, volume and dry weight (macroalgae removed from quadrats, washed and dried at 60°C for 24 hrs in a drying ovens). Macroalgal volume was quantified in the field based on methodology established by Robbins and Boese (2002). We fit linear relationships between 1) percent cover and dry weight: log macroalgae biomass = 1.61*log macroalgae percent cover -3.83, R^2 =0.85, $p < 0.001$, and 2) volume and dry weight: log macroalgae biomass = 1.08*log macroalgae volume – 3.49, R^2 =0.82, $p < 0.001$. We used these relationships to extrapolate our volume and percent cover time series to dry weight biomass (g dry wt 0.25m^2) values. The percent cover extrapolations were used for subsequent statistical analyses because they were more conservative than the volume-based extrapolations.

Appendix 3

Coos Bay inter-annual (2006-2010) producer and environmental trends. The mean macroalgae and eelgrass biomass (g dw 0.25 m^{-2} ± SE) are from surveys described in the Methods. The nutrient, water quality and meteorological time series data are acquired from the NERR SWMP (National Estuarine Research Reserve System Wide Monitoring Protocol) program (http://cdmo.baruch.sc.edu/). Data from the Marine (Charleston Bridge) and Riverine (Winchester Arm) water quality and nutrient stations used for analyses. The Marine station is located near the estuary mouth in the euhaline section of the estuary, and the Riverine station is located near the head of the estuary where salinities reflect freshwater inputs. All flagged data not meeting SWMP quality check protocol were removed. For all water quality and nutrient data mean values for June and July are reported. Nutrient concentrations are from water quality grab samples (3 samples $tide^{-1}month^{-1}$± SE) at each station. Salinity is recorded by permanent YSI Datasondes (Yellow Springs Instruments Model 6600) located at each station that record data at 15 minute intervals. The marine nutrient vector was composed of high tide dissolved inorganic nitrogen (mgL^{-1} ± SE DIN) and phosphate values (mgL^{-1} ± SE P) from the Marine station. The high tide nutrient values at this station were correlated with mean salinity data (ppt ± SE), which is also included as a descriptor of marine nutrients. The terrestrial nutrient vector was composed of low tide nutrient concentrations at both the Marine and Riverine stations which are negatively correlated with salinity values and indicative of freshwater inputs. The local climate vector was composed of total June and July precipitation (mm) and photosynthetically active radiation (PAR) flux per 15 minutes (mmol m^{-2}) recorded by the South Slough automated meteorological station (Campbell CR-10/CR-1000) located at the Oregon Institute of Marine Biology near to the Marine station.

Appendix 3 continued. Coos Bay inter-annual (2006-2010) producer and environmental trends.

		2006	**2007**	**2008**	**2009**	**2010**
Macrophyte Production	Macroalgae (g dw $0.25m^{-2}$)	24.4± 3.09	45.19± 4.00	62.64± 4.99	42.75± 4.11	12.29± 2.17
	Eelgrass (g dw $0.25m^{-2}$)	22.49± 2.30	17.21± 1.24	15.12± 2.59	8.49± 1.70	13.65± 1.59
Marine Nutrient Vector	Marine High tide DIN (µM)	0.246± 0.017	0.242± 0.002	0.242± 0.018	0.194± 0.006	0.200± 0.004
	Marine High tide P (µM)	0.046± 0.002	0.047± 0.000	0.056± 0.002	0.044± 0.001	0.036± 0.001
	Salinity (ppt)	31.79± 0.02	33.40± 0.01	33.00± 0.01	32.93± 0.01	30.91± 0.03
Terrestrial Nutrient Vector	Marine Low tide DIN(µM)	0.124± 0.001	0.064± 0.009	0.102± 0.032	0.086± 0.007	0.117± 0.000
	Marine Low tide P (µM)	0.043± 0.001	0.038± 0.000	0.050± 0.002	0.055± 0.003	0.037± 0.001
	Riverine Low DIN (µM)	0.140± 0.00	0.076± 0.004	0.102± 0.001	0.125± 0.000	0.201± 0.009
	Riverine Low P (µM)	0.008± 0.000	0.003± 0.000	0.008± 0.000	0.008± 0.000	0.004± 0.000
Local Climate Vector	Total Precipitation (mm)	29.20	54.22	32.00	32.52	134.1
	Photosynthetically active radiation (mmol m-^{2})	2.45 x 10^{6}	2.30 x 10^{6}	4.30 x 10^{6}	2.73 x 10^{6}	2.58 x 10^{6}

Appendix 4

Post hoc linear comparisons (Tukey test) from linear mixed effect model for spatial variation in eelgrass and macroalgal production between estuaries (biomass ~ producer-by-site (fixed) + year/site/quadrat (random). Matrix of z-values are from producer comparisons. P-values reported as 0 = ***, 0.001= **, <0.05 = *, >0.05 = ns.

<table>
<tr><th></th><th colspan="3">z-values P (>|z|)</th></tr>
<tr><th></th><th>Macroalgae vs. Eelgrass</th><th>Macroalgae</th><th>Eelgrass</th></tr>
<tr><td>Willapa Bay</td><td>-15.688***</td><td></td><td></td></tr>
<tr><td>vs. Netarts Bay</td><td></td><td>1.935 ns</td><td>-8.785**</td></tr>
<tr><td>vs. Coos Bay</td><td></td><td>-10.384***</td><td>9.259**</td></tr>
<tr><td>vs. Yaquina Bay</td><td></td><td>11.893***</td><td>-7.756*</td></tr>
<tr><td>Netarts Bay</td><td>-21.636***</td><td></td><td></td></tr>
<tr><td>vs. Coos Bay</td><td></td><td>-9.809***</td><td>17.716***</td></tr>
<tr><td>vs. Yaquina Bay</td><td></td><td>11.179***</td><td>-16.344***</td></tr>
<tr><td>Yaquina Bay</td><td>7.797***</td><td></td><td></td></tr>
<tr><td>vs. Coos Bay</td><td></td><td>1.601 ns</td><td>1.983 ns</td></tr>
<tr><td>Coos Bay</td><td>7.050***</td><td></td><td></td></tr>
</table>

Appendix 5

Ordination outputs for A) Principal Component Analysis (PCA) and B) Redundancy Analysis (RDA). A) Principal component analysis was used to reduce the multiple vector parameters described in Appendix 1(latitudinal patterns) and Appendix 3 (Coos Bay patterns) to their primary components (we used PCA axis 1 scores). Proportion of variance explained by the first three ordination axes reported, as well as axis 1 scores from estuarine sites (latitudinal patterns) and years (Coos Bay patterns). PCA 1 axis scores were then used as environmental constraints for the RDA ordination of macrophyte biomass by site and year. Proportion of variance explained by RDA axes 1 and 2 reported, along with RDA axis 1 and 2 scores for macrophytes and estuary sites (latitudinal patterns) or macrophytes and years (Coos Bay patterns)

Appendix 5A. Principle Component Analysis

	Environmental Factors	Proportion of Variance Explained			PCA axis 1 scores				
Spatial Latitudinal Patterns		**PC1**	**PC2**	**PC3**	**Willapa Bay**	**Netarts Bay**	**Yaquina Bay**	**Coos Bay**	
	High Marine Vectors	0.78	0.20	0.02	1.88	0.64	-0.20	-2.32	
	High Terrestrial Vectors	0.63	0.27	0.10	1.78	2.29	-1.97	-2.10	
	High Terrestrial Nutrients	1.0			1.17	1.22	-0.81	-1.58	
Coos Bay Inter-annual Patterns		**PC1**	**PC2**	**PC3**	**2006**	**2007**	**2008**	**2009**	**2010**
	High Marine Nutrients	0.78	0.22		0.60	0.56	1.52	-1.07	-1.62
	High Terrestrial Nutrients	0.87	0.13		0.67	-2.15	1.38	1.93	-1.81
	High Rain, Low Light	0.64	0.36		-0.06	0.45	-1.62	-0.25	1.49

Appendix 5B. Redundancy Analysis

	Proportion of Variance Explained		**RDA axis 1 scores**		**RDA axis 2 score**	
Spatial Latitudinal Patterns	RDA 1	RDA 2	Macroalgae	-1.07	Macroalgae	-0.27
	0.94	0.06	Eelgrass	1.07	Eelgrass	-0.27
			Coos Bay	-0.72	Coos Bay	0.15
			Yaquina Bay	-0.77	Yaquina Bay	-0.52
			Netarts Bay	1.04	Netarts Bay	-0.83
			Willapa Bay	0.47	Willapa Bay	1.21
Coos Bay Inter-annual Patterns	RDA 1	RDA 2	Macroalgae	1.07	Macroalgae	0.16
	0.43	0.14	Eelgrass	0.29	Eelgrass	-0.61
			2006	-0.26	2006	-2.38
			2007	0.43	2007	-0.38
			2008	1.11	2008	0.63
			2009	-0.08	2009	2.17