EXPERIMENTAL EFFECTS OF AMMONIUM ON EELGRASS (ZOSTERA MARINA L.) SHOOT DENSITY IN HUMBOLDT BAY, CALIFORNIA

By

Ginger Tennant

A Thesis

Presented to

The Faculty of Humboldt State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

In Biology

(May, 2006)

EXPERIMENTAL EFFECTS OF AMMONIUM ON EELGRASS (ZOSTERA MARINA L.) SHOOT DENSITY IN HUMBOLDT BAY, CALIFORNIA

by

Ginger Tennant

Approved by the Master's Thesis Committee:

Frank J. Shaughnessy, Major Professor Date

Edward C. Metz, Committee Member Date

Erik S. Jules, Committee Member Date

William L. Bigg, Committee Member Date

Michael R. Mesler, Graduate Coordinator Date

Donna E. Schafer, Dean for Research and Graduate Studies Date

ABSTRACT

Experimental Effects of Ammonium on Eelgrass (*Zostera marina,* L.) Shoot Density in Humboldt Bay, California

Ginger Tennant

Humboldt Bay contains two populations of eelgrass; *Zostera marina* L., a sparse population in North Bay and a significantly denser population in South Bay. No study has attempted to determine the cause of this density difference despite the importance of shoot density for protecting eelgrass community members. My objectives were to describe some of the ambient conditions in the water column and sediment of Humboldt Bay relevant to eelgrass, to experimentally determine if the variation in shoot density is caused by either ammonium limitation or toxicity, and to determine if ambient ammonium levels are affecting other eelgrass metrics. Ambient conditions were determined through monthly water samples, sediment cores, and data loggers. The effect of ammonium on density and other eelgrass metrics was examined through the *in situ* application of ammonium/phosphate fertilizer. The water column in Humboldt Bay contained insufficient ammonium to saturate eelgrass uptake and in the summer was nitrate limited as well. However, the sediment contained sufficient ammonium and the experiment demonstrated that the density difference between bays was not due to ammonium limitation. The application of fertilizer to the sediment of North Bay caused a significant decrease in eelgrass density and below ground biomass. The high levels of phosphate in North Bay treatment plots indicated phosphate not ammonium, toxicity.

North Bay eelgrass may be vulnerable to an increase in nutrient loading. The abundance of *Phyllaplysia taylori* L. was a significant predictor of the change in density indicating that light levels may also be impacting densities between the bays.

ACKNOWLEDGMENTS

This thesis was far too large to be accomplished single-handed, and I was amazed at the outpouring of help I received from all quarters. I would like to start by thanking Frank Shaughnessy for all the hours he put into this thesis, including the time spent slogging mudflats, and his tireless attempts to find sources of funding for me. I would also like to thank the other members of my committee, Ed Metz, Erik Jules and Bill Bigg for all their support and advice. A special thanks to the honorary member of my committee, Susan Schlosser, for her generous donation of equipment, data and personnel. There are whole sections of my thesis that could never have been accomplished without her.

Thank you to those who helped support this thesis financially, the Friends of the Arcata Marsh, the Redwood Region Audubon Society, the Department of Biological Sciences at Humboldt State University, and California Sea Grant.

I would like to acknowledge those many people who gave me or loaned me equipment and supplies. Thanks: John Mello, Dave Hoskins, Anthony Baker, Grant Eberle, Susannah Ferson, Jeff Black, and Tristen Joy. You made it possible to accomplish a lot on very little money.

Thank you to those who shared their knowledge, skills and data with me, especially Randy Klein, Clark Fenton, Matthew Bracken, Andrew Stevens, Susan Marshall, Greg Crawford, Marie De Angelis, and Fred Short.

A huge thank you to all the volunteers who got up in the dark and waited outside the gate to South Spit just so we could slog mudflats and measure eelgrass. Thanks: Tyler Smith, Michelle Koury, Ken Henderson, Stephanie Clemens, Melissa Batka, Josh Neely, and a special thank you to my "rocks" Neil Kalson, Martha Walker, Cara Witte, and Mark Watson. I would also like to thank Jessica Oliver for helping me in the lab for endless hours.

I would like to acknowledge those who gave me permission to tromp all over their territory and take samples. Thanks: Greg Dale, California Department of Fish and Game and the Humboldt Bay National Wildlife Refuge.

Finally, a big hug and kiss for Skip Shoemaker for his unfailing support both on the mudflats and from as far away as Dutch Harbor, Alaska. Skip, you were the only one who shared my joy at finishing the field work and told me all along that I could do this, even when there was evidence to the contrary–especially when there was evidence to the contrary. Thank you.

Thank you all! I could never have done this on my own.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Eelgrass, *Zostera marina* L., is a marine monocotyledonous angiosperm found in low intertidal to shallow subtidal habitats within temperate to subtropical bays and estuaries in the northern hemisphere (McRoy and Barsdate 1970; Green and Short 2004). It is perennial in temperate regions, annual in subtropical regions, and reproduces primarily through rhizomatous cloning (Phillips et al. 1983; Ramage and Schiel 1999; Santamaria-Gallegos et al. 2000). Roots and rhizomes are anchored in small-grained, organic-rich sediment that is often anoxic due to high microbial oxygen demands (Terrados et al. 1999). Physiologically, seagrasses and eelgrass in particular, are noted for being eurythermal, euryhaline and having high light but low nitrogen requirements relative to phytoplankton and seaweeds (Biebl and McRoy 1971; Wium-Andersen and Borum 1984; Gallegos 2001). Eelgrass survives long term at water temperatures ranging from -6-34° C, but the optimal temperature range for eelgrass is 20-25º C and both density and biomass are greater when temperatures are colder (Biebl and McRoy 1971; Wium-Andersen and Borum 1984; Orth and Moore 1986; Zimmerman et al. 1989; Bintz et al. 2003; Touchette et al. 2003). The natural salinity range for eelgrass is 9 to 42 ppt, but it has been maintained under laboratory conditions at 0 ppt (Biebl and McRoy 1971; Wium-Andersen and Borum 1984). The minimum light requirement for eelgrass is 10 - 22% of surface light, which is an order of magnitude higher than the minimum light requirement for phytoplankton, but the Redfield ratio for eelgrass (the ratio of C: N: P) is 106:16:1, so eelgrass requires approximately four times less nitrogen and phosphorous per carbon atom than phytoplankton (Duarte 1995; Hemminga and Duarte 2000;

Gallegos 2001). Eelgrass is a clonal plant that often forms monospecific beds which lack the genetic diversity necessary to withstand stochastic events such as the "wasting disease" caused by *Labyrinthula zosterae,* Porter and Muehlstein, that destroyed entire North Atlantic eelgrass populations in the 1930's (Vergeer et al. 1995; Hemminga and Duarte 2000). The lack of seagrass species diversity and so lack of functional redundancy makes the entire *Z. marina* community vulnerable to any stress suffered by the eelgrass.

Zostera marina is an important component of marine, shallow-water ecosystems. It is a major contributor to primary productivity, with peak values during the summer and lowest values in the winter (Sand-Jensen 1975; Jacobs 1979; Aioi 1980; Wium-Andersen and Borum 1984; Kentula and McIntire 1986; Orth and Moore 1986; Thom and Albright 1990; Olesen and Sand-Jensen 1994; Hansen et al. 2000; Risgaard-Peterson and Ottosen 2000). Eelgrass provides this trophic support through two separate carbon pathways; grazing and detritus. While living, eelgrass can be directly consumed by waterfowl and amphipods and epiphytic diatoms and macroalgae are grazed by a wide diversity of invertebrates (Henry 1980; Perkins-Visser et al. 1996; Pinckney and Micheli 1998; Duffy and Harvilicz 2001; Moore et al. 2004). The second pathway for carbon occurs when eelgrass and the algae die and are consumed by detritivores (Irlandi 1996).

Eelgrass beds also have nursery, and refuge functions, both of which are dependent on the vegetation complexity of eelgrass. The hypothesis that eelgrass beds function as "nursery grounds" defined by Williams and Heck (2001) as a place where juvenile animals survive and grow at higher rates than on nearby unvegetated substrate is based on observations that the juveniles of many fish and invertebrate species are found in higher abundance in eelgrass beds than on nearby unvegetated substrate (Heck and Thoman 1984; Hoffman 1986; Pohle et al. 1991; Perkins-Visser et al. 1996; Mattilla et al. 1999; Valle et al. 1999; Murphy et al. 2000; Beck et al. 2001; Peterson 2001; Hovel et al. 2002; Heck et al. 2003; Hovel 2003; Pastén et al. 2003). The effectiveness of eelgrass beds at increasing juvenile survival may stem from the increased complexity of the habitat contributed by the eelgrass, protecting juveniles from predation (Hoffman 1986; Prescott 1990; Mattilla et al. 1999; Hovel 2003; Lazzari et al. 2003). For example, juvenile bay scallops climb eelgrass blades to avoid predation from benthic predators, post-settlement predation on juvenile bivalves has been shown to be lower in eelgrass beds, and juvenile winter flounder (*Pseudopleuronectes americanus,* Walbaum) mortality is significantly lower in eelgrass beds than bare substrate (Pohle et al.1991; Bostrom and Bonsdorff 2000; Manderson et al. 2000). The effectiveness of eelgrass beds at increasing juvenile growth rates may stem from the higher quality and quantity of food resources within the eelgrass canopy (Connolly 1994; Perkins-Visser et al. 1996).

Adult fish and invertebrates use the complex structure of eelgrass beds as a refuge. Some species live their entire lives in eelgrass beds and are found solely on eelgrass blades, such as the opisthobranch *Phyllaplysia taylori* L (Dykhouse 1976). Other species are only found in eelgrass beds during vulnerable stages, such as adult blue crabs (*Callinectes sapidus* Rathbun) undergoing molting, or are found in higher abundances close to eelgrass beds and use the eelgrass canopy as an occasional refuge to avoid predators (Perkins-Visser et al. 1996; Jenkins et al. 1997).

Shoot density is one of the most important aspects of eelgrass bed structure. Eelgrass beds with lower shoot density provide fish and invertebrates with less protection from predation (Heck and Thoman 1981; Irlandi 1997; Bostrom and Bonsdorff 2000; Bell et al. 2001; Bostrom et al. 2002). Lower shoot density decreases juvenile growth rates and allows faster water flow through the eelgrass bed increasing erosion, altering the species composition of bivalves and decreasing larval recruitment (Fonseca and Cahalan 1992; Perkins-Visser et al. 1996; Webster 1998; Irlandi et al. 1999; Bostrom and Bonsdorff 2000; Peterson et al. 2004).

Resource managers are therefore interested in the causes of shoot density variation since eelgrass bed structure is connected to several major functions of this ecosystem. Humboldt Bay, California, has a substantial stock of eelgrass (≈1890 ha; Schlosser et al. *accepted*) that inhabits the intertidal and subtidal mudflats of both North Bay (Arcata Bay) and South Bay. North Bay eelgrass has significantly lower shoot densities than South Bay (Figure 1). This significant difference in density exists in both winter and summer and is not of recent origin. As far back as 1963, Masters Theses from Humboldt State University have remarked on the disparity of the two populations but no study has attempted to determine the cause (Keller 1963; Harding 1973). Such a study could be useful to local resource managers as several animals of commercial importance, such as Pacific herring (*Clupea pallasii* Valenciennes 1847), Dungeness crab (*Cancer magister,* Dana 1852), and rockfish (*Sebastes* spp., Cuvier 1829) utilize the Humboldt Bay eelgrass beds.

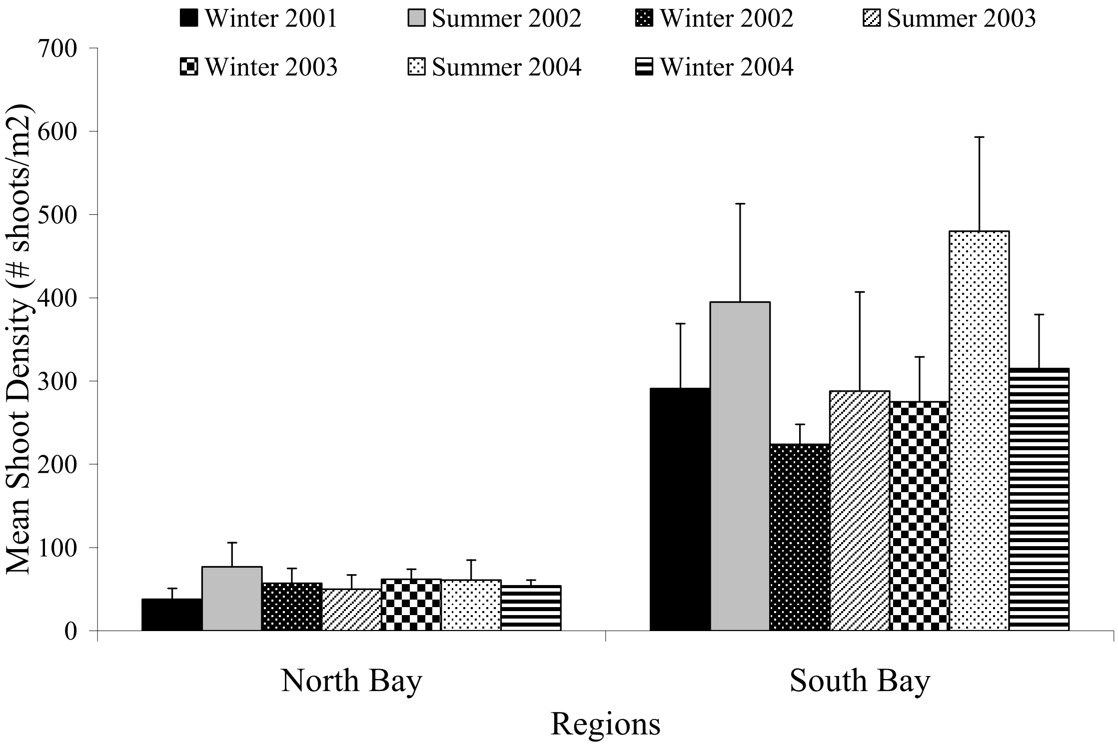


Figure 1. Mean shoot density in North Bay and South Bay over time. Values shown are means + 1SD (modified from Schlosser et al. accepted).

There are many environmental factors, that can affect the shoot density of *Z. marina*, including anoxia (Holmer and Nielsen 1997; Holmer and Bondgaard 2001), current speeds and wave exposure (Nelson and Lee 2001; Schanz and Asmus 2003; Bostrom et al. 2004; Polte et al. 2005), desiccation (Boese et al. 2003), epiphyte and grazer loads (Howard and Short 1986; Williams and Ruckelshaus 1993), grazing by water fowl (Moore et al. 2004), light limitation (Backman and Barilotti 1976; Zimmerman et al. 1987; Olesen and Sand-Jensen 1993; Duarte1995; De Casabianca et al. 1997; Moore et al. 1997; Havens et al. 2001; Nelson and Lee 2001), nutrient limitation (Orth 1977; Harlin and Thorne-Miller 1981; Short 1983; Roberts et al. 1984; Williams and Ruckelshaus 1993; Udy and Dennison 1997; van Katwijk et al. 1997; Worm and Reusch 2000), nutrient toxicity (Short 1983; van Katwijk et al. 1997; Nixon et al. 2001; Touchette et al. 2003; Lee et al. 2004), and sediment grain size (Aioi 1980; Bostrom et al. 2004). Upon examining the specific environments of North Bay and South Bay, a subset of likely hypotheses includes grazing by black brant geese (*Branta bernicla nigricans* Lawrence 1846), light limitation, and the effects of ammonium limitation and ammonium toxicity.

The first hypothesis, grazing by black brant, is feasible because South Bay, where the shoot density is greatest, hosts 60% of the total black brant population (approximately 17,000 birds) from Nov - May of each year while brant seldom graze in North Bay (Henry 1980; Moore et al. 2004). Black brant obtain most of their diet from eelgrass and their grazing could lead to compensatory growth in the form of either an increased shoot density or leaf growth rate response (Cebrian et al. 1998; Moore et al. 2004). The second hypothesis, light limitation, is also feasible because the mean tidal elevation of the eelgrass beds in North Bay, where shoot densities are lower, appears to be deeper than the mean tidal elevation of the eelgrass beds in South Bay (S. Schlosser, pers. comm.). Also, more watersheds with their suspended sediment loads empty directly into North Bay than to South Bay and the water turbidity in North Bay has been measured by secchi disk to be greater than the water turbidity in South Bay (0.80 m vs. 1.10m; Pequegnat and Butler 1981). As both water depth and turbidity in the water column affects light attenuation, the eelgrass beds in North Bay could be expected, on average, to contend with a larger degree of light limitation.

I chose to focus my thesis on the third hypothesis, that the difference in shoot densities is caused by ambient nutrient concentrations despite the fact that seagrasses are generally adapted to low nitrogen conditions (Hemminga and Duarte 2000). Multiple studies have demonstrated nutrient limitation and others have shown that high nutrient concentrations can be toxic. Both nutrient limitation and nutrient toxicity may decrease *Z. marina* shoot density (Orth 1977; Harlin and Thorne-Miller 1981; Roberts et al. 1984; Udy and Dennison 1997; van Katwijk et al. 1997; Worm and Reusch 2000; Nixon et al. 2001; Brun et al. 2002; Touchette et al. 2003; Lee et al. 2004). Nutrient limitation also reduces canopy height, leaf width and leaf area (Short 1987). Nutrients that can be limiting to eelgrass include nitrogen, phosphorus and carbon (Moore and Wetzel 2000).

Regarding the marine environment, it is generally thought that nitrogen is limited world-wide due to the loss of fixed nitrogen back into the air as diatomic nitrogen, whereas phosphorus is only limiting in tropical regions due to the binding of phosphorus in the carbonate sediments (Smith 1984; Short 1987). However, several studies have detected phosphate limitation of eelgrass in temperate regions (Harlin and Thorne-Miller 1981; Udy and Dennison 1997; Bostrom et al. 2004). As photosynthetic organisms require both nitrogen and phosphate many researchers use the nitrogen/phosphorous (N/P) ratio as an indicator of nutrient limitation. *Z. marina* tissues contain an N/P ratio of 23:1 (Short 1983). Ambient environmental N/P ratios should ideally approximate the Redfield ratio (16:1) and ratios of < 5:1 are considered limiting for eelgrass (Thom and Albright 1990; Murray et al. 1992).

Unlike some terrestrial plants, *Z. marina* is able to use nitrogen in two forms, nitrate and ammonium. Both forms are present in the water column and the sediment. Water column ammonium and nitrate concentrations originate from anthropological sources such as agriculture, and natural processes such as nitrogen fixation or seasonal upwelling (Castro et al. 2003). Sediment that is anoxic produces ammonium through the decomposition of organic matter. Sediment that is aerobic produces nitrate through bacterial nitrification that converts ammonium to nitrate and through nitrogen fixation (Iiuzumi et al. 1982; McGlathery et al. 1998).

Eelgrass is able to take up nitrogen from the water column through the leaves and from the sediment through the roots (McRoy and Barsdate 1970; Thursby and Harlin 1982). It is estimated that over 50% of the uptake occurs through the roots although the exact ratio depends on the light and temperature environment (Zimmerman et al. 1987; Hansen et al. 2000). Ammonium is the preferred form of nitrogen for eelgrass, especially in the sediment because it can be used in metabolism directly via the GOGAT pathway (the formation of glutamine from glutamate and ammonium), whereas nitrate must be converted to ammonium prior to glutamine formation (McRoy and Barsdate 1970; Flindt et al. 1999; Moore and Wetzel 2000; Bostrom et al. 2004; Romero et al. 2006). Furthermore, ammonium is the most common form of nitrogen in the sediment of estuaries due to anoxic conditions in the sediment retarding the oxidation of ammonium to nitrate (Joye and Hollibaugh 1995; Hemminga and Duarte 2000; Touchette and Burkholder 2001).

It is not clear how much ammonium or nitrate saturates eelgrass growth rates although the Redfield ratio of 16:1 (N: P) provides some indication of the nitrogen and phosphate requirements for seagrasses. Concentrations of ammonium experimentally or analytically determined to saturate eelgrass growth differ by a factor of 10 (Table 1). Growth saturation concentrations of nitrate in the water column are also debated and are undetermined for the sediment.

Previous Humboldt Bay studies reported ammonium concentrations in the water column during an upwelling event ranging from 2.78 - 4.22 μM (Pequegnat and Butler 1981) and < 1 μM (Althaus et al. 1997). Nitrate ranged from 1.39 - 5.23 μM (Pequegnat and Butler 1981) and 2 - 12 μM (Althaus et al. 1997). These summer water concentrations of ammonium are insufficient to saturate eelgrass growth. The ammonium limitation present in the water column in the summer could be compensated for by adequate ammonium or nitrate concentrations in the sediment, or in the water column

Table 1. Ammonium and nitrate concentrations at which growth or uptake saturate or a toxic response occurs.

Parameter		Concentration (µM)	Source
Growth saturation	NH_4^+ sediment	10 - 30	Zimmerman et al. 1987
		100	Williams and Ruckelshaus 1993
		100	Dennison et al. 1987
	PO_4^{3-} sediment	5 - 15	Murray et al. 1992
	NO_3^- water	4*	Zimmerman et al. 1987
		8	Thom and Albright 1990
Uptake saturation	NH_4^+ roots	211	Thursby and Harlin 1982
	NH_4^+ leaves	15	Thursby and Harlin 1982
		>20	Iiuzumi and Hattori 1982
	NO_3^- leaves	>23	Iiuzumi and Hattori 1982
Toxicity	NH_4^+ sediment	>500*	Zimmerman et al. 1987
	NH_4^+ water	25	Van Katwijk et al. 1997
	NO_3^- sediment	200	Peralta et al. 2003
	NO_3^- water	35	Burkholder et al. 1992

* Correlative value

during the winter, but there are no studies on ammonium or nitrate concentrations in the sediment or in the water column of Humboldt Bay during winter months. Without complete information on the annual cycle of nitrogen concentrations in the water and sediment of Humboldt Bay, it is presently not possible to determine if nitrogen limitation is affecting eelgrass shoot densities in Humboldt Bay.

Ammonium limitation can decrease shoot density, but so can ammonium toxicity, which occurs if the eelgrass is also carbon and/or light limited (Peralta et al. 2003). Carbon limitation is common in the marine environment because carbon dioxide concentrations are relatively low in seawater and diffusion rates of carbon dioxide are ≈10,000 times slower in seawater than in the air (Hemminga and Duarte 2000). Ammonium is toxic to plant cells and must be immediately fixed into amino acids, a process which requires carbon skeletons. The carbon skeletons are provided by photosynthesis. Insufficient supplies of carbon dioxide or insufficient rates of photosynthesis allow toxic ammonium to build up in the plant tissues, leading to the death of the shoot (Harlin and Thorne-Miller 1981; Borum et al. 1989; Burkholder et al. 1992; van Katwijk et al. 1997).

The toxicity level of ammonium and nitrate is considered to be lower in the water column than in the sediment because eelgrass roots can regulate the uptake of nitrogen in the sediment by controlling the number of root hairs, while eelgrass leaves do not have any means for regulating uptake (McRoy and Barsdate 1970; Iizumi et al. 1982; Zimmerman et al. 1987; Peralta et al. 2003). Ammonium in the water column has been shown to cause death in eelgrass at levels of 25 µM whereas ammonium concentrations in sediment inhabited by eelgrass ranges from 50 - 500 µM without obvious toxic effect (Zimmerman et al. 1987; van Katwijk et al. 1997; Brun et al. 2002, Table 1). Nitrate in the water column has been shown to cause death of eelgrass shoots within 4-5 weeks at levels of 35 µM versus toxicity thresholds of 200 µM in the sediment (Burkholder et al. 1992; Peralta et al. 2003).

I decided to pursue the hypothesis that the difference in shoot density between North Bay and South Bay is caused by ambient nitrogen concentrations because North Bay and South Bay have several abiotic differences that allow an argument to be made for both nitrogen limitation and nitrogen toxicity. South Bay is much closer to the entrance of Humboldt Bay and its flushing time, or the time it takes to completely replace all the water, is thought to be much shorter than the flushing time of North Bay, although the exact time is debated (Barnhart et al. 1992). A shorter residence time would give phytoplankton in the water less time to absorb the ammonium and nitrate in the water column before they reach the eelgrass (Twilley et al. 1985; Touchette and Burkholder 2001; Touchette et al. 2003). Finally, short flushing times bring in more upwelled nitrogen in the form of nitrate from the open ocean, refreshing the average nitrate concentration on a daily basis. All of these environmental factors in combination with the low nitrogen adaptations in eelgrass reduce the possibility of nitrogen limitation for the eelgrass in South Bay. Short flushing times also reduce the possibility of nitrogen toxicity by removing ammonium and nitrate released from the sediment.

South Bay interchanges a greater volume of water with the ocean each day than does North Bay, but it has fewer freshwater sources to bring in ammonium and nitrate from runoff during the rainy season. The only sizeable source of freshwater input to South Bay is Salmon Creek, whereas North Bay receives appreciable runoff from Freshwater/Eureka Slough, Washington Slough, Jacoby Creek, Janes Creek, Jolly Giant Creek and McDaniel Slough. It was reasonable to assume, therefore, that South Bay would receive more nitrogen in summer/fall from the ocean due to seasonal upwelling and so have sufficient light and nitrogen at the same time, while North Bay would receive more nitrogen in the winter/spring from runoff when light is limiting and suffer from nitrogen limitation in the summer when there is sufficient light.

My first thesis objective was to describe some of the ambient conditions in the water column and sediment of Humboldt Bay that are relevant to eelgrass and could be affecting its shoot density, productivity, and biomass. My second objective was to experimentally determine if the variation in shoot density between the two populations of eelgrass in Humboldt Bay is caused by either ammonium limitation or ammonium toxicity. My third objective was to determine if ammonium levels are affecting other aspects of eelgrass morphology.

To address the first objective, I took monthly measurements of water column salinity, temperature and nutrient concentrations around Humboldt Bay and compiled measurements of other environmental factors from both North and South bays. For the last two objectives, I studied the effect of experimental *in situ* ammonium loading on eelgrass density and eelgrass morphology to test the hypotheses that either North Bay or South Bay was ammonium limited or ammonium overloaded. I predicted that ammonium enrichment would lessen shoot density variation and morphological variation between the two regions.

METHODS

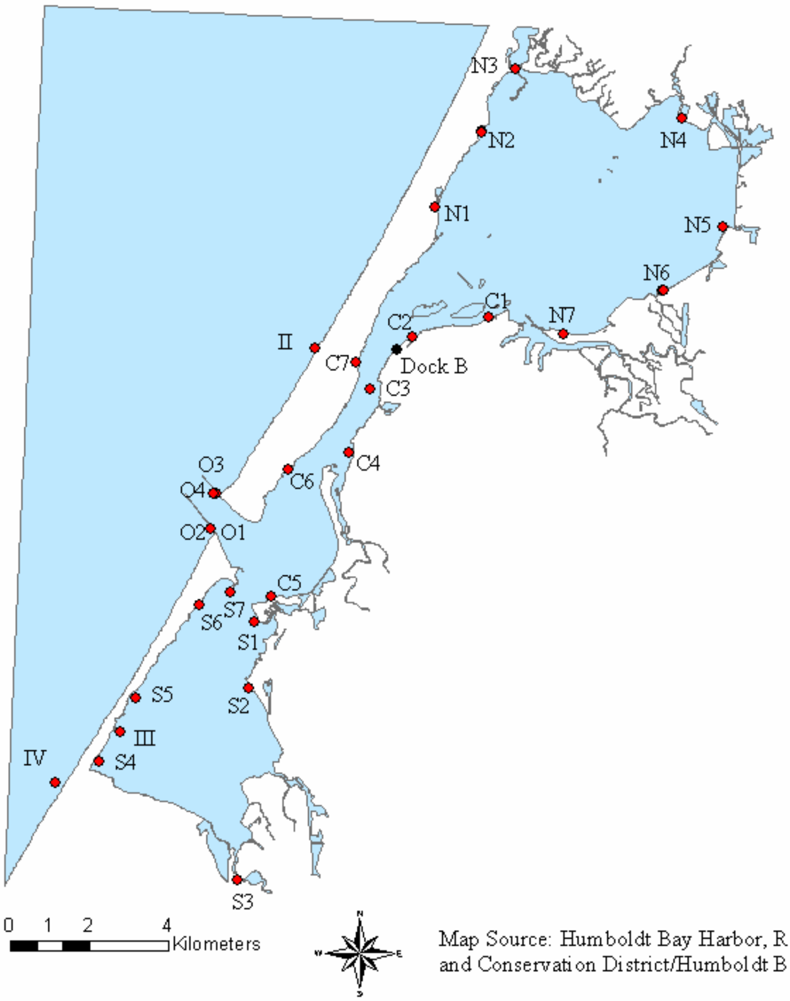
Study Site

Humboldt Bay, California (40° 44’ 59”N, 124° 12’ 34”W; Figure 2) is a protected embayment with a single entrance maintained by rock jetties. It has mixed diurnal tides, a surface area at mean high water of 6240 ha and a surface area at mean low water of 2800 ha (Barnhart et al. 1992). The water column is well mixed and seldom stratifies (Costa 1982). Much of the intertidal of Humboldt Bay is comprised of mudflats with large eelgrass meadows totaling 1890 ha of eelgrass, with 1037 ha in North Bay and 801 ha in South Bay (Schlosser et al. *accepted*). These meadows are exposed during the lowest spring tides each month. The bay is divided into three regions: North Bay, that is farthest from the entrance, Central Bay (the central area of Humboldt Bay which connects North Bay to the entrance), and South Bay. North Bay contains several commercial oyster and clam mariculture operations whereas South Bay is the site of recreational clamming during low tides.

Ambient Environmental Conditions

Water column nutrient concentrations

Water samples were collected in polyethylene vials from the surface of the water column at seven locations each in North Bay, Central Bay, and South Bay and four ocean locations to address the lack of knowledge regarding the ambient nutrient concentrations in Humboldt Bay (n = 25, Figure 2). Sampling was done once each month on the ebb tide. Water samples were placed on ice during transport to the lab and analyzed for ammonium, nitrate/nitrite (the nitrite fraction was negligible and will not be mentioned



Map Source: Humboldt Bay Harbor, Recreation and Conservation District/Humboldt Bay Atlas

Figure 2. Map of Humboldt Bay, California showing the location of the 25 water sampling sites (O1 - O4 = ocean sites, C1 - C7 = Central Bay sites, N1 - N7 = North Bay sites, and S1 - S7 = South Bay sites), the CICORE water quality data logger at Dock B, and the 12 hypersalinity sampling sites (N2, N4, N6, N7, S2, III, S5, S6, II, O2, O3, and IV).

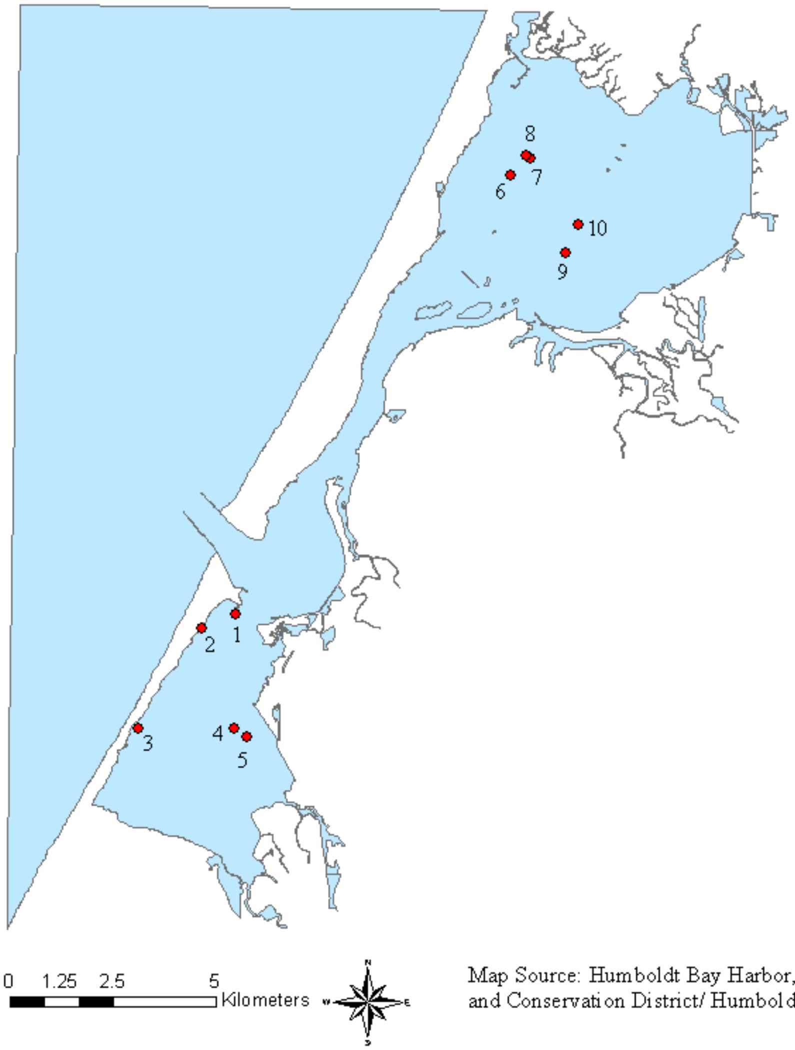
again) and phosphate concentrations, using colorimetric methods described by Solorzano (1969) for ammonium, Jones (1984) for nitrate, and Murphy and Riley (1962) for phosphate. Ammonium analysis was done within three hours of sampling. Nitrate and phosphate analyses were done within three weeks of sampling and water samples were stored at 4° C until analysis was completed. The temperature of the surface of the water was measured at the time of sampling. Only surface temperatures were taken, but Humboldt Bay is well mixed and seldom stratifies (Costa 1982). Well-mixed basins generally display the same water temperature throughout the water column (Hearn and Sidhu 2003). Water sampling was done monthly for a full year (March 2005-February 2006) so that temporal nutrient, temperature and salinity curves could be constructed for the entire bay. Total nitrogen/phosphate (N/P) ratios for the water column were calculated as well as another indicator of nutrient limitation (Smith 1984).

Sediment temperature

The temperature of the mudflats was monitored from April 2005 to August 2005 by using four iButton® temperature loggers at sites 3 and 4 in South Bay and sites 7 and 9 in North Bay (Figure 3). The loggers were set at mud level and took temperature readings every 1.5 hours. Water depth data from the CICORE data logger at Dock B (Figure 3) were used to distinguish emergent from submergent temperatures.

Salinity

The salinity of each water sample in parts per thousand (ppt) was measured in the lab using a refractometer. Precipitation data were obtained from the University of



Map Source: Humboldt Bay Harbor, Recreation and Conservation District/ Humboldt Bay Atlas

Figure 3. Map of Humboldt Bay, California, showing the location of the ten eelgrass sampling sites. Sites 1-5 are in South Bay and sites 6-10 are in North Bay. Mudflat temperature loggers were located at sites 3, 4, 7, and 9.

California Sea Grant weather station courtesy of S. Schlosser and from Salmon Forever-Watershed Watch courtesy of C. Fenton. Average freshwater input data were acquired from Humboldt Bay researchers (Jacoby Creek, R. Klein, hydrologist, unpublished data; Elk River and Freshwater Creek, C. Fenton, unpublished data). Hypersalinity, which is defined by Largier et al. (1997) as an increase in salinity larger than the standard deviation of the salinity flux of local ocean water, can occur when freshwater input and new seawater input from flushing does not exceed evaporation. Humboldt Bay, with a relatively small watershed, a Mediterranean climate and a narrow basin would be especially prone to hypersaline conditions (Largier et al. 1997). The possibility of Humboldt Bay becoming hypersaline in late summer was tested by taking water samples at four locations each in North Bay, South Bay and the ocean (n = 12, Figure 2) each week from August 13th, 2005 through October 2nd, 2005, when precipitation made further sampling unnecessary.

Sediment grain size

The mud from three of the core samples taken from each site during the baseline (T_0) sampling for the experimental part of this study (n = 30) was analyzed for sediment grain size by calculating the percent of sand, clay and silt, through the hydrometer analysis method described by Liu and Evett (2003) and by calculating the mean weighted diameter (MWD) using the method proposed by Bathke et al. (1991). This was done by measuring the amount, by weight, of grains of 11 different diameters (diameter determined by hydrometer analysis) and using those values in the formula:

$$d_a = \frac{\sum^{N}_{i=1} w_i d_i}{\sum^{N}_{i=1} w_i}$$

where w_i is the weight of the particles of diameter d_i, and N is the number of size intervals used. The result is a single value for each plot that allows sizes to be compared at different sites using inferential statistics. Percentage of organic material in the sediment samples taken during the baseline (T_0) sampling was analyzed as ash free weight following combustion at 375 ºC (Short 1983).

Manipulative Experiment

The hypothesis that the shoot density variation is due to ammonium limitation or ammonium toxicity was addressed by a randomized block design. Eight treatments (n = 5/treatment/ bay) were applied; fertilizing the sediment, fertilizing the water column, and in each case fertilizing with 3 different levels of ammonium (low water treatment = 51g NH_4^+, medium water treatment = 105g NH_4^+ , high water treatment = 210g NH_4^+ , low sediment treatment = 51g NH_4^+, and 8.5g PO_4^{3-}, medium sediment treatment = 210g NH_4^+, and 35g PO_4^{3-}, and high sediment treatment = 510g NH_4^+, and 85g PO_4^{3-}). The last two treatments were sediment and water column controls. Ammonium was used because it is preferred over nitrate by eelgrass (Iizumi et al. 1982; Short 1983; Zimmerman et al. 1987).

The fertilizer applied to the sediment contained 30% ammonium, and 5% phosphate as well as small amounts of potassium, sulfur, iron, copper, manganese and zinc. The fertilizer applied to the water column contained 21% ammonium, 24% sulfur and no phosphate. Lower levels of nutrients were added to the water column than to the sediment because ammonium toxicity is believed to occur at lower ammonium concentrations in the water (25 µM vs. >500 µM, van Katwijk et al. 1997; Zimmerman et al. 1987).

Five study sites were set up in North Bay and five in South Bay (Figure 3). Because *Z. marina* is a clonal plant, these sites were scattered across each region to avoid pseudoreplication due to physiological integration (Bostrom et al. 2004). Scattering randomly picked sites also allowed me to report on the entire region. In North Bay all sites were placed outside of mariculture operations to avoid the fertilizing effects of bivalve feces, and in South Bay all sites were placed outside of the most heavily utilized black brant grazing areas to avoid the fertilizing effects of brant feces.

Each of the ten sites, or blocks, contained one replicate of all eight treatments, randomly placed. The treatment plots within a block (Figure 4) were 1 m^2 and separated by at least 10 m to avoid cross-contamination (Harlin and Thorne-Miller 1981; Williams and Ruckelshaus 1993; Invers et al. 2004). All sites were placed at least 10 m from the nearest edge of the bed to avoid edge or channel effects (Nelson and Waaland 1997; Nelson and Lee 2001) and were placed in a straight line parallel to the nearest channel so the distance from the channel was a constant across treatments and sites. One pathway was used in each site to minimize disturbance (Williams and Ruckelshaus 1993).

The sediment was fertilized with slow-release fertilizer pellets pushed into the mud (Orth 1977; van Lent et al. 1995; Udy and Dennison 1997; Terrados et al. 1999;

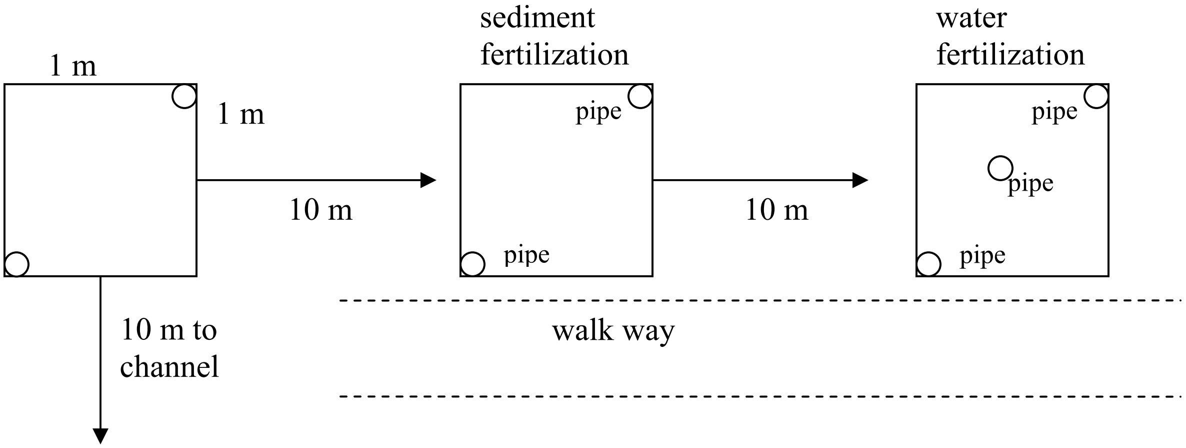


Figure 2. The arrangement of treatment plots within one block. The plots were marked by two PVC pipes at opposing corners and water fertilization and water control plots had a third pipe in the middle to hold the plaster block above the substrate.

Worm and Reusch 2000). Slow-release fertilizer was used because it has been shown that steady application of a lower level of fertilizer is more realistic than large, short pulses of nutrients (Twilley et al. 1985; Moore and Wetzel 2000; Touchette and Burkholder 2001; Brun et al. 2002; Touchette et al. 2003). The sediment control plot was “massaged” in a similar fashion without the addition of fertilizer pellets. The water column was enriched with fast-dissolving fertilizer mixed into plaster blocks so the release would be slow and steady as the plaster dissolved over several months (modified from Invers et al. 2004 by the substitution of larger plaster blocks uncoated by wax). Unfertilized plaster cubes were applied to the water column control to test for shading effects from the turbidity added by the plaster dissolving into the water column.

Starting Conditions

The sites were set up and baseline data (T_0) were taken in February 2005. A 0.1m² quadrat (in South Bay) or a 0.25m² quadrat (in North Bay) was randomly placed within each plot and all shoots within the quadrat were counted and converted to no. shoots/m² for shoot density (Orth 1977; Harlin and Thorne-Miller 1981). Quadrat sizes differed to lessen the amount of lab work while still adequately sampling the shoot density in each bay (Keiser 2004; Schlosser et al. *accepted*). The same quadrat was placed halfway between plots for shoot morphology, above-ground biomass, epiphyte loads, and grazer loads (n =4/ site). All eelgrass shoots within the quadrat were removed at mud level and placed in ziplock bags for transportation to a refrigerator. A core (10 cm wide and 30 cm deep) was taken at the center of each quadrat in order to measure below-ground biomass, rhizome diameter, sediment grain size, and percent organic matter in the sediment (Short 1983).

Eelgrass was refrigerated immediately after sampling and underwent laboratory analysis within a week of collection. Epiphytes were scraped off all eelgrass leaves within each quadrat with a razor blade, dried, weighed, and converted to g/shoot. Since grazers have an effect on the amount of epiphytes present, all epiphyte grazers adhering to eelgrass leaves were counted and identified to the lowest possible taxon (Borum 1985; Williams and Ruckelshaus 1993; Duarte 1995; Lin et al. 1996; Moore and Wetzel 2000; Schanz et al. 2002).

Response variables other than shoot density were also assessed since they might respond to the ammonium treatments and ultimately affect shoot density. These included measurements of rhizome diameters to determine the relative amount of starch stored in the rhizome (Wear et al. 1999). Rhizomes were then dried, weighed, and the dry weight converted to kg/m^2 for below-ground biomass. The length and width of all leaves from ten shoots in each quadrat were also measured (Short 1983). Leaf length, width and no. of leaves/shoot were used to calculate leaf area index (LAI; mean one-sided leaf area / m^2 x no. shoots / m^2). Canopy height was calculated as the mean length (mm) of the ten measured shoots in each quadrat. The eelgrass shoots were dried, weighed and the dry weight converted to kg/m^2 for above-ground biomass.

Time 1 - Time 3 Sampling

Treatments were applied immediately after setting up the sites. The study then followed a repeated measures design (Worm and Reusch 2000). Non-destructive sampling for shoot density as well as leaf length and width took place at re-randomized locations within each plot after two months (T_1, April 2005), five months (T_2, July 2005), and the final destructive sampling took place after six months (T_3, August 2005). Sampling at T_1 and T_2 consisted of counting shoots within the quadrat and measuring the length and width of the longest leaf of ten shoots. Sampling at T_3 repeated T_0 sampling procedures except destructive sampling took place within each plot (n = 8/site).

A sediment sample of 35-80g was taken from the high sediment treatment plot at each site to test the effectiveness of the fertilizer application at raising the sediment ammonium and phosphate concentrations and two more samples were taken between plots at each site to test ambient ammonium, nitrate and phosphate concentrations. The pore water was extracted following the method described by van Katwijk et al. (1999) and the pore water was then analyzed for ammonium, nitrate/nitrite and phosphate using colorimetric methods described by Solorzano (1969) for ammonium, Jones (1984) for nitrate, and Murphy and Riley (1962) for phosphate. Total-nitrogen/phosphorous (N/P) ratios were also calculated from the sediment nutrient concentrations.

Leaf Area Index

The lengths and widths of all leaves in each shoot were only measured at T_0 and T_3. A single-leaf LAI was calculated from T_0 and T_3 separately, utilizing only the length and width of the longest leaf in each measured shoot to ascertain if an LAI could be calculated for T_1 and T_2 when only the length and width of the longest leaf in each shoot was measured. The single-leaf LAI from T_0 and T_3 was then compared to the full LAI from T_0 and T_3, using a two-sample t-test. The difference between the two was significant at both T_0 and T_3 (T = -4.0514, P = 0.0000 for T_0 and T = -7.0763, p = 0.0000 for T_3; all variables log transformed for normality). However, when the single-leaf LAI was transformed by multiplying each value by 3, the difference was no longer significant (T= -0.8382, p = 0.4045 for T_0 and T = -1.1445, p = 0.2542 for T_3; T_0 variables log transformed for normality). As this was true of both winter (T_0) LAI and summer (T_3) LAI, transformed LAI for T_1 and T_2 were used in all statistical analyses.

Statistical Analysis

Inferential statistics were performed in Number Cruncher Statistical System (NCSS; Hintze 2001) software. Differences in ambient conditions between North Bay and South Bay were analyzed using a two-sample t-test ($\alpha = 0.05$). Normality assumptions were checked with skewness normality, kurtosis and omnibus normality, and a modified-Levene equal-variance test was also used to check for unequal variances. Differences in environmental variable means between regions (North Bay, Central Bay, and South Bay) and months were analyzed using a two-way ANOVA ($\alpha = 0.05$).

Starting conditions for the experimental study were analyzed using a two-sample t-test ($\alpha = 0.05$). Normality assumptions were checked with skewness normality, kurtosis and omnibus normality, and a modified-Levene equal-variance test was also used to check for unequal variances. Treatment responses to the application of fertilizer were tested by using plot data to calculate four time periods (period 1 =T_1-T_0, period 2 =T_2-T_1, period 3 = T_3-T_2, and period 4 =T_3-T_0). A one-way ANOVA ($\alpha = 0.05$) was run on each time period in each region to determine if there were treatment effects on the change in shoot density, or change in shoot morphology, with treatment as the only factor. Assumptions were checked as previously described and multiple comparisons were made with the Tukey-Kramer test. Only period 4 proved to be significant and the other three periods were not analyzed further. A multiple regression was run on period 4 shoot density changes to pinpoint other significant predictors of shoot density besides the treatments. Independent variables for the multiple regression analysis were selected by running the NCSS procedures all possible regression, multivariate variable selection, and stepwise regression.

RESULTS

Ambient Environmental Conditions

Water column nutrient concentrations

Precipitation from March, 2005 through February 2006 ranged from 0.13 to 32.31 cm per month with the highest values in winter and spring months and lowest values in the summer (Figure 5). Ebb tide nitrate concentrations in the rainy season were high across all regions (Table 2) North Bay nitrate concentrations were high at Washington Slough (site N5). In Central Bay, nitrate concentrations were high at Freshwater/Eureka Slough (site C1) and the Elk River (site C4). In South Bay nitrate concentrations were high at Hookton Slough (Salmon Creek; site S3; Figure 6a). All of these sites were sources of freshwater input into the bay. However, nitrate levels were also relatively high in the ocean. There were no significant differences in nitrate concentrations between the three regions of Humboldt Bay and the ocean (Table 3). Ebb tide nitrate concentrations were very low in North Bay and South Bay and very high in Central Bay during the dry season (Figure 6b). Nitrate levels in North Bay and South Bay were significantly lower than Central Bay and the ocean but not significantly different from each other (Table 3).

Ebb tide Ammonium concentrations were highly variable during the rainy season (Table 2). Large pulses of ammonium were loaded into Humboldt Bay, but there did not appear to be any consistent source for those pulses (Figure 7a). During the dry season, ebb tide ammonium levels dropped across all regions and only a few relatively small pulses were detected (Figure 7b). Again, there was no consistent source for those

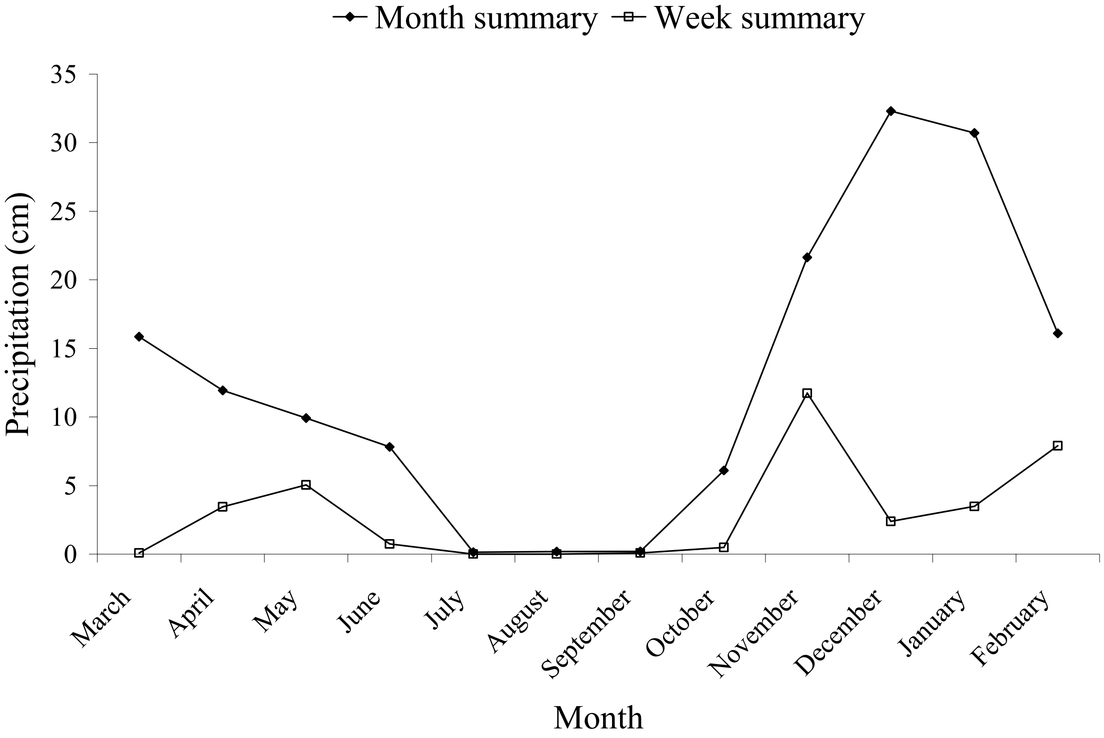


Figure 3. Summaries of precipitation (cm) for each month of water sampling and summaries of precipitation the week prior to water quality sampling from March 2005 – February 2006 (Monthly data source: California Department of Water Resources; weekly data source: S. Schlosser, Mar – Dec 2005; C. Fenton, Jan – Feb 2006).

Table 2. Rainy season and dry season water column concentrations of nitrate, ammonium and phosphate for all regions. Rainy season = March - May 2005 and November 2005 - February 2006, dry season = June - October 2005 (all values are means ± 1 SE).

Season	Nutrient	North Bay concentration (μM)	Central Bay concentration (μM)	South Bay concentration (μM)	Ocean concentration (μM)
Rainy	Nitrate	7.645 ± 0.811	8.908 ± 0.538	8.034 ± 0.718	8.918 ± 0.766
	Ammonium	6.511 ± 0.944	5.820 ± 1.282	8.093 ± 1.360	6.359 ±1.837
	Phosphate	3.187 ± 0.233	2.503 ± 0.239	3.175 ± 0.281	2.043 ± 0.157
Dry	Nitrate	1.720 ± 0.229	9.746 ± 0.786	2.743 ± 0.502	18.01 ± 0.971
	Ammonium	2.731 ± 0.624	2.384 ± 0.253	2.007 ± 0.405	3.110 ± 0.872
	Phosphate	3.759 ± 0.403	3.291 ± 0.218	3.634 ± 0.900	3.526 ± 0.321

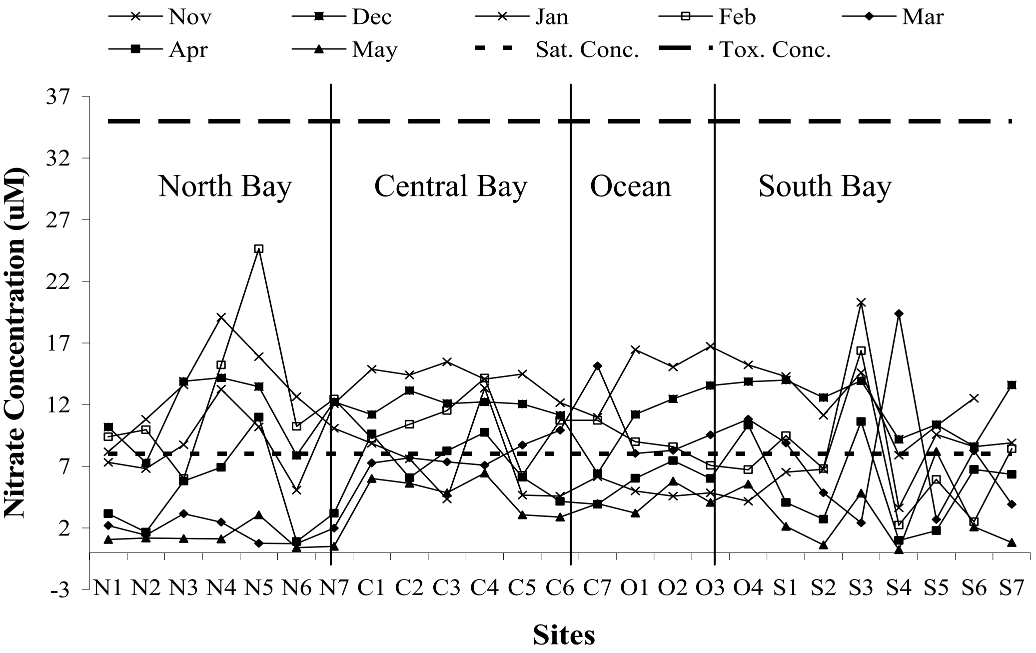


Figure 4a. Ebb tide ambient nitrate concentrations in the water column for the rainy season (winter and spring). Sat. Conc. = saturation concentration, Tox. Conc. = toxic concentration.

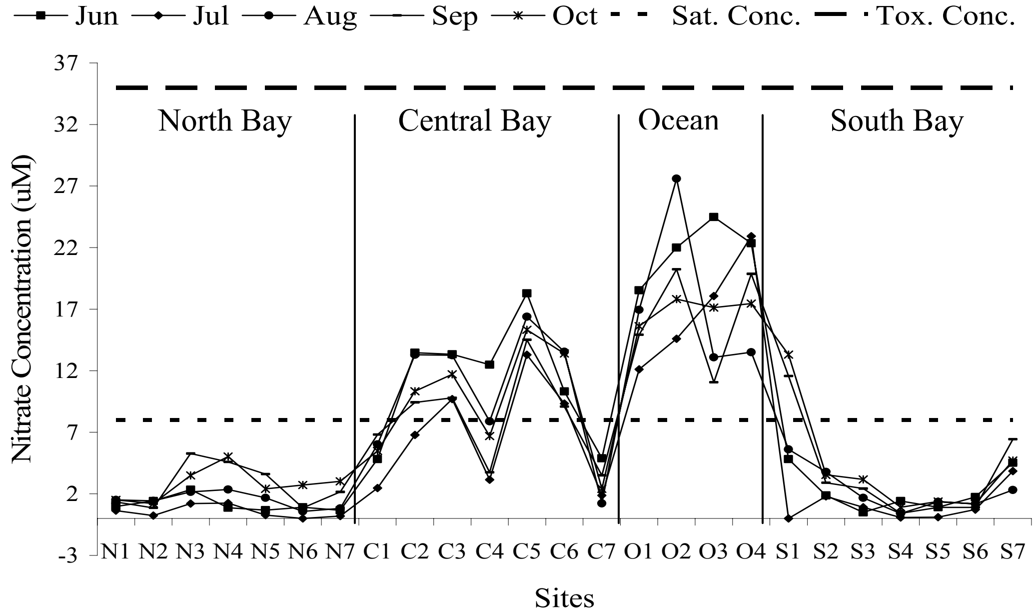


Figure 6b. Ebb tide ambient nitrate concentrations in the water column for the dry season (summer and fall). Sat. Conc. = saturation concentration, Tox. Conc. = toxic concentration.

Table 3. Two-way ANOVA results for ambient environmental factors (α = 0.05). Region = North Bay, South Bay, Central Bay and the ocean, time in the rainy season = March, April, May, November, December 2005, January, and February 2006, Time in the dry season = June – October 2005. Similar groups are underlined in the Tukey-Kramer column, numbers = months, NB = North Bay, SB = South Bay, CB = Central Bay, O = the ocean.

Factor		P	Tukey-Kramer
Nitrate in the water	Region	0.1115	
rainy season	Time	0.0000	11, 12, 1, 2, 3, 4, 5
	Interaction	0.0000	
Nitrate in the water	Region	0.0000	NB, SB, CB, O
dry season	Time	0.1016	
	Interaction	0.4456	
Ammonium in the water	Region	0.4448	
rainy season	Time	0.0000	11, 12, 1, 2, 5, 3, 4
	Interaction	0.3362	
Ammonium in the water	Region	0.4447	
dry season	Time	0.0012	7, 6, 8, 10, 9
	Interaction	0.0530	
Phosphate in the water	Region	0.0008	NB, SB, CB, O
rainy season	Time	0.0000	11, 12, 3, 5, 1, 2, 4
	Interaction	0.8215	
Phosphate in the water	Region	0.8961	
dry season	Time	0.0227	6, 7, 9, 10, 8
	Interaction	0.9994	
N/P ratio in the water	Region	0.0180	NB, SB, CB, O
rainy season	Time	0.0000	11, 12, 1, 5, 3, 2, 4
	Interaction	0.0060	

Table 3. *Continued*

Factor		P	Tukey-Kramer
N/P ratio in the water	Region	0.0000	NB, SB, CB, O
dry season	Time	0.0000	6, 7, 9, 10, 8
	Interaction	0.0000	
Water temperature	Region	0.000	NB, SB, CB, O
rainy season	Time	0.0000	12, 1, 2, 3, 11, 4, 5
	Interaction	0.0000	
Water temperature	Region	0.0000	NB, SB, CB, O
dry season	Time	0.0000	7, 8, 6, 10, 9
	Interaction	0.0000	
Salinity	Region	0.0000	NB, SB, CB, O
rainy season	Time	0.0000	3, 11, 12, 5, 1, 2, 4
	Interaction	0.3474	
Salinity	Region	0.3409	
dry season	Time	0.0037	8, 6, 9, 10
	Interaction	0.9588	

pulses. Ammonium levels were not significantly different between regions in the rainy or dry season (Table 3).

Ebb tide phosphate concentrations showed little variation across region, especially in the dry season (Table 2). Small amounts of loading were detected during the rainy season in North Bay at the Arcata Marsh (site N4), in Central Bay at the Elk River (site C4) and in South Bay at the foot of Table Bluff (site S4; Figure 8a). Phosphate concentrations were significantly higher in North Bay and South Bay than in the ocean but they were not significantly different from each other (Table 3). During the dry season, ebb tide phosphate concentrations increased but did not differ significantly between regions. Site S4 at the foot of Table Bluff in South Bay was a strong source for phosphate loading (Figure 8b).

The total-nitrogen/phosphorous (N/P) ratio in the water column ranged from 2.54 to 28.79 in the ocean and 0.00 to 29.66 in the bay. North Bay exhibited a significantly lower N/P ratio than the ocean during the rainy season (Table 3) but the interaction term was significant due to a high peak in N/P values in April that coincided with high runoff (Figure 9). During the dry season North Bay and South Bay displayed significantly lower N/P values than the ocean, but again the interaction term was significant due to another N/P peak in July and August that coincided with upwelling.

Water temperature

Ebb tide water surface temperatures ranged from 9.8 to 16.4 ºC in the ocean, and 6.0 to 24.3 ºC in the bay. In the spring and fall bay temperatures were similar to ocean

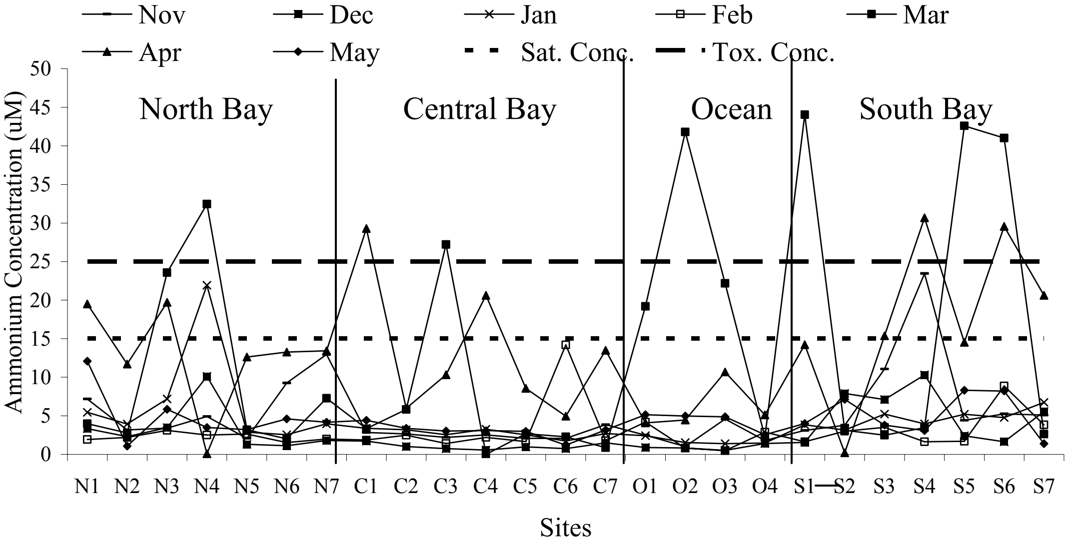


Figure 7a. Ebb tide ambient ammonium concentrations in the water column for the rainy season (winter and spring). Sat. Conc. = saturation concentration, Tox. Conc. = toxic concentration.

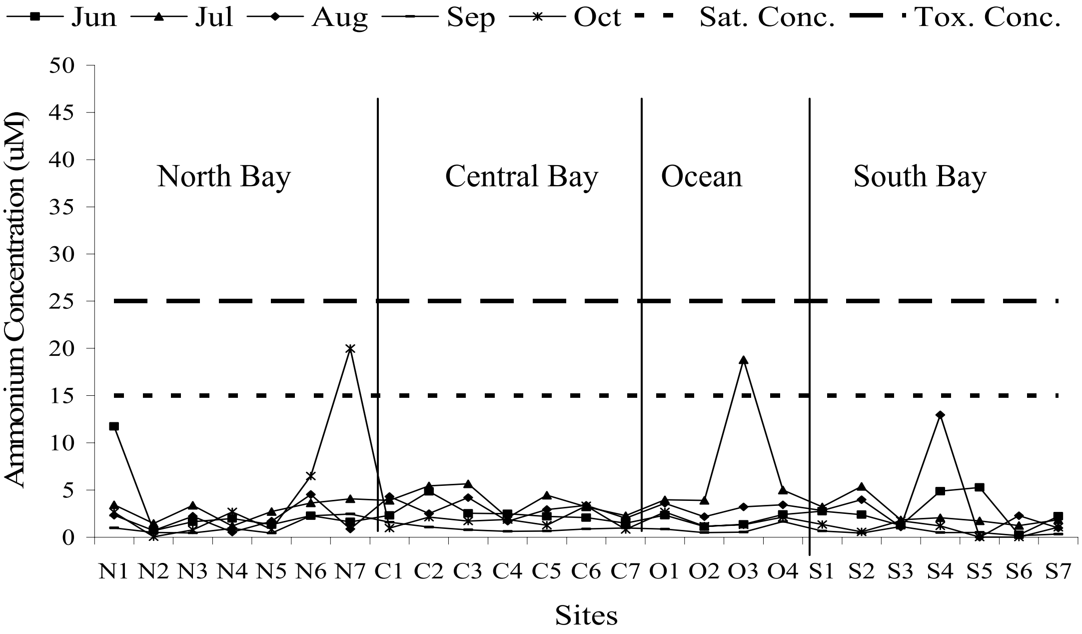


Figure 7b. Ebb tide ambient ammonium concentrations in the water column for the dry season (summer and fall). Sat. Conc. = saturation concentration, Tox. Conc. = toxic concentration.

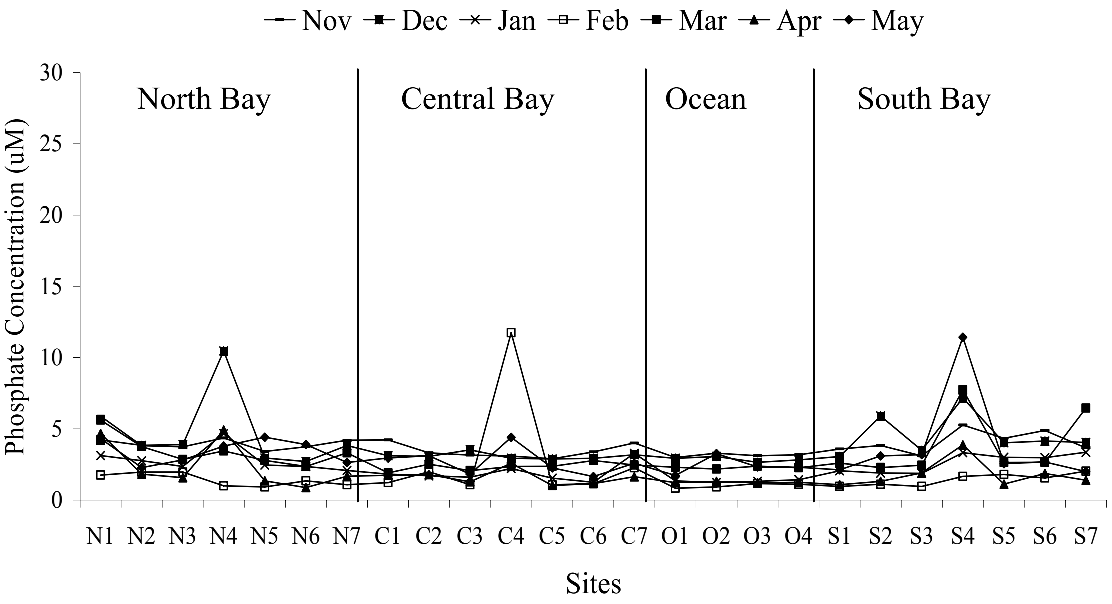


Figure 8a. Ebb tide ambient phosphate concentrations in the water column for the rainy season (winter and spring). Saturation and toxic concentrations are unknown.

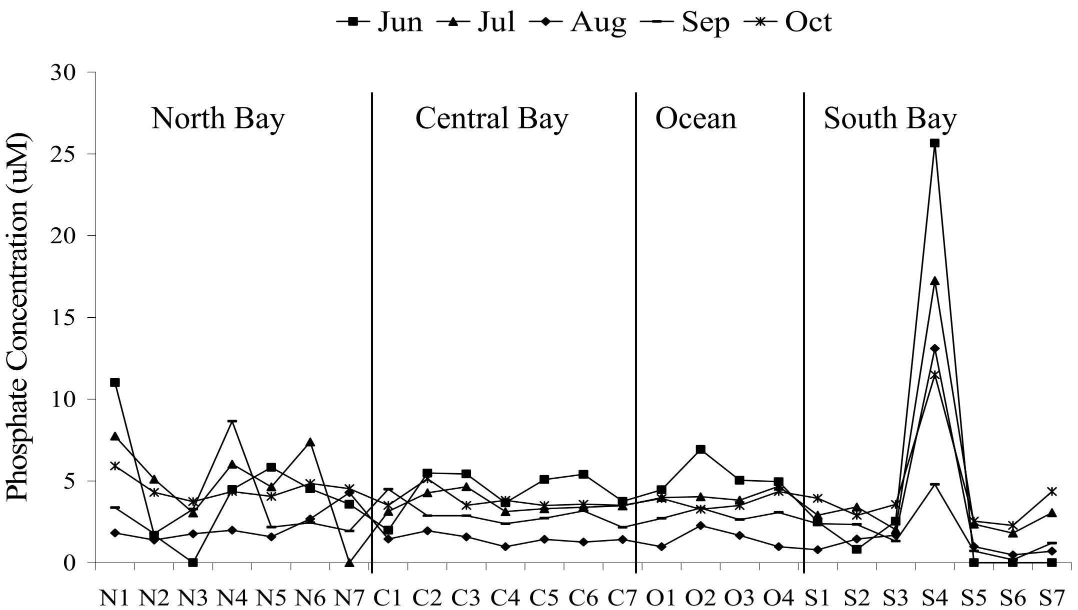


Figure 8b. Ebb tide ambient phosphate concentrations in the water column for the dry season (summer and fall). Saturation and toxic concentrations are unknown.

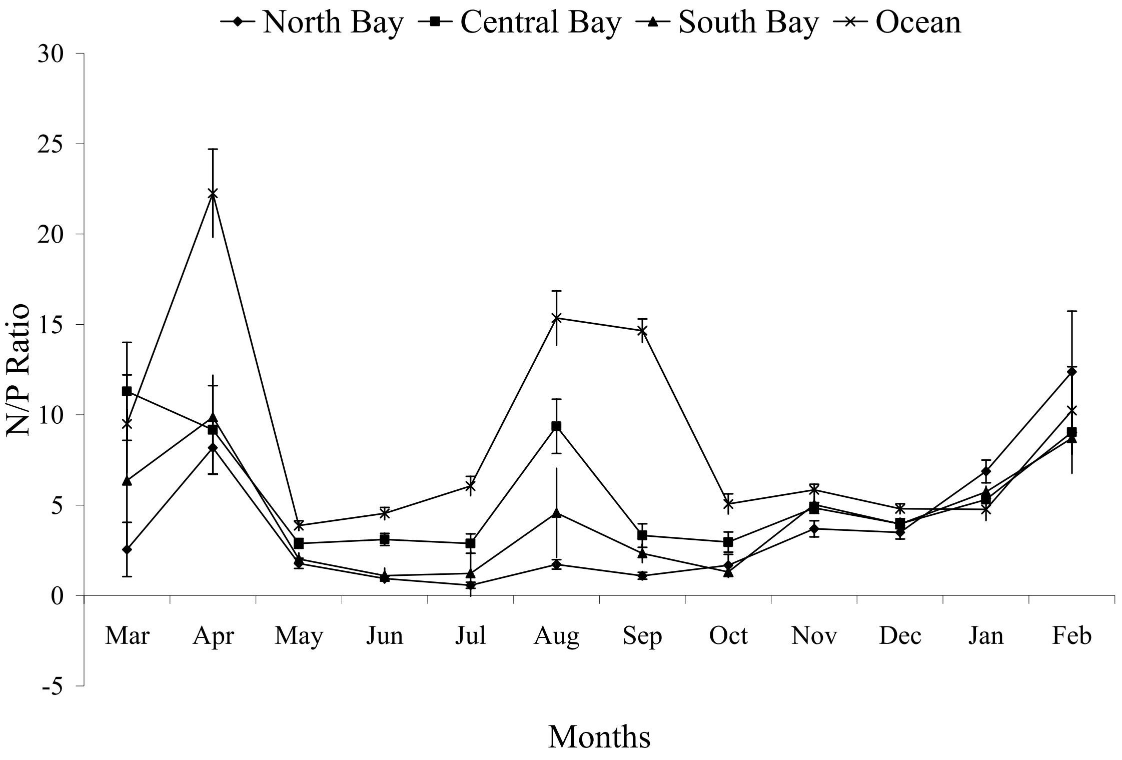


Figure 9. Ebb tide N/P ratio in the water column across regions. Error bars are ± 1 SE.

temperatures, whereas in the winter, bay temperatures decreased with distance from the entrance (Figure 10a) and in the summer bay temperatures increased with distance from the entrance (Figure 10b). North Bay had significantly colder water temperatures than South Bay in both the rainy and dry seasons (Table 3). In the rainy season there was a significant interaction term due to March water temperatures being similar across regions and in the dry season there was a significant interaction term because August was the only month in which water temperatures were warmer in North Bay than South Bay.

Emergent/Submergent eelgrass temperatures

The iButton® temperature logger at site 9 in North Bay was damaged in the field, and could not be downloaded so there was no site replication of temperatures within the eelgrass bed of North Bay. The air and water temperature on the mudflat surface averaged 16.8 ± 1.6 ºC at the single site in North Bay (Figure 11a) and 15.0 ± 1.6 ºC averaged across two sites in South Bay (Figure 11b), but South Bay mudflat surface temperatures covered a wider range (10.0 to 25.0 ºC, versus 12.5 to 22.0 ºC, respectively). Mudflat surface temperatures were significantly warmer in North Bay than South Bay (Table 4) and temperatures fluctuated on a daily basis in conjunction with the water depth as it fluctuated with the daily tide cycle in both North Bay (Figure 12a) and South Bay (Figure 12b).

Salinity

The average annual input of freshwater runoff was much higher in North Bay (Jacoby Creek average annual flow = 2.4 X 10^7 m^3, R. Klein, unpublished data;

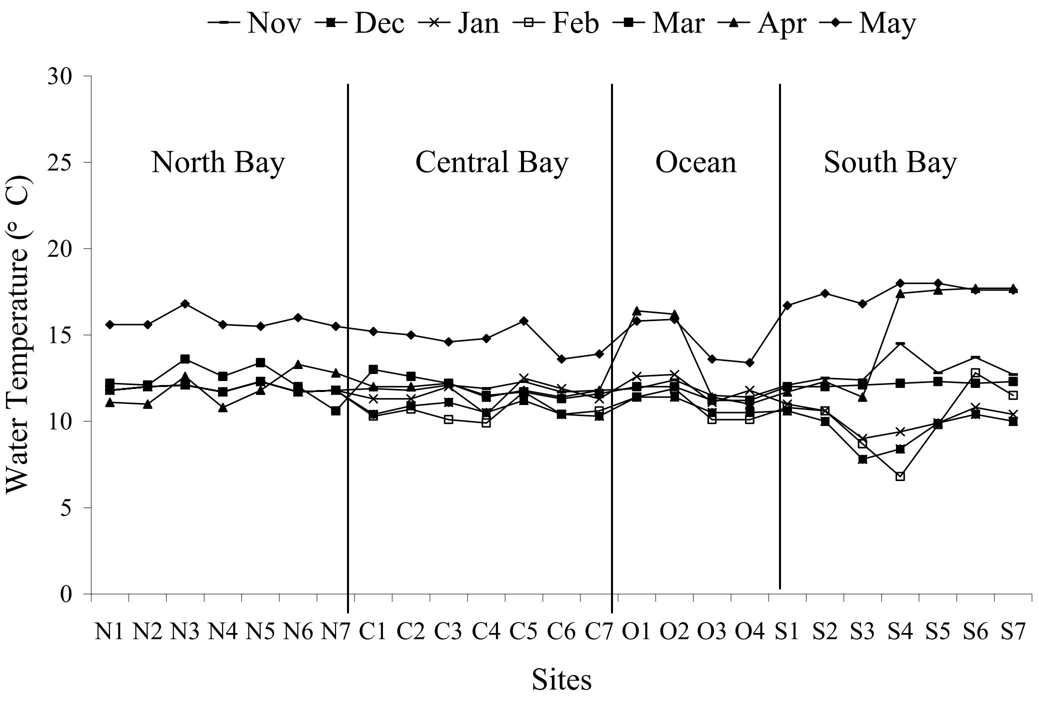


Figure 10a. Ebb tide water temperatures during the rainy season (winter and spring).

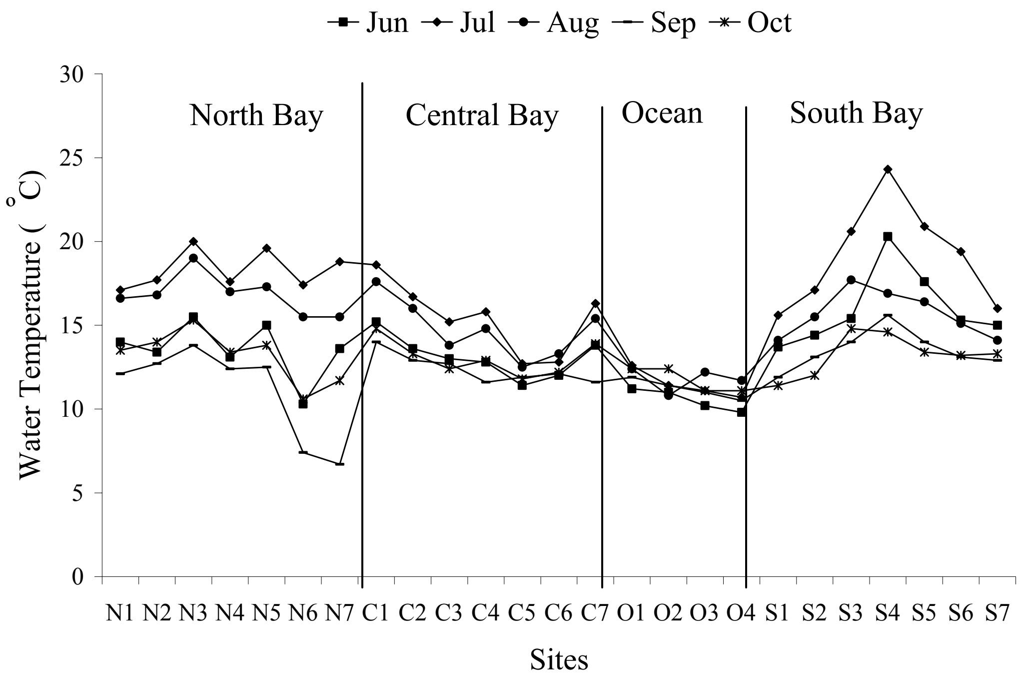


Figure 10b. Ebb tide water temperatures during the dry season (summer and fall).

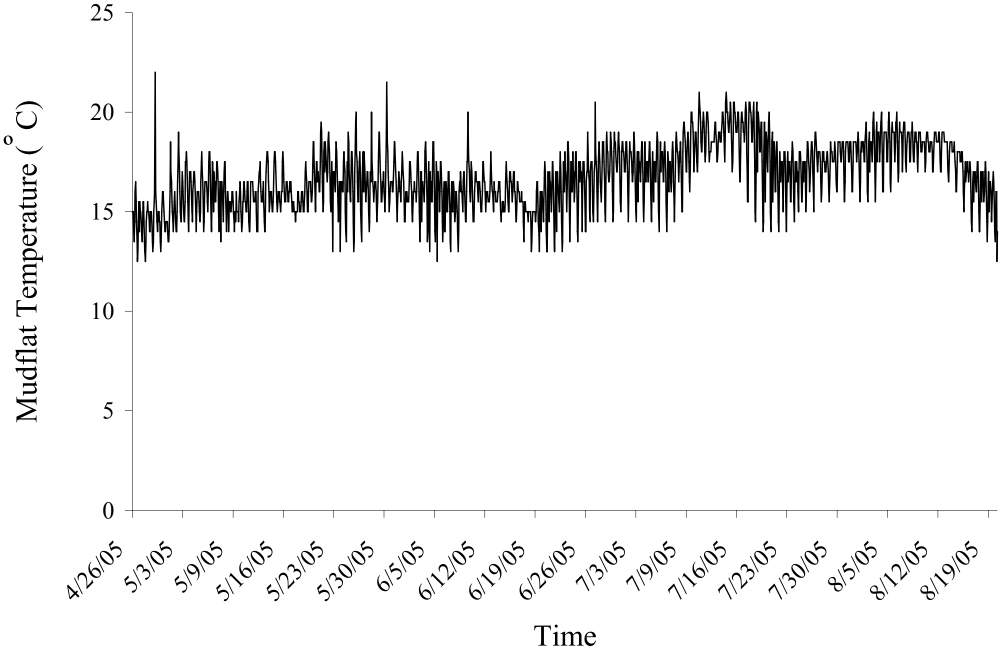


Figure 11a.Emergent/submergent temperatures within the eelgrass canopy in North Bay (site 7) from April 2005 to August 2005. Measurements were taken every 1.5 hours.

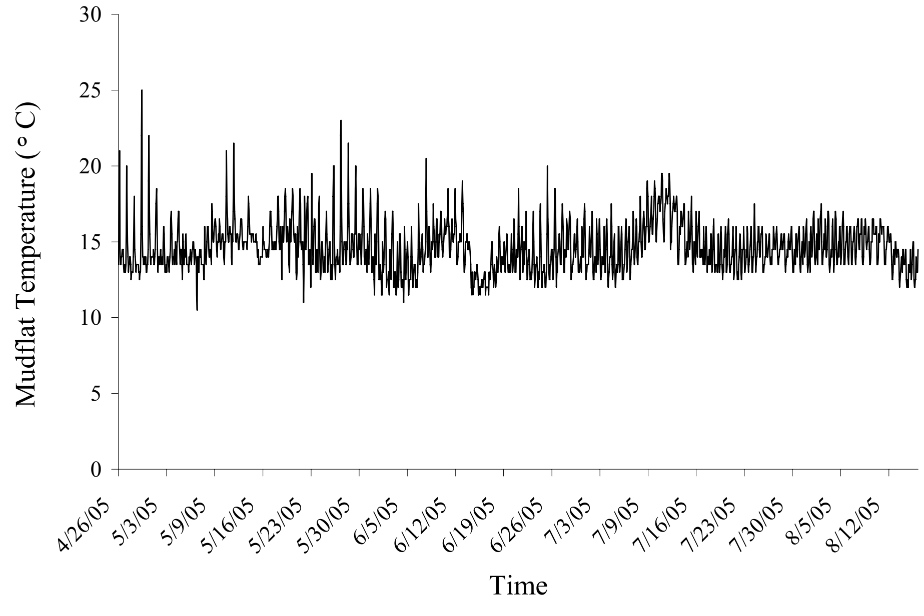


Figure 11b. Emergent/submergent temperatures within the eelgrass canopies in South Bay (site 4) from April 2005 to August 2005. Measurements were taken every 1.5 hours.

Table 4. Two sample t-test results comparing North Bay to South Bay ambient conditions during the course of the study ($\alpha = 0.05$).

Factor	North Bay mean ± 1SE	South Bay mean ±1SE	P
Ammonium sediment concentrations	76.14 ± 4.68 µM	92.29 ± 4.72 µM	0.0620
Nitrate sediment concentrations	52.29 ± 8.69 µM	139.59 ± 28.40 µM	0.0356
Phosphate sediment concentrations	91.51 ± 8.03 µM	100.22 ± 10.19 µM	0.3240
N/P ratio (sediment)	1.52 ± 0.23	2.94 ± 0.96	0.0395
Percent organic matter	1.90 ± 0.22 %	1.57 ± 0.22 %	0.0481
Sediment grain size (MWD)	1.95 ± 0.22	2.83 ± 0.61	0.0565
Mudflat temperature	16.8 ± 0.04 ºC	15.0 ± 0.04 ºC	0.0000

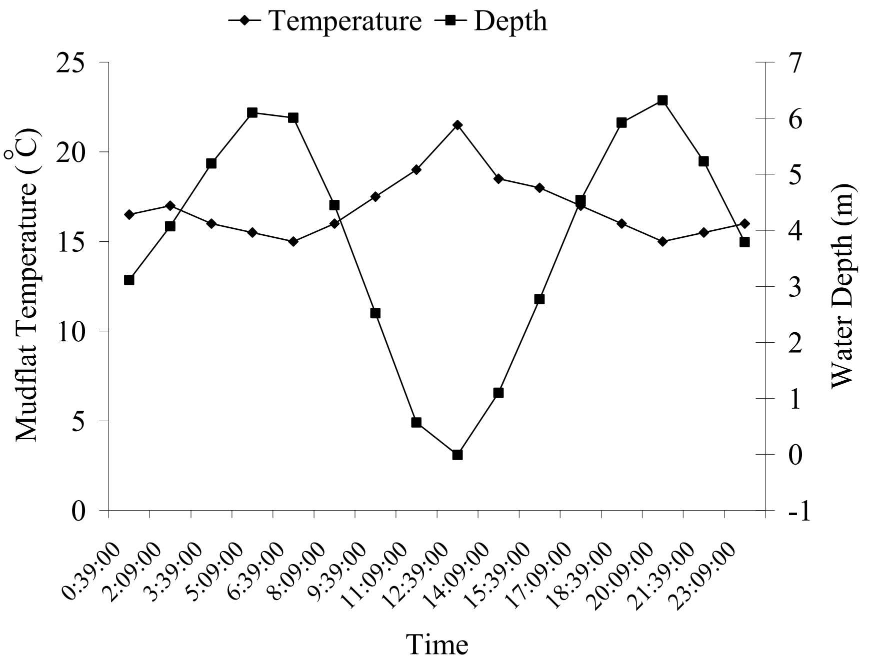


Figure 12a. North Bay emergent/submergent temperatures on the mudflat May 30th 2005 vs. water depth. Water depth obtained from the CICORE logger at Dock B.

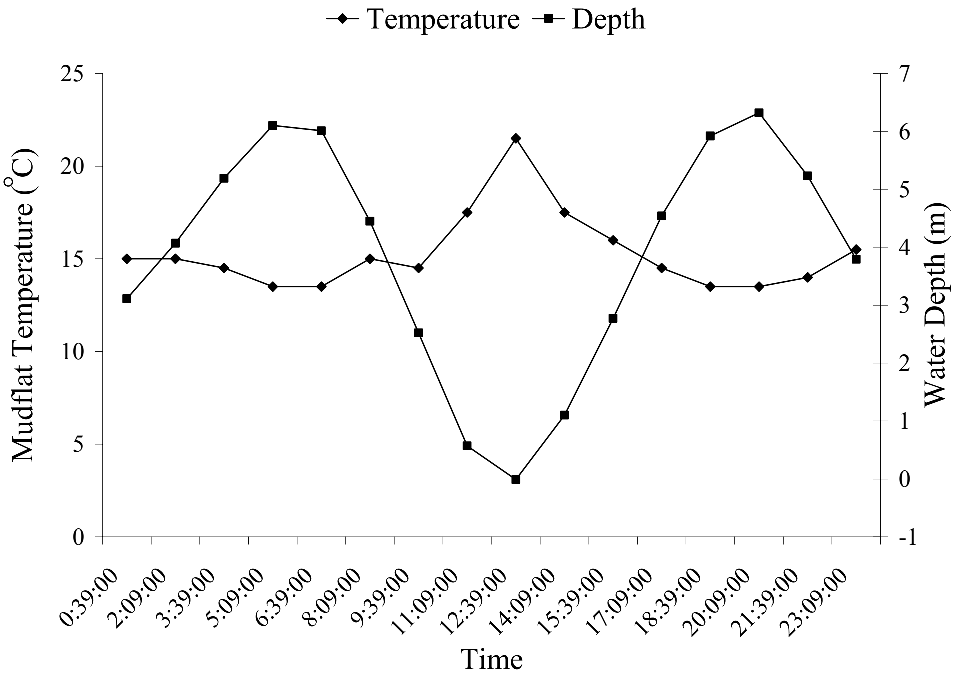


Figure 12b. South Bay emergent/submergent temperatures on the mudflat May 30th 2005 vs. water depth. Water depth obtained from the CICORE logger at Dock B.

Freshwater/Eureka Slough average annual discharge = 5.7 x 10^7 m^3, C. Fenton, unpublished data) and Central Bay (Elk River average annual discharge = 1.0 x 10^8 m^3, C. Fenton, unpublished data) than South Bay (Salmon Creek average annual discharge = 9.0 x 10^4 m^3, Barnhart et al. 1992). Even leaving out the sources of freshwater into North Bay for which there is little data such as Washington Slough, Jolly Giant Creek and McDaniels Slough, North Bay has an annual freshwater discharge around 8.1 x 10^7 m^3, Central Bay has an annual input of freshwater of ≈1.0 x 10^8 m^3 while South Bay has an annual input of 9.0 x 10^4 m^3.

Salinity ranged from 28-37 ppt in the ocean and 5-40 ppt in the bay. North Bay displayed low salinities during the rainy season at Vance Ave (site N1), Washington Slough (site N5), and Freshwater/Eureka Slough (site C1; Figure 13a). In Central Bay low salinities were measured at the Elk River (site C4). In South Bay, low salinities were measured at Hookton Slough (Salmon Creek; site S3). Hookton Slough was the last freshwater source to display appreciable runoff during the dry season, (Figure 13b). Two sites in east North Bay (sites N6 and N7) displayed high salinities.

Since salinity values for sites near freshwater sources were dependent upon the exact distance between the sampling site and the source, the site in each region with the lowest salinity was dropped from any statistical analysis of regional means (n = 6). The following sites were dropped: In North Bay, Washington Slough (site N5), in Central Bay the Elk River (site C4), and in South Bay, Hookton Slough (site S3). Humboldt Bay was significantly more brackish than the ocean during the rainy season and North Bay was

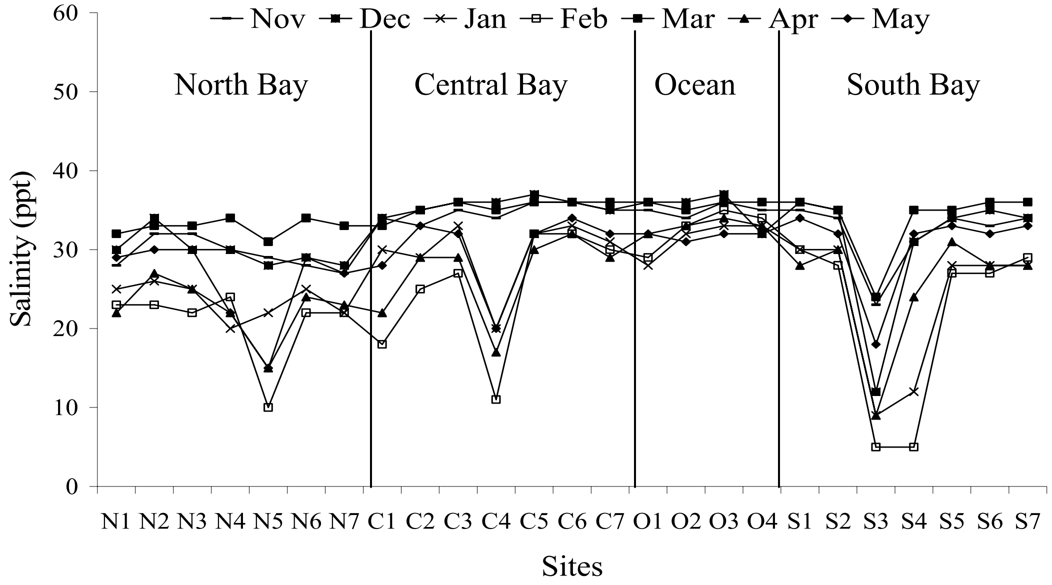


Figure 13a. Ebb tide salinity values in months with runoff (winter and spring). There are freshwater inputs at Vance Ave (N1), Washington Slough (N5), Freshwater/Eureka Slough (C1), Elk River (C4) and Hookton Slough (S3).

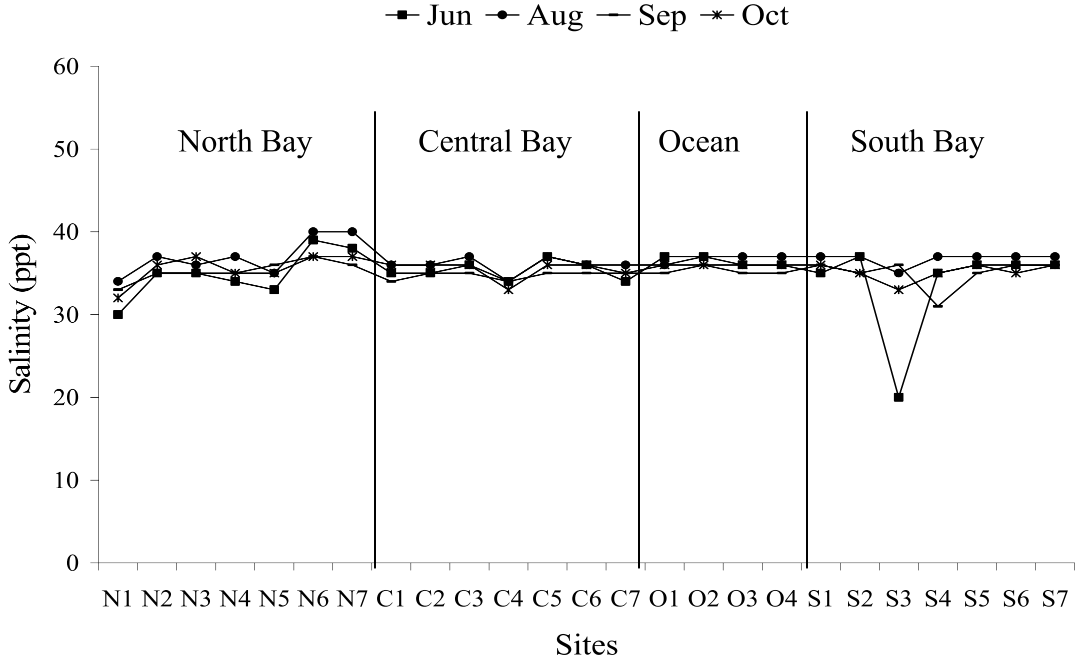


Figure 13b. Ebb tide salinity values for months without runoff (summer and fall).

significantly more brackish than South Bay (Table 3). During the dry season there were no significant differences between regions and Humboldt Bay salinities displayed less bay-wide variability. July salinity values were flawed and were not used in the analysis.

Hypersalinity

The mean ocean salinity from August 13 to October 2 ranged from 35-37 ppt. The standard deviation (SD) of the oceanic salinity fluctuations during the same time period was 0.583. Salinities in North Bay ranged from 34-40 ppt. Salinities in South Bay ranged from 31-37 ppt. Two sampling sites (N6 and N7) on the east side of North Bay (Fig 2) displayed salinities up to 4 points above the ocean and 3 points above the SD of the oceanic salinity fluctuation and could therefore be considered hypersaline (Figure 14). During the week of September 11-17, Humboldt Bay received 0.15 cm of precipitation. Salinities at sites N6 and N7 dropped to 1 point above ocean salinity and were no longer hypersaline. Salinity at those two sites declined further on October 2 after a week with 0.46 cm of precipitation. Hypersalinity sampling was discontinued at that time.

Sediment grain size

The mean weighted diameter of sediment grain size ranged from 1.21 to 3.64 in North Bay and 0.86 to 5.91 in South Bay. Sediment grain size decreased with distance from the entrance (Figure 15). Sites 1 and 2 had the highest percentage of sand followed by site 8, while the other sites had higher percentages of silt and clay. There were no significant differences in the mean grain size between North Bay and South Bay sites (Table 4).

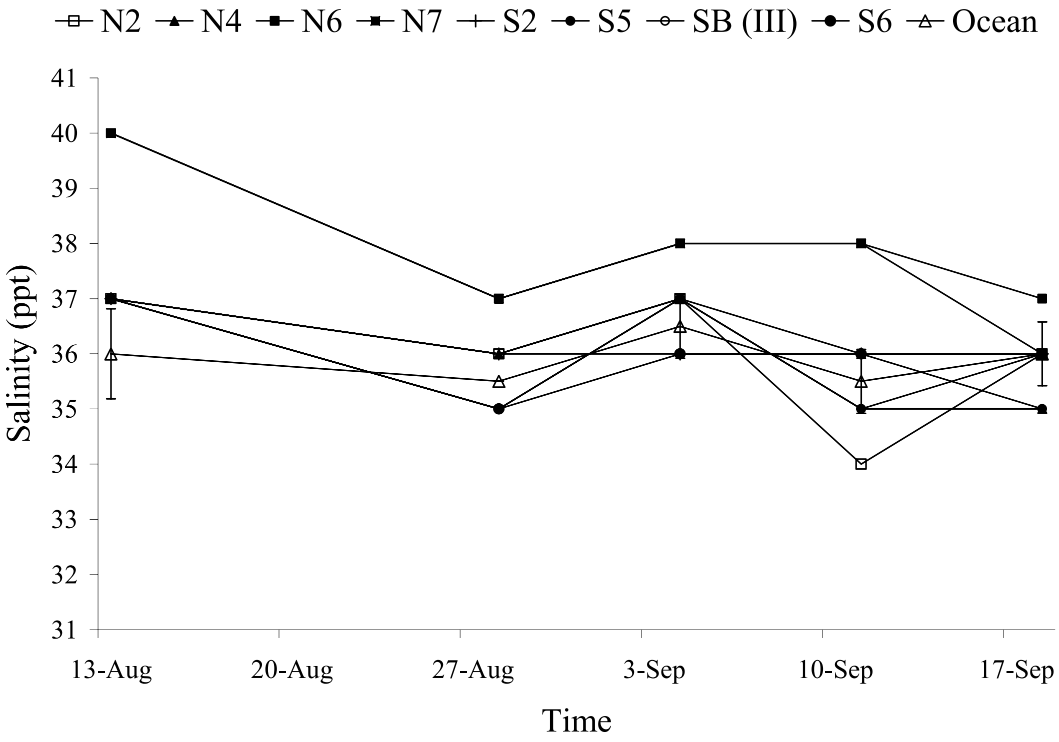


Figure 14. Weekly salinity values in North Bay (sites N2, N4, N6, and N7) and South Bay (sites S2, SB (III), S5, and S6) vs. the ocean from August 13th, 2005 through September 18th, 2005. The lines for sites N6 and N7 are displaying hypersalinity. SB = South Bay. Ocean values are means with error bars ± 1 SD.

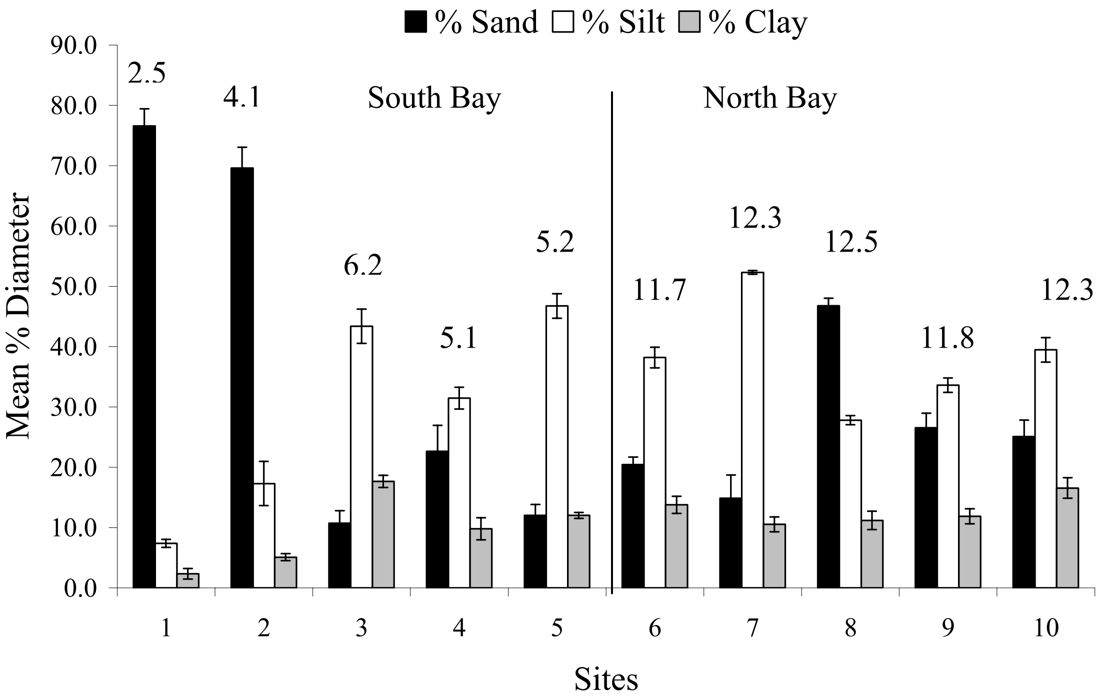


Figure 15. Mean sediment grain size across all sites. Sites 1 and 2 have the highest percentage of sand and are closest to the entrance of Humboldt Bay. Site 8 also has a high percentage of sand and is situated along the Mad River Slough channel. Labeling numbers are the distance from the bay entrance in km. Site numbers are manipulative study sites (Figure 3). Error bars are ± 1SE.

Percent organic matter ranged from 0.00- 3.76 % in North Bay and 0.20 to 3.23 % in South Bay. The organic matter content was significantly higher in North Bay sediment than South Bay sediment (Table 4).

Sediment nutrient concentrations

Ambient ammonium concentrations in the sediment ranged from 60.90 - 92.51 µM in North Bay and 66.53 - 103.19 µM in South Bay. Ammonium concentrations did not differ significantly between regions (Table 4) and were similar to concentrations determined by previous studies to saturate eelgrass growth (Figure 16). Ambient nitrate concentrations ranged from 31.06 - 96.59 µM in North Bay and 70.31 - 297.10 µM in South Bay. Nitrate concentrations were significantly higher in South Bay than in North Bay. Ambient phosphate concentrations ranged from 61.13 - 119.39 µM in North Bay and 63.36 - 144.65 µM in South Bay. Phosphate concentrations did not differ significantly between regions.

Manipulative Experiment

Starting conditions

South Bay eelgrass was significantly denser than North Bay at T_0 as well as having significantly more *Phyllaplysia taylori* and other grazers (Table 5). North Bay eelgrass had significantly taller canopy height, wider leaf width, more leaves per shoot, larger rhizome diameter, and more epiphytes per shoot than South Bay. Above ground biomass, below ground biomass, total biomass and LAI were not significantly different between bays.

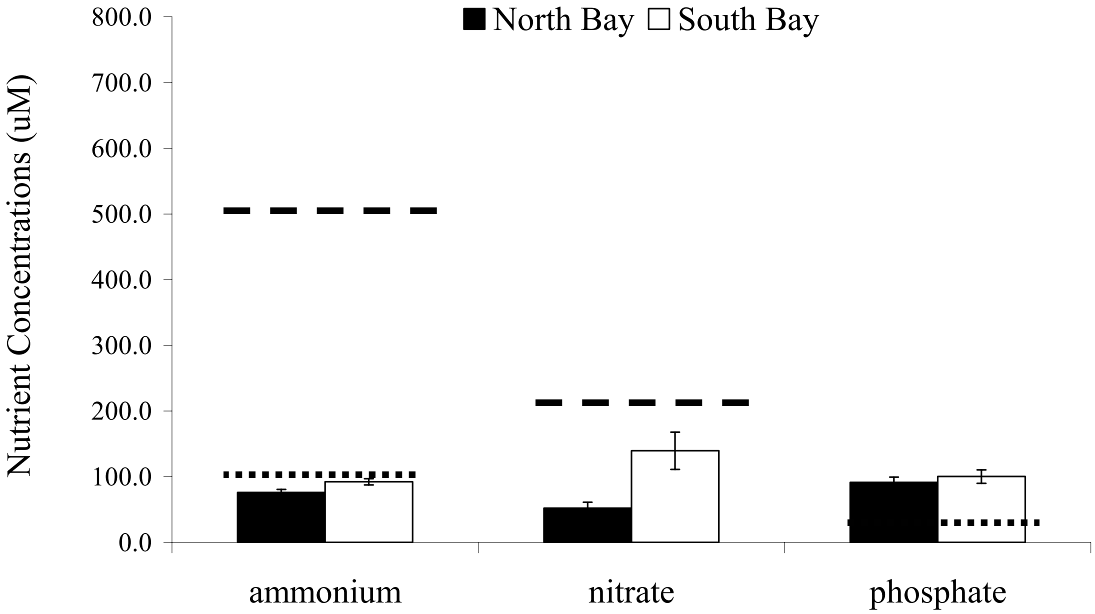


Figure 16. Ambient nutrient concentrations in the sediment of North Bay and South Bay. Error bars are ± 1 SE. Dotted line = saturation concentration for eelgrass, dashed line = toxic concentration for eelgrass. The saturation concentration for nitrate and the toxic concentration for phosphate are unknown

Table 5. Two-sample t-test results comparing baseline (T_0) differences between North Bay and South Bay ($\alpha = 0.05$).

Factor	North Bay mean ± 1SE	South Bay mean ±1SE	P
Density	51 ± 4 shoots/m²	393 ± 39 shoots/m²	0.0280
Canopy height	490 ± 9 mm	311 ± 8 mm	0.0007
Above ground biomass	0.028 ± 0.002 kg/m²	0.039 ± 0.005 kg/m²	0.2681
Below ground biomass	0.093 ± 0.003 kg/m²	0.172 ± 0.017 kg/m²	0.0855
Total biomass	0.151 ± 0.006 kg/m²	0.176 ± 0.017 kg/m²	0.0880
Leaf area index	0.58 ± 0.03	1.64 ± 0.21	0.0758
Leaf width	8 ± 0 mm	6 ± 1 mm	0.0008
# Leaves per shoot	5 ± 0	4 ± 1	0.0325
Rhizome diameter	6 ± 0 mm	4 ± 0 mm	0.0001
ag/bg ratio	1.032 ± 0.044	0.425 ± 0.044	0.0073
Epiphyte biomass	0.224 ± 0.014 g/sht	0.012 ± 0.002 g/sht	0.0000
Grazer abundance	20 ± 2 /m²	256 ± 36 /m²	0.0339
P. taylori abundance	14 ± 2 /m²	201 ± 25 /m²	0.0390

Treatment effects on shoot density

Shoot density in the summer (T_3) ranged from 4 - 184 shoots/m² in North Bay and 20 - 960 shoots/m² in South Bay. One medium water treatment plot in South Bay was overrun with *Rhizoclonium* sp. (Kutzing) and all eelgrass died. As that entire end of the site was covered in the macroalga, the loss of eelgrass in the plot was not considered a response to the treatments and that plot was left out of all analyses. There was a significant shoot density response in North Bay to the high sediment treatment (Table 6). Shoot density in the high sediment treatment plots decreased (Figure 17). There were no other significant treatment effects on density. The change in shoot density displayed extremely high variation between sites in South Bay, which kept most of the treatment effects in South Bay from being significant.

The high sediment fertilization treatments significantly increased the ammonium and phosphate concentrations in the sediment over ambient conditions ($p = 0.0000$, and $p = 0.0252$, respectively). Phosphate levels in the high sediment treatment plots in North Bay increased 3 to 14 times over the ambient levels (Figure 18) and were significantly higher in North Bay than South Bay ($p = 0.0236$). Phosphate levels in the high sediment treatments in South Bay increased by 1 to 2 times over ambient conditions. The difference in ammonium and nitrate concentration in the high sediment treatment plots was not significantly different between regions.

Table 6. One-way ANOVA results of treatment effects on period 4 shoot density ($\alpha = 0.05$). Response variable is the change in shoot density in either North Bay or South Bay and the factor is the eight treatments. Similar groups are underlined in the Tukey-Kramer column. HS = high sediment treatment, LS = low sediment treatment, MW = medium water treatment, CW = control water treatment, CS = control sediment treatment, MS = medium sediment treatment, LW = low water treatment, HW = high water treatment.

Response variable	P	Tukey-Kramer
North Bay period 4 density	0.0244	HS, MW, LS, CW, CS, MS, LW, HW
South Bay period 4 density	0.7682	

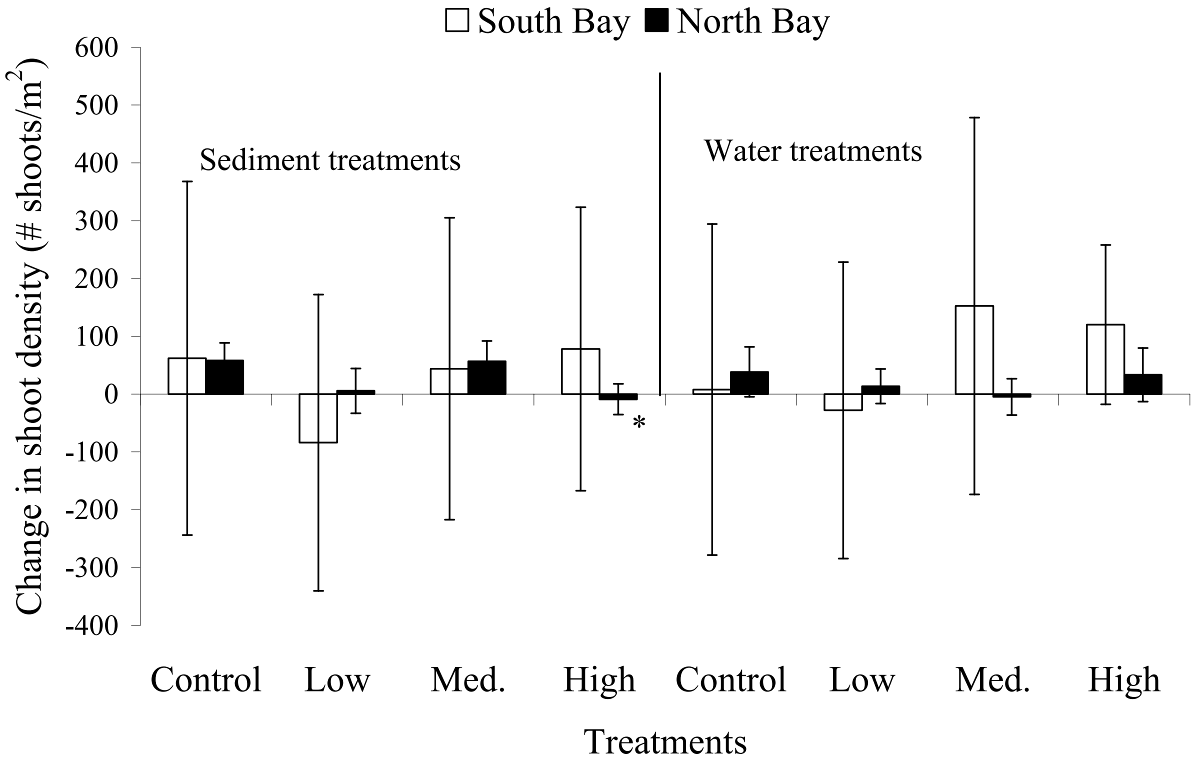


Figure 17. The change in mean shoot density over the course of the study, by treatment (* = $p<0.05$). Error bars are ± 1SD.

Environmental predictors of shoot density

Besides the application of fertilizer, there were many ambient environmental factors that could have affected eelgrass shoot density during the course of the study. The possible effects of these other factors were examined with a multiple regression of period 4 shoot density vs. region (bay), salinity, water temperature, ambient nitrate, ammonium and phosphate concentrations in the sediment and water column, the N/P ratio in the sediment and the water column, percent organic matter, sediment grain size, distance of the site from the bay entrance (Entrance Channel), epiphyte loads and the abundance of the grazer *Phyllaplysia taylori*. The resulting model showed that only the concentration of nitrate in the water column and the abundance of *P. taylori* were significant predictors of the change in shoot density over the course of the study (Table 7). The abundance of *P. taylori* was the most important contributor to the model and displayed a positive relationship with the change in shoot density but the model only explained 40% of the variation in the change in shoot density.

The abundance of *P. taylori* ranged from 0 to 1520/m^2 and was significantly higher in South Bay than North Bay (Table 8). *P. taylori* was the dominant grazer with the rest of the grazer community being comprised of *Idotea resecata* (Stimpson 1857) and caprellids. *P. taylori* abundance was significantly higher in summer than winter, but increased more in South Bay than in North Bay leading to a significant interaction term (Figure 19a). The abundance of *P. taylori* was inversely correlated with epiphyte biomass, which was significantly higher in North Bay than South Bay (Table 8) but

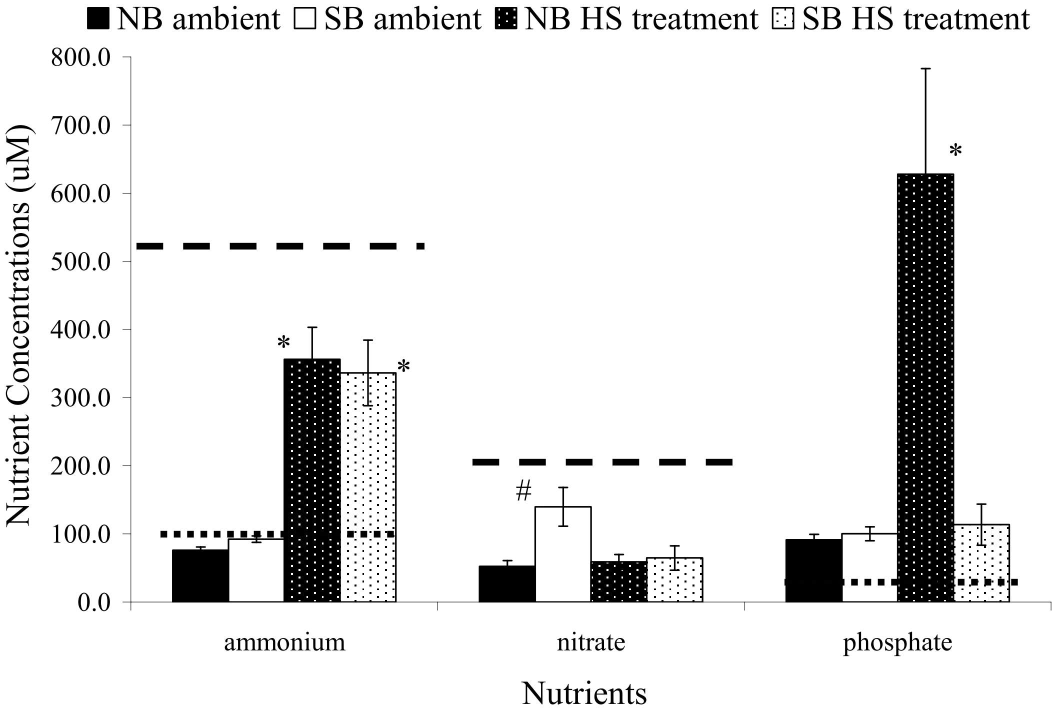


Figure 18. Ammonium, nitrate and phosphate concentrations in the in the high sediment (HS) treatment plots in North Bay (NB) and South Bay (SB) compared to ambient sediment concentrations of ammonium, nitrate and phosphate concentrations in both regions. * = treatment concentrations significantly higher than ambient concentrations, # = ambient South Bay concentrations significantly higher than ambient North Bay concentrations (α = 0.05). Error bars are ± 1SE. Dotted lines = growth saturation concentrations for eelgrass, dashed lines = toxic concentrations for eelgrass. The saturation concentration for nitrate and the toxic concentration for phosphate are unknown.

Table 7. Multiple regression analysis output for the change in shoot density over the course of the study (IV = independent variable, α = 0.05).

Factor	Standard coefficient	P	Model R^2	IV R^2
Nitrate concentrations in the water	-0.4233	0.0000	0.4018	0.0998
P. taylori abundance	0.5600	0.0000		0.2293

Table 8. Two-way ANOVA results of the effects of season and region on *Phyllaplysia taylori* abundance and epiphyte biomass ($\alpha = 0.05$). Region = North Bay and South Bay, season = winter (February 2005) and summer (August 2005). Similar groups are underlined in the Tukey-Kramer column. NB = North Bay, SB = South Bay, T_0 = February 2005, T_3 = August 2005.

Response variable		P	Tukey-Kramer
P. taylori abundance	Region	0.0000	NB, SB
	Season	0.0000	T_0, T_3
	Interaction	0.0043	
Epiphyte biomass	Region	0.0000	NB, SB
	Season	0.0000	T_0, T_3
	Interaction	0.0000	

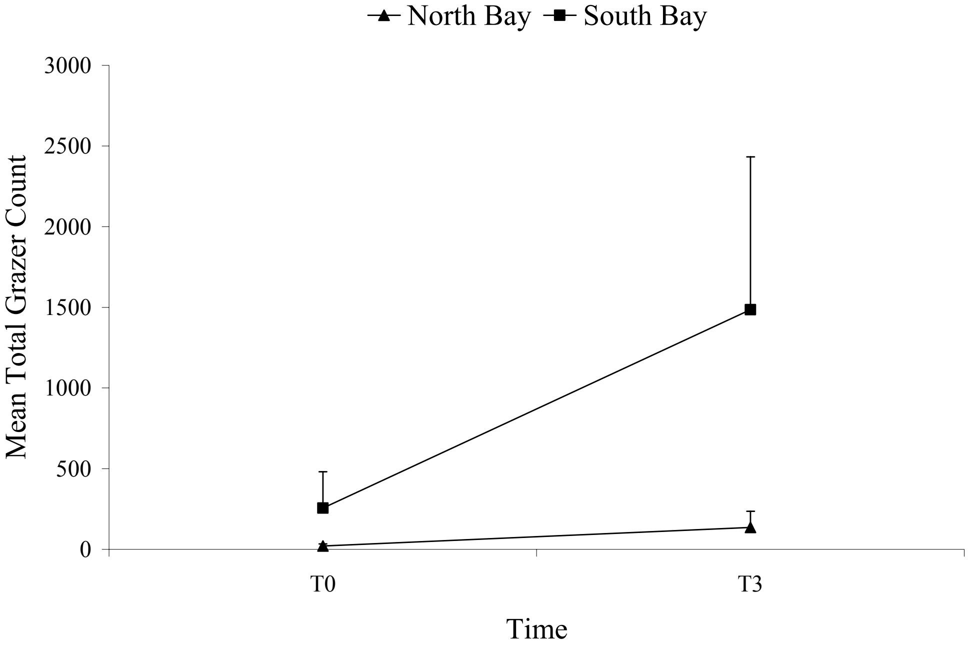


Figure 19a. Mean total grazer count over the course of the study. Grazer abundance increased more sharply in South Bay than in North Bay. Error bars are + 1 SD.

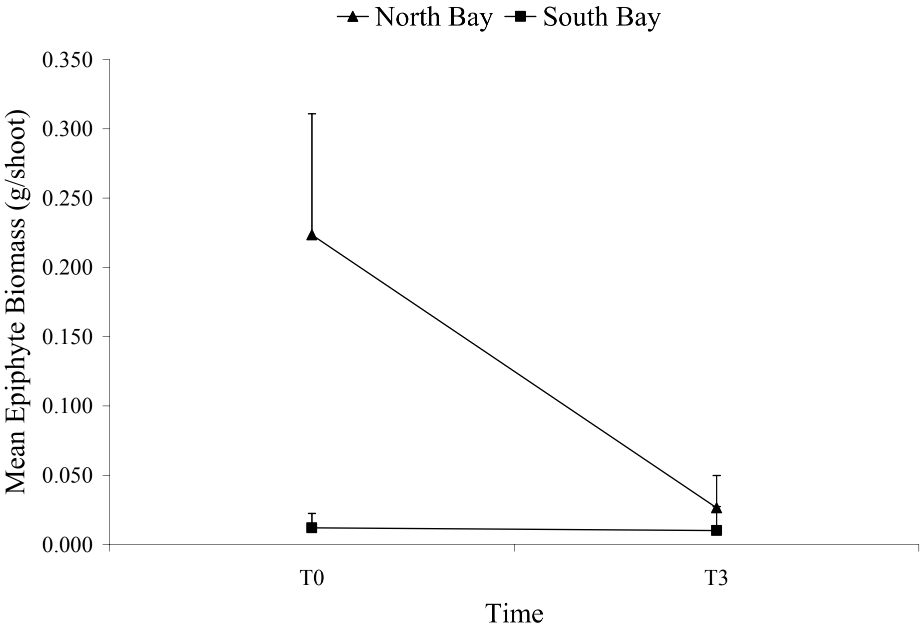


Figure 19b. Mean epiphyte biomass over the course of the study. Error bars are + 1 SD.

decreased more in North Bay than South Bay over the course of the study, leading to a significant interaction term (Figure 19b). The decrease in North Bay epiphyte biomass occurred despite low grazer loads. There were no significant treatment effects on *P. taylori* abundance, epiphyte biomass or total grazer abundance (Table 9).

Treatment Effects on Eelgrass Metrics

The high sediment treatment significantly decreased period 4 total biomass (Table 9), as well as period 4 below ground biomass in North Bay as it did for shoot density and as for shoot density there was a lot of variation between sites in South Bay (Figure 20). The high water and medium water treatments in North Bay significantly decreased rhizome diameter in North Bay over the course of the study (Table 9). There were no significant treatment responses in above ground biomass, canopy height, leaf width, number of leaves per shoot, LAI, epiphyte biomass or grazer loads.

Table 9. One-way ANOVA results of treatment effects on period 4 eelgrass metrics. Response variables are the change in plant metrics in either North Bay or South Bay and the factor is the eight treatments (NB = North Bay, SB = South Bay, BG = below ground, AG - above ground, * = log transformed for normality, α = 0.05). Similar treatment groups are underlined in the Tukey-Kramer column. HS = high sediment, LS = low sediment, MW = medium water, CW = control water, CS = control sediment, MS = medium sediment, LW = low water, HW = high water.

Response variable	P	Tukey-Kramer
Total biomass in NB	0.0211	HS, MW. HW, LW, CW, MS, LS, CS
Total biomass in SB	0.8270	
BG biomass in NB	0.0341	HS, HW, MW, CW, MS, LS, CS, LW
BG biomass in SB	0.8930	
AG biomass in NB	0.2840	
AG biomass in SB	0.5627	
Canopy height in NB	0.8449	
Canopy height in SB	0.8601	
Leaf width in NB	0.9969	
Leaf width in SB	0.8127	
No. leaves/ shoot in NB	0.8040	
No. leaves/ shoot in SB	0.7132	
LAI in NB	0.2710	
LAI in SB	0.8027	
Rhizome diameter in NB	0.0413	HS, HW, MW, CW, MS, LS, LW, CS
Rhizome diameter in SB	0.3732	
Epiphyte biomass in NB	0.9997	
Epiphyte biomass in SB	0.9797	
Total grazers in NB*	0.0708	
Total grazers in SB	0.9024	
P. taylori in NB	0.2395	
P. taylori in SB	0.9238	

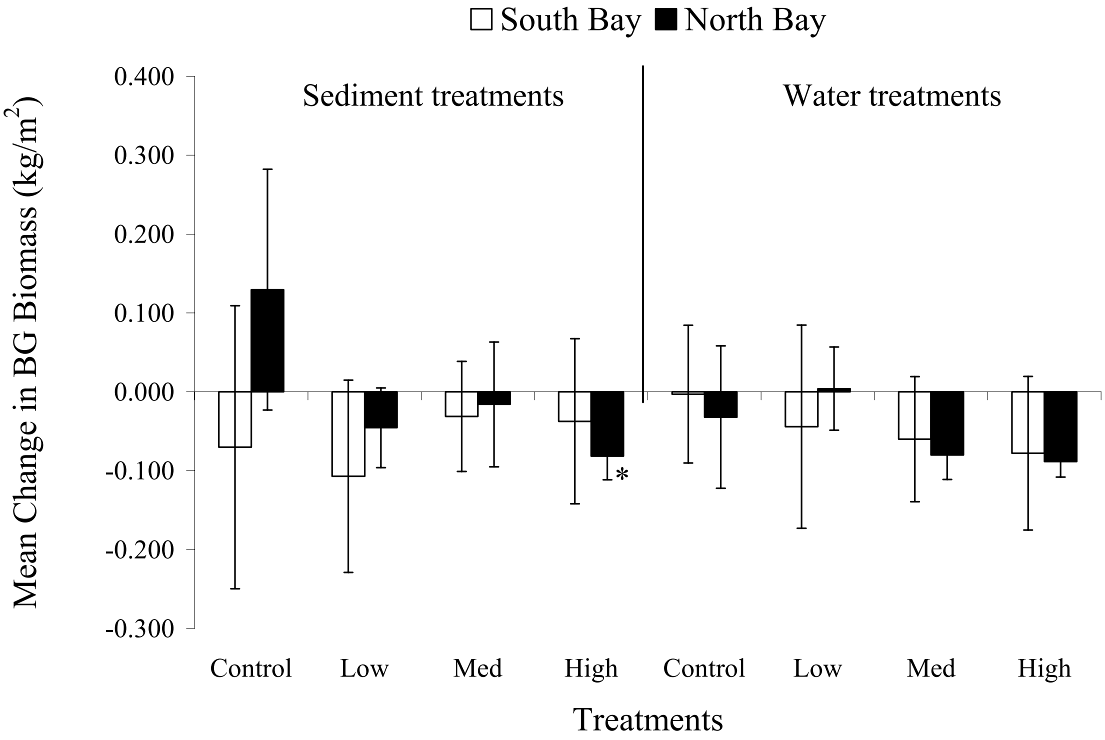


Figure 20. Treatment effects on the change in below ground biomass. * = $p<0.05$, BG = below ground. Error bars are ±1SD.

DISCUSSION

Shoot density is one of the most important aspects of eelgrass bed structure. Lower shoot density decreases the survival and growth rates of juvenile fish and invertebrates within the eelgrass bed (Irlandi 1997; Bostrom and Bonsdorff 2000; Bell et al. 2001; Peterson et al. 2004).The shoot density of *Z. marina* in Humboldt Bay, California has varied significantly between North Bay and South Bay since at least the late 1950's. Multiple studies have reported the difference, but no study has ever attempted to determine the cause of the difference. The present study has described some of the ambient conditions in Humboldt Bay, primarily nutrients, that could be affecting eelgrass shoot densities, as well as experimentally examining the effects of ammonium on these densities, and lastly the relationship of shoot density to a variety of environmental predictors was also evaluated. Measurements of ambient environmental conditions delineated several factors that could be affecting the shoot density of Humboldt Bay *Z. marina* populations.

Some spatial data for water column nitrate and ammonium existed for Humboldt Bay (Pequegnat and Butler 1981; Barnhart et al. 1992; Althaus et al. 1997), but no data of this kind existed for sediment nutrients and time series measurements of nitrogen or phosphorous had never been made in Humboldt Bay. The present study found that nitrate entered Humboldt Bay from freshwater sources during times of precipitation and from the ocean during times of upwelling. A previous Humboldt Bay study also noted that the ocean was a primary source of nitrate for Humboldt Bay during the summer (Althaus et al.1997). A comparison of Humboldt Bay water column nitrate concentrations to other estuaries, and archipelagos in the Pacific Northwest shows that Humboldt Bay has higher concentrations than have been reported for Puget Sound but lower concentrations than have been reported for Friday Harbor (1.72 - 8.918 µM for Humboldt Bay, 0.1 - 0.6 µM for Puget Sound and 22.9 - 24.2 µM for Friday Harbor; Thom and Albright 1990; Nelson 1997). Nitrate concentrations appear to be highly variable throughout the Pacific Northwest.

Ammonium entered the bay in large pulses from freshwater sources during times of precipitation and in small pulses of scattered origin during the dry season. Humboldt Bay had higher concentrations of ammonium in the rainy season than has been reported for other Pacific Northwest estuaries, and archipelagos throughout the year (2.007 - 8.093 µM for Humboldt Bay, 0.1 - 3.5 µM for Puget Sound, 0.312 - 0.723 µM for Friday Harbor; Thom and Albright 1990; Nelson 1997)

Phosphate concentrations in the bay were similar to phosphate concentrations in the ocean with the greatest loading occurring during the rainy season. Humboldt Bay phosphate concentrations are slightly higher than most other Pacific Northwest bays, estuaries, and archipelagos (2.043 - 3.759 µM for Humboldt Bay, 0.74 - 1.53 µM for Yaquina Bay, 1.74 - 2.05 µM for Friday Harbor, 1.0 - 4.4 µM for Puget Sound; Thom and Albright 1990; Nelson 1997; Kentula and DeWitt 2003). During the summer, the only strong phosphate source for Humboldt Bay was at the bottom of Table Bluff in South Bay (site S4). This site is the slowest flushing corner of South Bay and contains several rivulets that drain a salt marsh belonging to the Humboldt Bay National Wildlife Refuge. The phosphate loading from the salt marsh is presumably due to decaying organic matter that accumulates due to poor flushing. Longer flushing times slow the diffusion of nutrients into the water column from the sediment by allowing nutrients to build up in the substrate boundary layer of the water column lessening the concentration gradient between nutrients in the sediment and nutrients in the water column (Touchette et al. 2003).

Low ammonium concentrations and seasonally low nitrate concentrations led to an N/P ratio in the water column well below the Redfield ratio of 16:1. The N/P ratio was below the nutrient limitation threshold of ≈5.0 suggested by Thom and Albright (1990) except during March, April, January, and February, months with high runoff and high concentrations of ammonium and nitrate. The fluctuations of the N/P ratio appeared to follow the fluctuations of nitrogen concentrations as phosphate concentrations showed little variation throughout the year. All of the summer ammonium, nitrate and phosphate concentrations measured during my study were similar to the values found during Humboldt Bay studies in 1980 (Pequegnat and Butler 1981), and 1986 (Barnhart et al. 1992) so there is no evidence of increased anthropogenic nutrient loading.

The water column in North Bay and South Bay did not contain sufficient ammonium to saturate eelgrass uptake in any season (4.905 ± 0.433 µM vs. 15 µM or >20 µM; Iiuzumi and Hattori 1982; Thursby and Harlin 1982) and nitrate concentrations in the water column were insufficient to saturate eelgrass growth in the dry season (2.224 ± 0.236 µM vs. 4 µM or 8 µM; Zimmerman et al. 1987; Thom and Albright 1990). However, ambient ammonium levels in the sediment (60.90 - 103.19 µM) were well above the levels predicted by Zimmerman et al. (1987) to saturate eelgrass growth (10 -

30 µM) and equal to or slightly under the growth saturation levels reported by other studies (100 µM; Thursby and Harlin 1982; Dennison et al. 1987; Murray et al. 1992; Williams and Ruckelshaus 1993). Ambient phosphate levels in the sediment were also well above the concentration estimated to saturate growth (5 - 15 µM; Murray et al. 1992). The sediment in both regions contains sufficient ammonium and phosphate resources to support eelgrass growth suggesting that the low summer nutrient concentrations in the water column do not necessarily mean that the eelgrass in Humboldt Bay is nitrogen or phosphorous limited.

Other environmental differences between North Bay and South Bay that may be affecting eelgrass shoot density include temperature and salinity. The temperature at the surface of the mud within the eelgrass bed was warmer, on average, in North Bay but displayed a wider range in South Bay. A previous study in North Bay that measured the temperature 2 cm below the mud surface reported similar mudflat temperature ranges (15-20˚C; Rumrill and Poulton 2004). The higher temperatures in South Bay corresponded to emersion during minus tides and so indicate South Bay eelgrass is emersed for longer periods and therefore receives higher amounts of light energy. Higher levels of light energy often results in higher shoot density (Backman and Barilotti 1976; Olesen and Sand-Jensen 1993; Havens et al. 2001; Nelson and Lee 2001).

Water temperatures decreased with distance from the entrance during the rainy season, presumably due to the influx of cold freshwater. In the dry season, the trend was reversed and water temperatures warmed with distance from the bay entrance, a pattern that has been seen elsewhere in the Pacific Northwest (Kentula and DeWitt 2003).

However, North Bay water was colder than South Bay water in both seasons. The range of temperatures in both bays was within the optimal temperature range for *Z. marina* and probably is not a factor in the shoot density difference, as higher temperatures usually decrease shoot density and Humboldt Bay eelgrass is denser in the warmer regions (Bintz et al. 2003). Humboldt Bay displayed warmer temperatures in the summer than have been reported for other bays in the Pacific Northwest (6.7 - 24.3 ºC for Humboldt Bay, 16.0 - 17.8 ºC for Willapa Bay, 13.1 - 16.7 ºC for Coos Bay, 10.9 - 14.6 ºC in Yaquina Bay; Kentula and DeWitt 2003; Thom et al. 2003).

Salinity ranged widely in North Bay. More than twice as much freshwater discharge enters North Bay than South Bay so North Bay is significantly lass saline than South Bay during the rainy season (a finding also reported by Skeesick 1963). Low salinities in North Bay may be affecting the eelgrass shoot density in North Bay as eelgrass densities have been shown to decrease down a salinity gradient in estuaries (Kentula and DeWitt 2003; Thom et al. 2003). The low salinities in North Bay may also help protect the eelgrass from high nutrient loads that would otherwise be toxic. Immature *Z. marina* leaf tissue is sensitive to salinity and increased nutrient loads can increase growth rates, prematurely exposing young tissue to seawater (van Katwijk et al. 1999). Lower salinities would do less damage to the immature eelgrass leaves under those conditions. The eelgrass in East North Bay also experiences hypersalinity in late summer. Water column phosphate levels were especially high and nitrate levels especially low at the hypersaline sites in August, as predicted by Largier et al. (1997). Further study of hypersalinity in Humboldt Bay is suggested. Salinities in Humboldt Bay ranged more widely than has been reported for other Pacific Northwest bays and estuaries (5 - 40 ppt for Humboldt Bay, 21.8 - 29.2 ppt for Willapa Bay, 28.0 - 32.1 ppt in Coos Bay, 31.0 - 33.0 in Yaquina Bay, 25.7 - 30.2 for Puget Sound; Thom and Albright 1990; Kentula and DeWitt 2003; Thom et al. 2003). However, the salinity range seen in Humboldt Bay falls within the range reported to be acceptable for *Z. marina* growth (10-42 ppt; Biebl and McRoy 1971) except at Hookton Slough (site S3) in South Bay where short-term salinities as low as 5 ppt were measured during periods of heavy rainfall.

The experimental effect of ammonium on eelgrass shoot densities was addressed to determine if the eelgrass in Humboldt Bay is ammonium limited or dealing with ammonium toxicity. There was no evidence of ammonium limitation as shoot densities did not increase in either bay in response to the treatments. This verifies that the eelgrass in Humboldt Bay obtains sufficient ammonium from the sediment to saturate growth despite the ammonium limitation present in the water.

The application of ammonium/phosphate fertilizer to the sediment of Humboldt Bay had a toxic effect on North Bay eelgrass shoot density. No similar toxic effect was seen in South Bay, despite similar concentrations of ammonium in the treatment plots, but ammonium reaches toxic levels faster in low-light conditions due to lower rates of photosynthesis and the subsequent lack of carbon skeletons for fixing ammonium into glutamine (van Katwijk et al. 1997). North Bay is believed to suffer from greater light limitation than South Bay, and so would be likely to suffer from toxicity at lower nutrient levels.

North Bay is also believed to have a longer flushing time than South Bay (Barnhart et al. 1992). Longer flushing times increase the effects of nutrient loading through the retention of phytoplankton blooms which increase light attenuation and so light limitation and by slowing the diffusion of nutrients into the water column from the sediment (Valiela et al. 2000; Touchette et al. 2003). Bays like Humboldt Bay that are shallow and wind-mixed are especially sensitive to nutrient loading from resuspended sediment (Glasgow and Burkholder 2000). Sediment phosphate levels in the high sediment treatment plots were much higher in North Bay than South Bay, which may have been due to slower diffusion of phosphate out of the sediment and into the water column.

While ammonium increased to a similar degree in the high sediment treatment plots in both North Bay and South Bay, phosphate accumulated at much higher levels in North Bay than South Bay. Phosphate concentrations in the sediment are estimated to reach saturation levels for eelgrass at 5-15 µM (Murray et al. 1992). Phosphate levels in North Bay treatment plots reached concentrations of 1298 µM so it is possible phosphate was having a toxic effect on shoot density instead of ammonium. Phosphate concentrations have been reported to correlate negatively with eelgrass biomass (Thom and Albright 1990). However, it is not clear if phosphate toxicity is possible in *Z. marina.* Eelgrass acts as a link between phosphate in the sediment and the water column by taking up the nutrient through the roots and leaking it out through the leaves (McRoy and Barsdate 1970; Penhale and Thayer 1980) so phosphate may not build up in the tissues.

Eelgrass populations of North Bay and South Bay are not ammonium or phosphorous limited at ambient concentrations of those nutrients, as there were no positive treatment effects on shoot density but carbon limitation was not ruled out by my study. Carbon limitation could have been a factor in my experiment as plants must assimilate ammonium into organic compounds immediately to reduce its toxic effects and that requires carbon skeletons (Borum et al. 1989). Carbon limitation is more likely under conditions of low salinity due to lower concentrations of HCO_3^- and so could have been a factor in the negative eelgrass response to the fertilization of the sediment in North Bay (Hellblom and Bjork 1999).

The abundance of *P. taylori* and the concentration of nitrate in the water column were significant predictors of the change in shoot density. The importance of *P. taylori* to eelgrass shoot density is most likely connected to rates of photosynthesis. South Bay had higher abundances of *P. taylori* and lower epiphyte biomass. Epiphytic microalgae form a film over the eelgrass leaves and block light from reaching the chloroplasts, thereby slowing photosynthetic rates. When the microalgae are removed by grazers such as *P. taylori,* and *Idotea* spp., light levels and photosynthetic rates increase which has been shown to lead to growth rate increases (Williams and Ruckelshaus 1993; DeLorenzo 1999).

The importance of nitrate concentrations in the water column of North Bay during the summer may be partially explained by the relative abundance of epiphytic algae and epiphyte grazers between bays. Epiphytic algae maintained a low biomass in South Bay from winter to summer, but grazer abundance increased in this bay over the summer and was presumably reduced by grazing (Williams and Ruckelshaus 1993; Moore and Wetzel 2000). Epiphyte biomass in North Bay was relatively high in the winter, but dropped sharply in the summer without an increase in grazer abundance. However, the summer drop in North Bay epiphytes is correlated to lower concentrations of nitrate than South Bay water. These measurements were taken on ebb tides, but the same differences in nitrate concentrations have been reported between the two bays on flood tides as well (Pequegnat and Butler 1981; Barnhart et al. 1992). As light limitation differences between North Bay and South Bay are probably lower in the dry season than in the winter when turbidity is added by runoff, the lack of epiphytes on the eelgrass in the summer may be due to nitrogen limitation in the water column (Dennison et al. 1987, Thom and Albright 1990). Phytoplankton require four times as nitrogen per carbon atom as eelgrass, so nitrogen limitation would occur at lower levels for phytoplankton than eelgrass (Duarte 1995; Hemminga and Duarte 2000; Gallegos 2001).

The abundance of *P. taylori* and the concentration of nitrate in the water column only explained 40% of the variation in the change in shoot density witnessed by this study even though several other environmental parameters such as water temperature and salinity were evaluated in the multiple regression analysis. What other factors may be affecting the shoot density in Humboldt Bay? Light may be an important component of the eelgrass habitat in Humboldt Bay. Accurate tidal elevations for the mudflats in North Bay and South Bay are not yet available but there is anecdotal evidence that the eelgrass beds in North Bay are at a lower mean tidal elevation than South Bay eelgrass beds (S. Schlosser, pers. com.). As water absorbs light, deeper eelgrass beds would receive less irradiance. North Bay also receives higher levels of freshwater discharge during the rainy season. Runoff contains suspended sediment that increases turbidity in the water column. Turbidity spikes in North Bay during the rainy season have been captured by the CICORE sonde at Dock B. High winds during the spring and summer can resuspend sediment and cause turbidity events as well. Since North Bay has a longer fetch than South Bay under prevailing wind directions, wind-caused turbidity events may be of greater magnitude in North Bay than South Bay (Shaughnessy et al. *submitted*). Light limitation due to tidal elevation and water column turbidity could be having a strong effect on shoot density.

The response of other eelgrass metrics to the application of fertilizer was similar to the shoot density response. There was no evidence of ammonium or phosphate limitation and no treatment effects on epiphyte loads or grazer loads, so there is no evidence that the nutrients were absorbed by eelgrass epiphytes before the eelgrass could obtain them as has been previously seen (Williams and Ruckelshaus 1993). The reduction of below ground biomass in the high sediment treatment plots in North Bay also supported the possibility that phosphate concentrations reached toxic levels in the North Bay sediment. The significant decrease in rhizome diameters in the high water and medium water treatments in North Bay is probably not biologically significant as it was not accompanied by a leaf response

There are spatial and temporal patterns in the ambient concentrations of nitrate, ammonium, and phosphate in the water column of Humboldt Bay. Nitrate enters the bay primarily through freshwater runoff in the winter and primarily from the ocean in the summer. Ammonium is discharged across the bay in large pulses in the winter and smaller pulses in the summer and the bay receives phosphate loading through a few small point sources and one large point source in the summer at the foot of Table Bluff in South Bay. Water column ammonium concentrations are insufficient to saturate eelgrass growth throughout the year and nitrate concentrations are insufficient to saturate growth in the summer. Water temperatures decrease with distance from the bay entrance in the winter and increase with distance from the entrance in the summer. Humboldt Bay is more brackish than the ocean in the rainy season and has similar salinities to the ocean in the dry season. North Bay salinities span a wider range than South Bay and reach hypersaline levels in the late summer. Nitrogen and phosphorous concentrations, salinity, and mudflat temperatures may be affecting the shoot densities in North Bay and South Bay.

Humboldt Bay eelgrass populations are not suffering from nutrient limitation at ambient ammonium and phosphate levels although that is due to sufficient nutrient resources in the sediment making up for insufficient nutrient resources in the water column. There was a toxic response to the addition of fertilizer to the sediment that appears to be due to phosphate concentrations in the sediment as opposed to ammonium concentrations. However, there was no evidence of ammonium or phosphate toxicity at ambient levels. It required the largest amount of fertilizer applied to the slowest-flushing region of the bay to initiate a toxic response South Bay is apparently able to handle a sudden increase of nutrients to both the water column and the sediment and is relatively safe from human impacts. North Bay does not normally suffer from nutrient toxicity, but is more likely to accumulate nutrients in the sediment. A future increase in nutrient loading from freshwater sources could therefore be damaging to the eelgrass in North Bay.

The abundance of *Phyllaplysia taylori* was an important predictor of the change in shoot density. This may be due to the impact grazers have on the amount of light that can reach the chloroplasts of the eelgrass through the removal of epiphytic algae. The concentration of nitrate in the water column also predicted the change in shoot density which corresponds to epiphyte loads on the eelgrass blades. Between them, these two variables explained 40% of the variation in the change in shoot density. The rest of the variation may be due to other factors that influence the amount of photosynthetically active radiation the eelgrass receives, such as tidal elevation and water column turbidity.

LITERATURE CITED

Aioi, K. 1980. Seasonal change in the standing crop of eelgrass (*Zostera marina* L.) in Odawa Bay, Central Japan. Aquatic Botany 8: 343-354.

Althaus, A.M., S. Baiz, H. Cardenas, C.M. Dailey, B.D. Eisen, W.A. Heim, M.K.S. Hubbard, H. Ito, K.B. Kegel, S.L. Nastich, J.M. Riley, M. Villalobos, J.D. Whitaker, S.A. White, G. Crawford, M. de Angelis, J. Pequegnat. 1997. A comparative study of nutrient sources to Humboldt Bay, late spring through summer, 1997.Unpublished data. Humboldt State University Oceanography Dept.

Backman, T.W.H., and D.C. Barilotti. 1976. Irradiance reduction: effects on standing crops of the eelgrass *Zostera marina* in a coastal lagoon. Marine Biology 34: 33-40.

Barnhart, R.A., M.J. Boyd, and J.E. Pequegnat. 1992. The ecology of Humboldt Bay, California: an estuarine profile. U.S. Department of the Interior, Fish and Wildlife Service, Washington D.C., USA.

Bathke, G.R., A. Amoozegar, and D.K. Cassel. 1991. Description of soil pore size distribution with mean weighted pore diameter and coefficient of uniformity. Soil Science 152: 82-86.

Beck, M.W., K.L. Heck, Jr., K.W. Able, D.L. Childers, D.B. Eggleston, B.M. Gillanders, B. Halpern, C.G. Hays, K. Hoshino, T.J. Minello, R.J. Orth, P.F. Sheridan, M.P. Weinstein. 2001. The identification, conservation and management of estuarine and marine nurseries for fish and invertebrates. Bioscience 51: 0006-3568.

Bell, S.S., R.A. Brooks, B.D. Robbins, M.S. Fonseca, and M.O. Hall. 2001. Faunal responses to fragmentation in seagrass habitats: implications for seagrass conservation. Biological Conservation 100: 115-123

Biebl, R., and C.P. McRoy. 1971. Plasmatic resistance and rate of respiration and photosynthesis of *Zostera marina* at different salinities and temperatures. Marine Biology 8: 48-56.

Bintz, J.C., S.W. Nixon, B.A. Buckley, and S.L. Granger. 2003. Impacts of temperature and nutrients on coastal lagoon plant communities. Estuaries 26: 765-776.

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

Borum, J. 1985. Development of epiphytic communities on eelgrass (*Zostera marina* L.) along a nutrient gradient in a Danish estuary. Marine Biology 87: 211-218.

Borum, J., L. Murray, and W.M. Kemp. 1989. Aspects of nitrogen acquisition and conservation in eelgrass plants. Aquatic Botany 35: 289-300.

Bostrom, C., and E. Bonsdorff. 2000. Zoobenthic community establishment and habitat complexity–the importance of seagrass shoot density, morphology and physical disturbance for faunal recruitment. Marine Ecology Progress Series 205:123-138.

Bostrom, C., C. Roos, and O. Ronnberg. 2004. Shoot morphometry and production dynamics of eelgrass in the northern Baltic Sea. Aquatic Botany 79: 145-161.

Bostrom, C., E. Bonsdorff, P. Kangas, and A. Norkko. 2002. Long-term changes of a Brackish-water eelgrass (*Zostera marina* L.) community indicate effects of coastal eutrophication. Estuarine, Coastal and Shelf Science 55: 795-804.

Brun, F.G., I. Hernandez, J.J. Vergara, G. Peralta, and J.L. Perez-Llorens. 2002. Assessing the toxicity of ammonium pulses to the survival and growth of *Zostera noltii*. Marine Ecology Progress Series 225: 177-187.

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

Castro, M.S., C.T. Driscoll, T.E. Jordan, W.G. Reay, and W.R. Boynton. 2003. Sources of nitrogen to estuaries in the United States. Estuaries 26: 803-814.

Cebrian, J., C.M. Duarte, N.S.R. Agawin, and M. Merino. 1998. Leaf growth response to simulated herbivory: a comparison among seagrass species. Journal of Experimental Marine Biology and Ecology 220: 67-81.

Connolly, R.M. 1994. The role of seagrass as preferred habitat for juvenile *Sillaginoides punctata* (Cuv. & Val.) (Sillaginidae, Pisces): habitat selection or feeding? Journal of Experimental Marine Biology and Ecology 180: 39-47.

Costa, S.L. 1982. The physical oceanography of Humboldt Bay. In: Toole, C., and C. Diebel, editors. Proceedings of the Humboldt Bay Symposium. Eureka, California. 2-31.

De Casabianca, M.-L., T. Laugier, and D. Collart. 1997. Impact of shellfish farming eutrophication on benthic macrophyte communities in the Thau lagoon, France. Aquaculture International 5: 301-314.

DeLorenzo, A. 1999. A study of *Phyllaplysia taylori* in the eelgrass ecosystem of Padilla Bay. Washington State Department of Ecology, Padilla Bay National Estuarine Research Reserve: Mount Vernon, Washington. Padilla Bay National Estuarine Research Reserve Reprint Series No. 29.

Dennison, W.C., R.C. Aller, and R.S. Alberte. 1987. Sediment ammonium availability and eelgrass (*Zostera marina*) growth. Marine Biology 94: 469-477.

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

Duffy, J.E., and A.M. Harvilicz. 2001. Species-specific impacts of grazing amphipods in an eelgrass-bed community. Marine Ecology Progress Series 223: 201-211.

Dykhouse, J.D. 1976. Seasonal dynamics of dominant epiphytic invertebrates on eelgrass (*Zostera marina* L.) in South Humboldt Bay. Master's Thesis, Humboldt State University, Arcata, California, USA.

Flindt, M.R., M.A. Pardal, A.I. Lillebo, I. Martins, and J.C. Marques. 1999. Nutrient cycling and plant dynamics in estuaries: a brief review. Acta Oecologia 20: 237-248.

Fonseca, M.S., and J.A. Cahalan. 1992. A preliminary evaluation of wave attenuation by four species of seagrass. Estuarine, Coastal and Shelf Science 35: 565-576.

Gallegos, C.L. 2001. Calculating optical water quality targets to restore and protect submersed aquatic vegetation: Overcoming problems in partitioning the diffuse attenuation coefficient for photosynthetically active radiation. Estuaries 24: 381-397.

Glasgow, H.B., and J.M. Burkholder. 2000. Water quality trends and management implications from a five-year study of a eutrophic estuary. Ecological Applications 10: 1024-1046.

Green, E.P. and F.T. Short. 2004. Species range maps. Pages 262-286 *in* E.P. Green and F.T. Short, editors. World Atlas of Seagrasses, University of California Press, Berkeley, California.

Hansen, J.W., A.-G.U. Pedersen, J. Berntsen, I.S. Ronbog, L.S. Hansen, and B.A. Lomstein. 2000. Photosynthesis, respiration, and nitrogen uptake by different compartments of a *Zostera marina* community. Aquatic Botany 66: 281-295.

Harding, L.W. Jr. 1973. Primary production in Humboldt Bay. Master's Thesis, Humboldt State University, Arcata, California, USA.

Harlin, M.M., and B. Thorne-Miller. 1981. Nutrient enrichment of seagrass beds in a Rhode Island coastal lagoon. Marine Biology 65: 221-229.

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

Hearn, C.J., and H.S. Sidhu. 2003. Stommel transitions in shallow coastal basins. Continental Shelf Research 23: 1071-1085.

Heck, K.L., Jr., and T.A. Thoman. 1981. Experiment on predator-prey interactions in vegetated aquatic habitats. Journal of Experimental Marine Biology and Ecology 53: 125-134.

Heck, K.L., Jr. and T.A. Thoman. 1984. The nursery role of seagrass meadows in the upper and lower reaches of the Chesapeake Bay. Estuaries 7: 70-92.

Heck, Jr., K.L., G. Hays and R.J. Orth. 2003. Critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Progress Series 253: 123-136.

Hellblom, F., and M. Bjork. 1999. Photosynthetic responses in *Zostera marina* to decreasing salinity, inorganic carbon content and osmolality. Aquatic Botany 65: 97-104.

Hemminga, M.A., and C.M. Duarte. 2000. Seagrass ecology. Cambridge University Press, Cambridge, U.K.

Henry, W.G. 1980. Populations and behavior of black brant at Humboldt Bay, California. M. A. Thesis, Humboldt State University, Arcata, California, USA.

Hintze, J. 2001. NCSS and PASS. Number Cruncher Statistical Systems. Kaysville, Utah. WWW.NCSS.COM

Hoffman, R.S. 1986. Fishery utilization of eelgrass (*Zostera marina*) beds and non-vegetated shallow water areas in San Diego Bay. Southwest Region National Marine Fisheries Service, NOAA, Administrative Report.

Holmer, M., and E.J. Bondgaard. 2001. Photosynthetic and growth response of eelgrass to low oxygen and high sulfide concentrations during hypoxic events. Aquatic Botany 70: 29-38.

Holmer, M., and S.L. Nielsen. 1997. Sediment sulfur dynamics related to biomass-density patterns in *Zostera marina* (eelgrass) beds. Marine Ecology Progress Series 146: 163-171.

Hovel, K.A. 2003. Habitat fragmentation in marine landscapes: relative effects of habitat cover and configuration on juvenile crab survival in California and North Carolina seagrass beds. Biological Conservation 110: 401-412.

Hovel, K.A., M.S. Fonseca, D.L. Myer, W.J. Kenworthy, and P.E. Whitfield. 2002. Effects of seagrass landscape structure, structural complexity and hydrodynamic regime on macrofaunal densities in North Carolina seagrass beds. Marine Ecology Progress Series 243: 11-24.

Howard, R.K., and F.T. Short. 1986. Seagrass growth and survivorship under the influence of epiphyte grazers. Aquatic Botany 24: 287-302.

Iiuzumi, H., and A. Hattori. 1982. Growth and organic production of eelgrass (*Zostera marina* L.) in temperate waters of the Pacific Coast of Japan. III. The kinetics of nitrogen uptake. Aquatic Botany 12: 245-256.

Iiuzumi, H., A. Hattori, and C.P. McRoy. 1982. Ammonium regeneration and assimilation in eelgrass (*Zostera marina*) beds. Marine Biology 66: 59-65.

Invers, O., G.P. Kraemer, M. Perez, and J. Romero. 2004. Effects of nitrogen addition on nitrogen metabolism and carbon reserves in the temperate seagrass *Posidonia oceanica.* Journal of Experimental Marine Biology and Ecology 303: 97-114.

Irlandi, E.A. 1996. The effects of seagrass patch size and energy regime on growth of a suspension-feeding bivalve. Journal of Marine Research 54: 161-185.

Irlandi, E.A. 1997. Seagrass patch size and survivorship of an infaunal bivalve. Oikos 78: 511-518.

Irlandi, E.A., B.A. Orlando, and W.G. Ambrose, Jr. 1999. Influence of seagrass habitat patch size on growth and survival of juvenile bay scallops, *Argopecten irradians concentricus* (Say). Journal of Experimental Marine Biology and Ecology 235:21-43.

Jacobs, R.P.W.M. 1979. Distribution and aspects of the production and biomass of eelgrass, *Zostera marina* L., at Roscoff, France. Aquatic Botany 7: 151-172.

Jenkins, G.P., H.M.A. May, M.J. Wheatley and M.G. Holloway. 1997. Comparison of fish assemblages associated with seagrass and adjacent unvegetated habitats of Port Phillip Bay and Corner Inlet, Victoria, Australia, with emphasis on commercial species. Estuarine, Coastal and Shelf Science 44: 569-588.

Jones, M.N. 1984. Nitrate reduction by shaking with cadmium: alternative to cadmium columns. Water Research 18: 643-646.

Joye, S.B., and J.T. Hollibaugh. 1995. Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. Science 270: 623-627.

Keiser, A.L. 2004. A study of the spatial and temporal variation of eelgrass, *Zostera marina*, its epiphytes, and the grazer *Phyllaplysia taylori* in Arcata Bay, California, USA. Master’s Thesis, Humboldt State University, Arcata, California, USA.

Keller, M. 1963. The growth and distribution of eelgrass (*Zostera marina* L.) in Humboldt Bay, California. Master’s Thesis, Humboldt State University, Arcata, California, USA.

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

Kentula, M.E., and C.D. McIntire. 1986. The autecology and production dynamics of eelgrass (*Zostera marina* L.) in Netarts Bay, Oregon. Estuaries 9: 188-199.

Largier, J.L., J.T. Hollibaugh, and S.V. Smith. 1997. Seasonally hypersaline estuaries in Mediterranean-climate regions. Estuarine, Coastal and Shelf Science 45: 789-797.

Lazzari, M.A., S. Sherman, and J.K. Kanwit. 2003. Nursery use of shallow habitats by epibenthic fishes in Maine nearshore waters. Estuarine, Coastal and Shelf Science 56: 73-84.

Lee, K-S., F.T. Short, and D.M. Burdick. 2004. Development of a nutrient pollution indicator using the seagrass, *Zostera marina*, along nutrient gradients in three New England estuaries. Aquatic Botany 78: 197-216.

Lin, H-J., S.W. Nixon, D.I. Taylor, S.L. Granger, and B.A. Buckley. 1996. Responses of epiphytes on eelgrass, *Zostera marina* L., to separate and combined nitrogen and phosphorus enrichment. Aquatic Botany 52: 243-258.

Liu, C., and J.B. Evett. 2003. Soil properties: testing, measurement and evaluation. 5th edition. Prentice Hall, New Jersey.

Manderson, J.P., B.A. Phelan, A.W. Stoner, and J. Hilbert. 2000. Predator-prey relations Between age-1+ summer flounder (*Paralichthys dentatus*, Linnaeus) and age-0 winter flounder (*Pseudopleuronectes americanus*, Walbaum): predator diets, prey selection, and effects of sediments and macrophytes. Journal of Experimental Marine Biology and Ecology 251: 17-39.

Mattilla, J., G. Chaplin, M.R. Eilers, K.L. Heck, Jr., J.P. O'Neal, and J.F. Valentine. 1999. Spatial and diurnal distribution of invertebrate and fish fauna of a *Zostera marina* bed and nearby unvegetated sediments in Damariscotta River, Maine (USA). Journal of Sea Research 41: 321-332.

McGlathery, K.J., N. Risgaard-Petersen, and P.B. Christensen. 1998. Temporal and spatial variation in nitrogen fixing activity in the eelgrass *Zostera marina* rhizosphere. Marine Ecology Progress Series 168: 245-258.

McRoy, C.P., and R.J. Barsdate. 1970. Phosphate absorption in eelgrass. Limnology and Oceanography 15: 6-13.

Moore, J.E., M.A. Colwell, R.L. Mathis, and J.M. Black. 2004. Staging of Pacific flyway brant in relation to eelgrass abundance and site isolation, with special consideration of Humboldt Bay, California. Biological Conservation 115: 475-486.

Moore, K.A., and R.L. Wetzel. 2000. Seasonal variations in eelgrass (*Zostera marina* L.) responses to nutrient enrichment and reduced light availability in experimental ecosystems. Journal of Experimental Marine Biology and Ecology 244: 1-28.

Moore, K.A., R.L. Wetzel, 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: 115-134.

Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Analytical Chemistry, Acta 27: 21-36.

Murphy, M.L., S.W. Johnson and D.J. Csepp. 2000. A comparison of fish assemblages in eelgrass and adjacent subtidal habitats near Craig, Alaska. Alaska Fishery Research Bulletin 7: 11-21.

Murray, D.L., W.C. Dennison, and W.M. Kemp. 1992. Nitrogen vs. phosphorous limitation for growth of an estuarine population of eelgrass (*Zostera marina,* L.).Aquatic Botany 44: 83-100.

Nelson, T.A. 1997. Interannual variance in a subtidal eelgrass community. Aquatic Botany 56: 245-252.

Nelson, T.A., and A. Lee. 2001. A manipulative experiment demonstrates that blooms of the macroalga *Ulvaria obscura* can reduce eelgrass shoot density. Aquatic Botany 71: 149-154.

Nelson, T.A. and J.R. Waaland. 1997. Seasonality of eelgrass, epiphyte and grazer standing stock and productivity in subtidal eelgrass meadows subjected to moderate tidal amplitude. Aquatic Botany 56: 51-74.

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: 1457-1481.

Olesen, B., and K. Sand-Jensen. 1993. Seasonal acclimatization of *eelgrass Zostera marina* growth to light. Marine Ecology Progress Series 94: 91-99.

Olesen, B. and K. Sand-Jensen. 1994. Biomass-density patterns in the temperate seagrass *Zostera marina*. Marine Ecology Progress Series 109: 283-291.

Orth, R.J. 1977. Effect of nutrient enrichment on growth of the eelgrass *Zostera marina* in the Chesapeake Bay, Virginia, USA. Marine Biology 44: 187-194.

Orth, R.J., and K.A. Moore. 1986. Seasonal and year-to-year variations in the growth of *Zostera marina* L. (eelgrass) in the Lower Chesapeake Bay. Aquatic Botany 24: 335-341.

Pastén, G.P., S. Katayama and M. Omori. 2003. Timing of parturition, planktonic duration and settlement patterns of the black rockfish, *Sebastes inermis*. Environmental Biology of Fishes 68: 229-239.

Penhale, P.A., and G.W. Thayer. 1980. Uptake and transfer of carbon and phosphorus by eelgrass (*Zostera marina* L.) and its epiphytes. Journal of Experimental Marine Biology and Ecology 42: 113-123.

Pequegnat, J.E., and J.H. Butler. 1981. The role of nutrients in supporting phytoplankton productivity in Humboldt Bay, California. Sea Grant Coll. Progress Report R-CSGCP-004: 218-222.

Peralta, G., T.J. Bouma, J. van Soelen, J.L. Perez-Llorens, and I. Hernandez. 2003. On the use of sediment fertilization for seagrass restoration: a mesocosm study on *Zostera marina* L. Aquatic Botany 75: 95-110.

Perkins-Visser, E., T.G. Wolcott, and D.L. Wolcott. 1996. Nursery role of seagrass beds: enhanced growth of juvenile blue crabs (*Callinectes sapidus* Rathbun). Journal of Experimental Marine Biology and Ecology 198: 155-173.

Peterson, C.H. 2001. The Exxon Valdez oil spill in Alaska: acute, indirect and chronic effects on the ecosystem. Advances in Marine Biology 39: 1-103.

Peterson, C.H., R.A. Luttich Jr., F. Micheli, and G.A. Skilleter. 2004. Attenuation of water flow inside seagrass canopies of differing structure. Marine Ecology Progress Series 268: 81-92.

Phillips, R.C., W.S. Grant and C.P. McRoy. 1983. Reproductive strategies of eelgrass (*Zostera marina* L.). Aquatic Botany 16: 1-20.

Pinckney, J.L. and F. Micheli. 1998. Microalgae on seagrass mimics: does epiphyte community structure differ from live seagrasses? Journal of Experimental Marine Biology and Ecology 221: 59-70.

Pohle, D.G., V.M. Bricelj, and Z. Garcia-Esquivel. 1991. The eelgrass canopy: an above-bottom refuge from benthic predators for juvenile bay scallops *Argopecten irradians.* Marine Ecology Progress Series 74: 47-59.

Polte, P., A. Schanz, and H. Asmus. 2005. Effects of current exposure on habitat preference of mobile 0-group epibenthos for intertidal seagrass beds (*Zostera noltii*) in the northern Wadden Sea. Estuarine Coastal and Shelf Science 62: 627-635.

Prescott, R.C. 1990. Sources of predatory mortality in the bay scallop *Argopecten irradians* (Lamarck): interactions with seagrass and epibiotic coverage. Journal of Experimental Marine Biology and Ecology 144: 63-83.

Ramage, D.L., and D.R. Schiel. 1999. Patch dynamics and response to disturbance of the seagrass *Zostera novazelandica* on intertidal platforms in southern New Zealand. Marine Ecology Progress Series 189: 275-288.

Risgaard-Peterson, N., and L.D.M. Ottosen. 2000. Nitrogen cycling in two temperate *Zostera marina* beds: seasonal variation. Marine Ecology Progress Series 198: 93-107.

Roberts, M.H. Jr., R.J. Orth, and K.A. Moore. 1984. Growth of *Zostera marina* L. seedlings under laboratory conditions of nutrient enrichment. Aquatic Botany 20: 321-328.

Romero, J., K.-S. Lee, M. Perez, M.A. Mateo, and T. Alcoverro. 2006. Nutrient dynamics in seagrass ecosystems. In: Larkum, A.W.D., R.J. Orth, and C.M. Duarte, editors. Seagrasses: Biology, Ecology and Conservation. Springer, The Netherlands: 227-254.

Rumrill, S.S., and V.K. Poulton. 2004. Ecological role and potential impacts of molluscan shellfish culture in the estuarine environment on Humboldt Bay, Ca. Western Regional Aquaculture Center Annual Report. 44 pps.

Sand-Jensen, K. 1975. Biomass, net production and growth dynamics in an eelgrass (*Zostera marina* L.) population in Vellerup Vig, Denmark. Ophelia 14: 185-201.

Santamaria-Gallegos, N.A., J.L. Sanchez-Lizaso and E.F. Felix-Pico. 2000. Phenology and growth of annual subtidal eelgrass in a subtropical locality. Aquatic Botany 66: 329-339.

Schanz, A., and H. Asmus. 2003. Impact of hydrodynamics on development and morphology of intertidal seagrasses in the Wadden Sea. Marine Ecology Progress Series 261: 123-134.

Schanz, A., P. Polte, and H. Asmus. 2002. Cascading effects of hydrodynamics on an epiphyte-grazer system in intertidal seagrass beds of the Wadden Sea. Marine Biology 141: 287-297.

Schlosser, S., J. Mello, N. Kalson, V. Frey, J. Robinson, and G. Tennant. Accepted. Seasonal and temporal fluctuations in the abundance, size, and above ground leaf biomass of eelgrass in Humboldt Bay, California. CA Fish and Game.

Shaughnessy, F.J., C.L. McGary, A.J. Frimodig, C. Witte, G.B. Roberts, and A. Keiser. Submitted. A conceptual model of community regulation applied to the eelgrass beds of Humboldt Bay, California. Sea Grant.

Short, F.T. 1983. The seagrass *Zostera marina* L.: plant morphology and bed structure in relation to sediment ammonium in Izembek Lagoon, Alaska. Aquatic Botany 16: 149-161.

Short, F.T. 1987. Effects of sediment nutrients on seagrasses: literature review and mesocosm experiment. Aquatic Botany 27: 41-57.

Skeesick, D.G. 1963. A study of some physical-chemical characteristics of Humboldt Bay. Master's Thesis, Humboldt State University, Arcata, California, USA.

Smith, S.V. 1984. Phosphorus versus nitrogen limitation in the marine environment. Limnology and Oceanography 29: 1149-1160.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnology and Oceanography 14: 799-801.

Terrados, J., N.S.R. Agawin, C.M. Duarte, M.D. Fortes, L. Kamp-Nielsen, and J. Borum. 1999. Nutrient limitation of the tropical seagrass *Enhalus acoroides* (L.) Royle in Cape Bolinao, NW Philippines. Aquatic Botany 65: 123-139.

Thom, R.M., and R.G. Albright. 1990. Dynamics of benthic vegetation standing-stock, irradiance, and water properties in central Puget Sound. Marine Biology 104: 129-141.

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

Thursby, G.B., and M.M. Harlin. 1982. Leaf-root interaction in the uptake of ammonia by *Zostera marina*. Marine Biology 72: 109-112.

Touchette, B.W., and J. Burkholder. 2001. Nitrate reductase activity in a submersed marine angiosperm: controlling influences of environmental and physiological factors. Plant Physiology and Biochemistry 39: 583-593.

Touchette, B.W., J.M. Burkholder, and H.B. Glasgow, Jr. 2003. Variations in eelgrass (*Zostera marina* L.) morphology and internal nutrient composition as influenced by increased temperature and water column nitrate. Estuaries 26: 142-155.

Twilley, R.R., W.M. Kemp, K.W. Staver, J.C. Stevenson, and W.R. Boynton. 1985. Nutrient enrichment of estuarine submersed vascular plant communities. 1. algal growth and effects on production of plants and associated communities. Marine Ecology Progress Series 23: 179-191.

Udy, J.W., and W.C. Dennison. 1997. Growth and physiological responses of three seagrass species to elevated sediment nutrients in Moreton Bay, Australia. Journal of Experimental Marine Biology and Ecology 217: 253-277.

Valiela, I., G. Tomasky, J. Hauxwell, M.L. Cole, J. Cebrian, and K.D. Kroeger. 2000. Operationalizing sustainability: management and risk assessment of land-derived nitrogen loads to estuaries. Ecological Applications 10: 1006-1023.

Valle, C.F., J.W. O'Brien, and K.B. Wiese. 1999. Differential habitat use by California halibut, *Paralichthys californicus*, barred sand bass, *Paralabrax nebulifer*, and other juvenile fishes in Alamitos Bay, California. Fishery Bulletin 97: 646-660.

van Katwijk, M.M., G.H.W. Schmitz, A.P. Gasseling and P.H. van Avesaath. 1999. Effects of salinity and nutrient load and interaction on *Zostera marina*. Marine Ecology Progress Series 190: 155-165.

van Katwijk, M.M., L.H.T. Vergeer, G.H.W Schmitz, and J.G.M. Roelofs. 1997. Ammonium toxicity in eelgrass *Zostera marina*. Marine Ecology Progress Series 157: 159-173.

van Lent, F., J.M. Verschuure, and M.L.J. van Veghel. 1995. Comparative study on populations of *Zostera marina* L. (eelgrass*): in situ* nitrogen enrichment and light manipulation. Journal of Experimental Marine Biology and Ecology 185: 55-76.

Vergeer, L.H.T., T.L. Aarts, and D.D. de Groot. 1995. The 'wasting disease' and the effect of abiotic factors (light intensity, temperature, salinity) and infection with *Labyrinthula zosterae* on the phenolic content of *Zostera marina* shoots. Aquatic Botany 52: 35-44.

Wear, D.J., M.J. Sullivan, A.D. Moore, and D.F. Millie. 1999. Effects of water column enrichment on the production dynamics of three seagrass species and their epiphytic algae. Marine Ecological Progress Series 179: 201-213.

Webster, P.J., A.A. Rowden, and M.J. Attrill. 1998. Effect of shoot density on the infaunal macro-invertebrate community within a *Zostera marina* seagrass bed. Estuarine, Coastal and Shelf Science 47: 351-357.

Williams, S.L., and K.L. Heck, Jr. 2001. Seagrass community ecology. *in* Bertness, M.D., S.D. Gaines, and M.E. Hay, editors. Marine Community Ecology. Sinauer Assoc., Sunderland, MA. 317-337.

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

Wium-Andersen, S., and J. Borum. 1984. Biomass variation and autotrophic production of an epiphyte-macrophyte community in a coastal Danish area: I. Eelgrass (*Zostera marina* L.) biomass and net production. Ophelia 23: 33-46.

Worm, B., and T.B.H. Reusch. 2000. Do nutrient availability and plant density limit seagrass colonization in the Baltic Sea? Marine Ecology Progress Series 200: 159-166.

Zimmerman, R.C., R.D. Smith, and R.S. Alberte. 1987. Is growth of eelgrass nitrogen limited? A numerical simulation of the effects of light and nitrogen on the growth dynamics of *Zostera marina*. Marine Ecology Progress Series 41: 167-176.

Zimmerman, R.C., R.D. Smith, and R.S. Alberte. 1989. Thermal acclimation and whole-plant carbon balance in *Zostera marina* L. (eelgrass). Journal of Experimental Marine Biology and Ecology 130: 93-109.