AN ABSTRACT OF THE THESIS OF

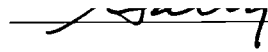Lorena Michelle Wisehart for the degree of

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Title: Impacts of Oysters on Eelgrass (*Zostera marina* L.): Importance of Early Life

History Stages in Response to Aquaculture Disturbance.

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The importance of seagrasses (families Posidoniaceae, Zosteraceae, Hydrocharitaceae and Cymodoceaceae) to estuarine communities is widely accepted and this, combined with their continued decline throughout the world, have resulted in a need to better understand the factors affecting their growth and reproduction. Conservation and restoration of seagrasses will require an understanding of their population biology including the role of seeds in maintaining populations following disturbance. On the US west coast, shellfish aquaculture can co-occur with protected eelgrass (*Zostera marina* L.). Many aquaculture practices constitute a pulse perturbation, and a key question concerns the ability of eelgrass to recover. This thesis addresses the impacts of oyster aquacultural disturbance on these early life history stages.

I studied seed production, germination, and seedling growth and survival of eelgrass under different oyster aquaculture practices: dredging and off-bottom longline culture. Germination of experimentally added seeds was highest in dredged areas, where adult shoot densities were lowest. Seedlings survived better and were bigger in plots where adult plants had been removed. Natural seedling recruitment and seed production were highest in dredged beds compared to longline beds and reference areas. From these data, I propose that the greater recruitment in dredged beds is due to both enhanced seed densities as well as removal of neighboring adult plants. Low success in longlines may be due to a combination of physical factors including increases in sediment accretion and significantly lower redox values. Dredging can enhance or at least maintain seed density and seed germination, but longline aquaculture appears to significantly reduce eelgrass recruitment.

I also address seed production and seed germination patterns across five sites in Willapa Bay, WA and discuss the relative roles of seed supply, physical factors, and biological factors in driving those patterns. Some of the variation in natural recruitment could be explained by patterns of flowering and seed production with some sites that had higher seedling numbers producing significantly more propagules. Recruitment was highest under colder water temperatures, while other physical factors (redox potential, sediment grain size, and sediment accretion) were less important. To understand what may be influencing seedling density, I conducted a seed addition experiment that showed differences in germination and early seedling survival were controlled in part by number of adult neighbors, although adult densities at the larger scale did not predict germination success. The results of this bay-wide study suggest that *Z. marina* recruitment patterns in

Willapa Bay are driven primarily by seed abundance, but differences in germination

success were also important.

Impacts of Oysters on Eelgrass (*Zostera marina* L.): Importance of Early Life History Stages in Response to Aquaculture Disturbance.

by
Lorena Michelle Wisehart

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CHAPTER 1. GENERAL INTRODUCTION

Seagrasses, a group of marine vascular plants from four families (Posidoniaceae, Zosteraceae, Hydrocharitaceae and Cymodoceaceae), are distributed worldwide and inhabit all types of substrate in both temperate and tropical regions. Seagrasses are among the most productive ecoystems in the world and they contribute to stabilization of sediments and nutrient transformation and also provide nursery and feeding habitat for many associated species (Phillips 1984, Thayer et al. 1984). Of the seagrasses, eelgrass (*Zostera marina* L.) is globally distributed, locally abundant, and is arguably one of the most important marine angiosperms worldwide. In coastal estuaries of the Pacific Northwest eelgrass plays a fundamental role in maintaining biotic and abiotic processes of estuaries (Williams and Heck 2001, Krause- Jensen et al. 2003). Eelgrass is an important source of detritus (Vahatalo and Sondergaard 2002); it cycles oxygen and carbon from the water column to the benthos (Vahatalo and Sondergaard 2002), and it functions as a nursery for commercial species and provides refuge and foraging opportunities for numerous other species (Phillips 1984, Wyllie-Echeverria et al. 1994, Williams and Heck 2001). The plant is also an important food source for migratory black brant (Derksen and Ward 1993) and other waterfowl. Many other organisms rely on eelgrass for food, shelter, and substrate (Ricketts et al. 1985, Little 2000, Duffy and Harvilicz 2001, Williams and Heck 2001, Zupo et al. 2001). Furthermore, many of the commercially valuable marine species in the United States occupy seagrass habitat at some stage in their life histories (Williams and Heck 2001).

North American and Western European eelgrass populations have declined significantly in the last 20-30 years (Short and Wyllie-Echeverria 1996). The declines have largely been the result of increasing anthropogenic activities that have included alterations of water quality or clarity, nutrient and sediment loading from runoff and sewage disposal, dredging and filling for navigation, pollution, upland development, and commercial fishing and aquaculture (Fonseca et al. 1984, Short and Burdick 1996, Short and Wyllie-Echeverria 1996).

Negative impacts of shellfish aquaculture activities on seagrasses are becoming increasingly more evident (Simenstad and Fresh 1995, Cabaço et al. 2005, Neckles et al. 2005). Recreation and commercial shellfish harvest has been shown to reduce seagrass biomass, density, and percent cover in harvested areas (Fonseca et al. 1984, Everett et al. 1995, Boese 2002, Thom et al. 2003, Cabaço et al. 2005, Neckles et al. 2005).

While there have been studies looking at the effects of aquaculture on the vegetative stages of eelgrass, the long-term effects of aquaculture on this species cannot be predicted without a better understanding of effects on this species reproductive rates. Understanding the interaction between shellfish aquaculture and early eelgrass life history stages is essential for developing sustainable aquaculture practices. In Chapter Two, **Impacts of oysters on eelgrass (*Zostera marina* L.): Importance of early life history stages in response to aquaculture disturbance**, I explore the interactions between oyster aquaculture practices and eelgrass recruitment and germination in Willapa Bay, Washington. I present results from both observational and experimental studies that address the role of early life history stages in the maintenance of eelgrass populations subject to aquaculture disturbance. In this chapter, I propose mechanisms

responsible for high seed production and germination in dredged oyster aquaculture beds and provide insight about the potential for recovery via sexual reproduction in oyster aquaculture areas.

Chapter Three, **Spatial variation in eelgrass recruitment from seed: influence of seed production, physical factors and adult neighbors**, describes eelgrass recruitment patterns at five different sites in Willapa Bay, WA. In this chapter I discuss the relative role of seed supply, physical factors, and biological interactions in determining early life history stage patterns. I present the results of an observational study describing patterns of natural seedling recruitment in Willapa Bay, WA, and I relate those patterns to differences in environmental factors and possible adult-seedling interactions. I discuss the results of a seed sowing experiment designed to determine whether once a seed arrives at a site, seed germination and seedling survival are dependent on local factors such as sediment type and/or biological interactions.

In Chapter Three I also discuss the use of seeds and seedlings in restoration projects. Although preservation of existing eelgrass beds and implementation of sustainable practices affecting eelgrass are critical to preventing further population decline, replacement of lost eelgrass may also be possible through effective restoration. Most restoration projects to date have consisted of transplanting adult plants from a donor site. However, many researchers now encourage the use of seeds and/or seedlings in restoration projects because seeds are generally abundant and the impacts to existing beds are lessened. In order for restoration via seeding to succeed, the habitat needs of seeds and seedlings must be addressed. While past research on eelgrass habitat requirements has focused on mature vegetative plants, this thesis provides much needed information

describing habitat requirements of eelgrass seeds and seedlings in a Pacific Northwest estuary.

I conclude this thesis in Chapter Four by summarizing the general conclusions from each of the two data chapters. I combine results describing the impacts of oyster aquaculture activities on early life history stages from Chapter Two with findings from Chapter Three describing patterns of spatial variation in eelgrass recruitment to discuss implications for conservation and management of eelgrass populations in the Pacific Northwest. I also describe some of the limitations of the two studies and provide suggestions for further research.

CHAPTER 2. IMPACTS OF OYSTERS ON EELGRASS (*ZOSTERA MARINA* L.): IMPORTANCE OF EARLY LIFE HISTORY STAGES IN RESPONSE TO AQUACULTURE DISTURBANCE

Abstract

Aquaculture is increasing worldwide, however we have little understanding of its impacts on marine communities. A critical element of many marine communities are seagrasses, a group of globally distributed marine angiosperms that are drivers of many abiotic and biotic processes in estuarine and marine communities. As with other seagrasses, the globally distributed eelgrass (*Zostera marina* L.) has been declining throughout its range. On the US west coast, shellfish aquaculture co-occurs with protected eelgrass. Many aquaculture practices constitute a pulse perturbation, and a key question concerns the ability of eelgrass to recover. We studied seed production, germination, and seedling growth and survival of eelgrass under different oyster aquaculture practices: dredging and off-bottom longline culture. To study germination, we added seeds to two different aquaculture types, as well as eelgrass reference areas, in paired control and eelgrass removal plots. Germination of experimentally added seeds was highest in dredged areas, where adult shoot densities were lowest. Seedlings survived better and were bigger in plots where adult plants had been removed. We also found natural seedling recruitment and seed production to be highest in dredged beds and lowest in longlines. We propose that greater recruitment in dredged beds is due to both enhanced seed densities as well as removal of neighboring adult plants. Low success in longlines may be due to a combination of physical factors including increases in sediment accretion and significantly lower redox values. Harvest dredging can enhance or at least

maintain seed density and seed germination, but longline aquaculture appears to significantly reduce eelgrass recruitment.

Introduction

Seagrasses, a group of marine angiosperms in the families Posidoniaceae, Zosteraceae, Hydrocharitaceae and Cymodoceaceae, are important components of estuarine communities, because they are among the most productive plants on earth (Duarte et al. 1999) and influence community structure and function through a combination of physical, chemical and biological mechanisms (Phillips 1984, Thayer et al. 1984). Additionally, many commercially and ecologically valuable species utilize seagrass beds as nurseries and/or refuge areas (Orth et al. 1984, Phillips 1984, Thayer et al. 1984, Jackson et al. 2001, Johnson et al. 2003). Seagrasses have been declining worldwide, largely due to increases in anthropogenic disturbance (Short and Burdick 1996) such as lowered water quality or clarity, nutrient and sediment loading from runoff and sewage disposal, dredging and filling for navigation, pollution, upland development, and commercial fishing (Fonseca et al. 1984, Short and Burdick 1996, Short and Wyllie-Echeverria 1996).

The impacts that aquaculture activities may have on seagrass populations are also of increasing concern (Simenstad and Fresh 1995, Neckles et al. 2005) because of the potential negative effects resulting from both recreational and commercial shellfish harvest, including reduced seagrass biomass, density, and percent cover in harvested areas (Fonseca et al. 1984, Everett et al. 1995, Boese 2002, Orth et al. 2002a, Thom et al. 2003, Cabaço et al. 2005, Neckles et al. 2005). Hydraulic dredges used to harvest oysters

can cause long-term disruption of eelgrass (*Zostera marina*) beds, reducing or even eliminating beds in some cases (Waddell 1964, Phillips 1984). Additional aquaculture activities, including the dumping of shell on eelgrass beds and mechanical raking or trampling, are also of concern, though few studies have addressed these impacts (NMFS 2001).

There are also examples of positive interactions between shellfish and seagrasses (Reusch et al. 1994, Peterson and Heck 1999, 2001). When a suspension-feeding mussel *Modiolus americanus* (Leach) was added to meadows of *Thalassia testudinum* (Banks ex König) an increase in sediment nutrients that were biologically available to the seagrass was noted (Peterson and Heck 2001). The response of *T. testudinum* included increased leaf widths and lengths as well as greater productivity in mussel addition areas, largely due to reduced epiphyte loads on the leaves (Peterson and Heck 2001).

Shellfish farming is an important industry along the northwest coast of the U.S. Washington State leads the nation in production of farmed bivalve shellfish, and Willapa Bay (WA) produces nearly 9% of US total oyster harvest (Ruesink et al. 2006). The two most common oyster aquaculture methods used in Willapa Bay are ground culture and longline culture (Quayle and Newkirk 1989, Conte et al. 1996, Feldman et al. 2000), although the former is much more abundant than the latter. Ground culture involves the placement of oysters directly on the sediment in a single layer (Feldman et al. 2000). These oysters may then be harvested by hand or by mechanical dredging. When harvest dredging occurs in areas with eelgrass, it is sometimes preceded by manual removal of above-ground eelgrass biomass (plants are often clipped at the surface to facilitate oyster removal), and below-ground roots and rhizomes may be removed inadvertently during

the harvest dredging operation (Simenstad and Fresh 1995). Additional management activities include mechanical harrowing and leveling of the culture areas (Quayle and Newkirk 1989, Conte et al. 1996). In contrast, longline culture entails suspending oysters above the sediment surface (poly vinyl chloride (PVC) pipe-stakes and rope). While in some areas "off-bottom" methods (e.g., stakes, racks) are implemented due to unsuitable conditions for ground culture (Simenstad and Fresh 1995), in Willapa Bay many beds have been switched recently from ground- to off-bottom culture (Ruesink personal observation). In other regions of the country (e.g., in Humboldt Bay, CA), regulators have required that use of ground culture be discontinued, and these areas have been replaced with longline culture due to a perceived reduction in negative impacts.

Although little is known about the response of vegetative stages to aquaculture (but see Tallis et al. in review), even less is known about the influence on seed recruitment and germination. The few studies that have addressed recruitment have reported contrasting effects. For example, Fonseca et al. (1984) proposed that dredging for scallops in isolated eelgrass beds could adversely affect the continuation and/or recovery of that meadow due to a reduction in the number of seeds and seedlings available to aid in recolonization. They ultimately concluded that the sexual and vegetative reproduction of eelgrass meadows could be severely hindered by repeated scallop dredging. More recently, Alexandre et al. (2005) reported an increase in *Z. noltii* reproductive effort that coincided with a decrease in shoot density following experimental clam harvest.

Although most eelgrass meadows persist by vegetative growth (Bell and Tomlinson 1980), the production and dispersal of seeds are still important for

maintaining eelgrass populations. Despite low seedling densities and growth rates, seedling recruitment is thought to be a regular and reliable source of recovery following disturbance, especially in larger areas where vegetative regrowth from the perimeter is limited (Whitfield 2004). Seeds are especially important in maintaining meadows that are subject to seasonal perturbations or other disturbances (Thayer et al 1984). Thus, understanding the interaction between shellfish aquaculture and early eelgrass life history stages is essential for developing sustainable oyster aquaculture practices.

Dredge harvesting of oysters typically reduces eelgrass biomass and shoot density (Tallis et al. in review), however, oyster growers have reported that eelgrass rapidly reappears in areas planted with oysters, which requires high levels of eelgrass recruitment into these disturbed areas. There are two potential mechanisms to explain high recruitment. One is that oysters influence eelgrass seed production, seed germination and/or seedling survival by altering the nutrient or light environment through filter feeding and feces/pseudofeces deposition or by trapping/protecting seeds. The other is that disturbance resulting from aquaculture activities (e.g., dredge harvest) affects eelgrass seed production, seed germination and/or seedling survival by removing eelgrass neighbors or modifying the physical characteristics of the environment (e.g. loss of fine sediments following dredge harvest). In this paper we explore these mechanisms by looking at the interaction between oyster aquaculture practices and eelgrass recruitment and germination in Willapa Bay, Washington. We present results from both observational and experimental studies that address the variable impacts of two different aquaculture types as well as the relative importance of seed supply, germination success and adult-seedling interactions in determining patterns of natural recruitment.

Methods

Study site

Willapa Bay (46°40'N, 124 ° 0'W) is a coastal estuary (260 km^2 at mean high

water) characterized by broad extensive tideflats and large tidal amplitude.

Approximately 21% of the bay's intertidal ground is commercially farmed and managed

for oysters (Feldman et al. 2000), the dominant species being the Pacific oyster

(*Crassostrea gigas*), which is native to Japan (Feldman et al. 2000). Oyster aquaculture

occurs from -1 m to +1 m, which overlaps with eelgrass beds that are usually below 0m

MLLW (Thom et al. 2003). Although it is generally restricted to the highest intertidal

zones, *Zostera japonica*, a non-native seagrass species, can also overlap with *Z. marina*

(Wisehart pers. obs.).

We conducted our studies at Stony Point, a nearshore site in the northeastern

section of Willapa Bay. This site is heavily influenced by both the Willapa and Palix

rivers and it's proximity to the bay mouth results in channel salinities that are oceanic (25

– 32ppt; Ruesink et al. 2003) . As a result, sediments at the site have high silt to sand

ratios and salinity is highly variable. Dredged ground culture beds and longlines are

present at Stony Point. These aquaculture areas are separated from clam beds in the high

intertidal by a large relatively un-manipulated eelgrass meadow.

Patterns of natural eelgrass seedling recruitment and seed supply

To understand the pattern of eelgrass seedling recruitment under different

aquaculture types, we surveyed eelgrass seedlings in March, April, and August 2005.

Thirty quadrats ($0.25m^2$) were haphazardly tossed in three spatially separate areas ($\approx 30m$ apart) within each aquaculture type (longlines, dredged, and eelgrass reference sites; n = 90) and the number of *Z. marina* seedlings, the number of vegetative *Z. marina* adult shoots, the percent cover of oysters, the percent cover of *Z. japonica* and the percent cover of macroalgae were recorded.

In July 2005, we surveyed flowering shoot densities by haphazardly tossing 50 quadrats ($0.25 \ m^2$) in three separate areas in the three aquaculture types (n = 150). In addition, a total of 30 flowering shoots were selected from the quadrats in order to estimate the number of seeds produced. In the lab, we estimated average seed production per flowering shoot by counting the number of spathes per shoot and multiplying that by the average number of seeds per spathe [seeds included total number of female flowers (ovaries) and fruits (fertilized ovaries)]. We then estimated the number of seeds produced per $0.25m^2$ by multiplying the number of flowering (seed producing) shoots per quadrat by the mean number of seeds produced per plant for that aquaculture type.

In January 2006, we surveyed *Z. marina* seeds in the sediment (after seed rain but prior to germination). Five cores (10 cm diameter x 15 cm depth) were haphazardly collected in each of three areas for each aquaculture type (n = 15). Oysters were harvested from the dredged areas just prior to the survey. The samples were first sieved in the field using a 0.5 mm sieve then materials left in the sieve from each sample were bagged individually and taken to the lab where they were visually inspected for the number of fully developed seeds and empty seed coats of *Z. japonica* and *Z. marina*.

Experimental manipulations of eelgrass recruitment

To understand whether germination would vary under different aquaculture types if seed supply were equivalent, we conducted an eelgrass seed addition experiment. We collected seed-bearing shoots from several sites throughout Willapa Bay and transferred them to large outdoor tanks partially filled with seawater. Once seeds fell from the parent plants, they were collected from the bottom of the tanks and stored at 4 deg C in filtered seawater in the dark until used for the experiment. Packets (10 cm dia.), designed to contain the seeds and allow them to remain on the sediment surface, were constructed using mesh fabric with an inner envelope of biodegradable paper (Dethier et al. 2005). Seeds were sown in the two aquaculture types (dredged and longlines) as well as non-cultured eelgrass meadows (reference sites). Five paired control and eelgrass removal plots (0.25 m^2) were created in each of three areas and one packet, containing 30 seeds, was secured with wire stakes directly on top of the sediment in each plot (n = 15 per treatment per aquaculture type). To maintain the eelgrass removal treatment, adult plants were pulled from the eelgrass removal plots in August 2004, March 2005, and April 2005.

Seed germination and seedling survival in the packets were monitored in March, April, and August of 2005. In addition, the number of *Z. marina* shoots, seedlings, and the percent cover of oysters and algae were determined for each of the 0.25 m^2 quadrats surrounding the packets. In March, we discovered that 15 packets were destroyed following oyster harvest and we were unable to locate an additional 15 packets in the dredged aquaculture type. To monitor seedling emergence and survival, a "table" was constructed with PVC pipe as the frame and a transparent Plexiglass sheet as the top (22

x 25 cm). The table was placed over the mesh packets at each plot, an acetate sheet was laid on the Plexiglass sheet, and the location and number of *Z. marina* seedlings and adults were marked with permanent ink. This mapping allowed for the determination of the date of emergence, branching, flowering, and disappearance of seedlings. In August 2005, all of the remaining seedlings were collected, measured for their length (cm), and dried to determine their biomass (g).

Physical characteristics

Sediment characteristics were measured in the different aquaculture types in April, 2005. Sediment redox potential was measured adjacent to each of the seed addition plots (n = 15 per treatment per aquaculture type) using a redox meter fitted with a platinum redox electrode and filled with AgCl solution (Thermo Orion Company, Beverly, MA, USA). Sediment grain size (silt to sand ratio) was determined by collecting three sediment samples (~50 g) from the sediment surface of three areas in each of the aquaculture types (n = 9), weighing before and after drying and wet sieving through a 63 μm sieve followed by a 2 mm sieve (0-63 μm = silt, 63 μm ≥ 2mm = sand). In addition, sediment accretion was determined by measuring depth of sediment deposition on the seed packets in June 2005, based on three measurements per packet with a ruler.

Light intensity ($\log$ Lum m^{-2}) was measured in January 2006 using HOBO® light intensity loggers (Onset Computer Corp., Pocasset, MA, USA). Three loggers were vacuum sealed and placed at the sediment surface of each aquaculture type by attaching them to L-shaped PVC pipes anchored in the sediment. The loggers recorded intensity every 3 minutes for a 24-hour period.

Statistical analyses

Differences in natural seedling density, adult eelgrass shoot density, and percent cover of oysters and macroalgae among aquaculture types were tested using a non-parametric Kruskal-Wallis test because the data were not normally distributed. The relationship between oyster cover and seedling density was analyzed with linear regression. The Kruskal-Wallis one-way ANOVA was also used to test for differences in seed production (per $0.25m^{2)}$ between aquaculture types, while a one factor ANOVA was used to test for differences in seed density in sediments. A two factor ANOVA was used to test for the effects of eelgrass treatment, aquaculture type, and their possible interaction, on seedling density in the seed addition experiments. Since many seedlings died by the end of the seed addition experiment, one factor ANOVA's were used to test for differences in seedling length and biomass among the removal and control treatments in dredged areas as well as the eelgrass removal treatment. One factor ANOVAs were also used to test for differences in redox potential, light intensity, sediment grain size and sedimentation among the aquaculture types. Scheffe's multiple comparison test was used as a post hoc test to examine all significant factors ($P < 0.05$). Data were transformed when necessary (Gotelli and Ellison 2004) although untransformed values are shown in figures. Analyses were run using NCSS 2001 (NCSS, Kaysville, UT, USA).

Results

Patterns of natural eelgrass seedling recruitment and seed supply

The density of *Z. marina* seedlings varied significantly among the three aquaculture types surveyed (Figure 1A; Kruskal Wallis one factor ANOVA; df = 2, H = 61.2, p < 0.001). Significantly fewer seedlings were observed in the longlines compared to dredged and reference areas, which did not significantly differ. In contrast, the density of adult eelgrass shoots was greatest in the reference areas compared to the longline and dredged areas, which did not differ (Figure 1B; Kruskal Wallis one factor ANOVA; df = 2, H = 107.5, p < 0.001). Macroalgal cover was also greatest in the dredged beds (Figure 1C; Kruskal-Wallis one factor ANOVA; df = 2, H = 55.9, p < 0.001). Benthic oyster abundance was greatest in the dredged beds compared to the longline beds and eelgrass beds, which did not differ (Figure 1D; Kruskal Wallis one factor ANOVA; df = 2, H = 181.9, p < 0.001), however, oyster (*C. gigas*) biomass may be underestimated by cover because longline oysters hang in clumps. The number of naturally occurring eelgrass seedlings was negatively correlated with percent cover of *C. gigas* in dredged areas (Figure 2).

Seed production varied significantly among aquaculture types (Figure 3A; Kruskal-Wallis one factor ANOVA: df = 2, H = 7.81, p = 0.020). More seeds were produced in the dredged beds than in the reference and longline areas, and lowest seed production occurred in the longlines. Seed bank densities also differed significantly among aquaculture types (Figure 3B; one factor ANOVA: $F_{2,41}$ = 28.56, p < 0.001). The greatest number of seeds was found in the dredged areas with similar but lower seed densities observed in the longline and reference areas (~15% less than dredged areas).

Experimental manipulations of recruitment

The number of seedlings in the packets varied between control and eelgrass removal treatments and among aquaculture types depending on date (Figure 4 A-C). In March, seedling densities were 146% higher in eelgrass removal treatments compared to control plots (averaged across all aquaculture types). Statistically, a two factor ANOVA revealed an eelgrass removal effect ($F_{1,82} = 11.9$, $p < 0.001$) but no aquaculture type effect ($F_{2,82} = 2.6$, $p = 0.085$) or interaction between the two factors (Figure 4A; $F_{2,82} = 1.3$, $p = 0.288$). By April, the eelgrass removal effect had disappeared (Figure 4B; $F_{1,104} = 0.2$, $p = 0.678$), however, seedling densities varied significantly among aquaculture types ($F_{2,104} = 9.8$, $p < 0.001$). The interaction between culture type and eelgrass removal was not significant ($F_{2,104} = 0.3$, $p = 0.767$). There were significantly more seedlings in the reference areas compared to the longline beds (Scheffe's $p < 0.05$). These results remained fairly consistent through August, when seedling densities varied with aquaculture type ($F_2 = 4.0$, $p = 0.021$), but not with eelgrass removal ($F_1 = 3.0$, $p = 0.087$) or the interaction of these factors (Figure 4C; $F_2 = 1.6$, $p = 0.204$). Significantly more seeds were present in dredged beds and reference areas compared to longline beds where no seedlings survived (Scheffe's $p < 0.05$).

Abundances of other macrophytes and oysters in the seed addition plots varied among the longline beds, dredged beds, and reference areas. In April 2005, benthic oysters were absent or nearly so in longline beds (but overall abundance was undersampled because oysters on lines were not sampled) and reference areas but abundant in dredged beds (2 factor ANOVA: aquaculture type $F_{2,103} = 109.9$, $p < 0.01$, treatment $F_{1,103} = 2.2$, $p = 0.14$, interaction $F_{2,103} = 2.2$, $p = 0.11$). Cover of macroalgae

was greatest in dredged beds compared to reference and longline areas (site: $F_{2,103} = 18.2$, $p < 0.01$, treatment: $F_{1,103} = 0.2$, $p = 0.662$, interaction: $F_{2,103} = 1.5$, $p = 0.223$). Finally, there was substantial spatial variation in adult eelgrass density, only part of which was related to the removal treatment. Densities varied among aquaculture types and eelgrass removal treatments (Table 1; 2 factor ANOVA, site: $F_{2,103} = 11.8$, $p < 0.01$, treatment: $F_{1,103} = 41.9$, $p < 0.01$) with a significant interaction between the two factors ($F_{2,103} = 7.2$, $p < 0.01$). The eelgrass removal treatment led to significant differences in adult shoot density in reference and longline areas. However, adult shoot density did not differ significantly between the control and eelgrass removal treatments (one factor ANOVAs; reference: $F_1 = 36.7$, $p < 0.01$, longlines: $F_1 = 69.3$, $p < 0.01$, dredged: $F_1 = 0.7$, $p = 0.432$). Overall, adult eelgrass densities were lowest in dredged beds, even relative to removal treatments within reference areas.

By August, seedling length and biomass in eelgrass removal treatments were similar between reference and dredged areas whereas the control plots in the dredged areas had smaller seedlings (Figure 5A, B). Seedling length did not differ significantly between dredged and reference areas or between control and eelgrass removal treatments (one factor ANOVA: dredged control vs. dredged removal, $F_{1,4} = 3.4$, $p = 0.139$; dredged removal vs. reference removal, $F_{1,9} = 0.1$, $p = 0.761$; dredged control vs. reference removal, $F_{1,9} = 2.3$, $p = 0.166$). The difference in seedling biomass between dredged control and eelgrass removal treatments was nearly significant (one factor ANOVA $F_{1,4} = 6.8$, $p = 0.059$) while seedling biomass did not differ significantly between dredged and reference areas (one factor ANOVA (dredged eelgrass removal vs. reference eelgrass removal, $F_{1,5} = 0.3$, $p = 0.617$; dredged control vs. reference eelgrass removal, $F_{1,5} = 2.4$,

p = 0.179). A trend toward decreasing seedling biomass with increasing density of adult shoots (Figure 5C) suggests that seedling biomass (per individual) is negatively correlated with adult density.

Physical characteristics

Sediment characteristics varied among aquaculture types. Redox potential was lowest under longlines, intermediate in dredged beds, and highest in eelgrass (Figure 6A; one factor ANOVA, $F_{2,105}$ = 13.0, p < 0.001, Scheffe's p < 0.05). This result was not driven exclusively by sediment grain size, which showed a different pattern. Silt:sand ratios were higher in longline and reference areas than dredged beds (Figure 6C; one factor ANOVA, $F_{2,24}$ = 7.3, p < 0.001, Scheffe's p < 0.05). Grain size was likely influenced by sediment accretion, which was significantly higher in longline and reference areas compared to dredged beds (Figure 6D; one factor ANOVA, $F_{2,105}$ = 8.9, p < 0.001, Scheffe's p < 0.05). Light intensity also varied significantly with aquaculture type (Figure 6B; one factor ANOVA, $F_{2,987}$ = 158.9, p < 0.001). There was significantly less light in the reference area compared to the longline and dredged aquaculture areas (Scheffe's p < 0.05).

Discussion

Our data suggest there is a strong relationship between oyster aquaculture type and eelgrass seedling success. Observational and experimental results show that seedlings are most abundant and have higher initial success in dredged beds compared to longline culture areas (Figure 1A, Figure 4A-C). While we found slightly more seedlings in the

dredged beds compared to reference areas, the difference was not significant. Several factors may be driving this pattern but the most important is likely the presence of adult eelgrass neighbors. We found higher seedling densities in dredged beds that had low density of adult plants compared to reference areas where adult density was significantly greater (Figure 1B). Furthermore, when neighbors were removed; seedlings survived better (Figure 4) and were significantly larger (Figure 5A-C).

Dredge harvest of oysters, which results in decreased eelgrass density due to the removal of above ground plant structures (Tallis et al. in review), may facilitate seed germination by reducing competition for light. Our results suggest that light, mediated by eelgrass blade density, is important for germination in all aquaculture types, but the importance disappears as summer progresses, probably due to an increase in light availability with increasing day length. Furthermore, light may determine seedling size, but it likely does not impact population size, as natural seedling densities were unrelated to adult shoot densities. Similar patterns have been observed in eelgrass populations responding to natural disturbances. For example, Robertson and Mann (1984) found that seed germination was greater in areas where ice scour removed large amounts of above ground eelgrass biomass, compared to areas where plants were left undisturbed. Although these authors did not find an effect of canopy shading on seedling survival, they showed that competition for light with adult plants negatively affected morphology, growth and the allocation of seedling biomass to below-ground plant parts.

Light may also be involved in controlling low success of eelgrass seedlings in longline beds (Figures 1A, 4A-C). We observed very few naturally recruiting seedlings in longline areas and survivorship of seedlings in longline seed addition plots was zero.

Although adult eelgrass densities in the longlines were similar to dredged beds, we know light was reduced (Figure 6) most likely due to the PVC structures and suspended oysters. Previous studies have shown that longline structures and algal mats that form around them may shade seedlings to the point that productivity is severely limited and the seedlings are unable to persist (Pregnall 1993, Everett et al. 1995).

The pattern of low seedling abundance in the longlines and higher densities in dredged areas may also be due to differences in seed supply. Our data suggest that seed production and seed bank densities are both high in dredged areas compared to longlines. Everett et al. (1995) also reported significantly greater recruitment in eelgrass reference areas compared to plots in stake culture areas. The increase in seed production within dredged areas relative to the reference sites may also be related to light availability or other factors associated with dredging disturbance. The effects of light availability on flowering and seed production have not been well studied, but flowering generally occurs in areas higher in the intertidal where there is more light available as well as an increase in physical stressors (e.g., desiccation, temperature extremes, etc.) (Thayer et al. 1984). The longline and reference areas at Stony Point did not typically drain at low tide, while the dredged areas were often exposed (personal observation LMW). Thus plants in the dredged areas may be responding to desiccation stress by flowering at higher percentages. Additionally, seed deposition may be influenced by the removal of adult plants. For example, Inglis (2000) reported high seedling numbers in dugong feeding trails and proposed that this was due to larger densities of seeds in the feeding depressions rather than enhanced germination. In the longlines, seed dispersal may be limited due to altered water flow around the PVC structures. Longlines may also act as

"clotheslines", causing plants to become entwined in the ropes at high tide resulting in severe desiccation at low tide, thus reducing the density of both vegetative and flowering shoots (Pregnall 1993, Everett et al. 1995). Future studies should address the role of these off-bottom structures in limiting dispersal of seeds and influencing water flow as well as causing physical damage to the eelgrass plants.

Above ground structures in longline areas have the potential to significantly alter hydrology and sedimentation within the culture area. Grant and Bacher (2001) developed a numerical model to assess the flow modification induced by suspended aquaculture in China and found that the structures could result in up to a 54% reduction in current speed. The reduction in flow causes longline areas to accrete sediment at much greater rates than would naturally occur (Everett et al. 1995) and could lead to burial of seeds and young seedlings. Seeds that are buried too deeply may not be able to germinate and are then lost to the population (Bigley 1981).

Sediment at the reference and longline plots was composed of a greater percentage of silt than dredged aquaculture areas. This pattern is likely due to a combination of factors. Frequent disturbance in the dredged areas stirs up the sediment and causes a reduction in the amount of fines present. Pregnall (1993) reported a significant decline in sediment porosity and fine grain sized sediments in ground cultured oyster beds and suggested that they are depositional environments and that the harvest of oysters produces an episodic release of sediments to the estuary. Additionally, the longline structures and eelgrass blades may cause increased sedimentation by slowing water movement. However, sedimentation and sediment grain size are likely not the driving factor in germination and seedling survival differences among aquaculture

treatments. In contrast, redox potential, a measure of oxygen content in sediments, could contribute to trends in germination and seedling survival as dredged beds had lower redox than eelgrass but higher seedling densities. Oxygenated sediments may delay or even prevent germination (Moore et al. 1993), which may explain why natural seedling densities were lower in the reference areas. The lowest redox values were observed in longlines, which could possibly be the result of oyster deposition of organic-rich particles from feces and pseudofeces changing the microbial community and increasing silt, which, combined could result in increased anoxia or buildup of sediment sulfides which can be toxic for seedlings. This may not occur in dredged beds because water flow in those areas is relatively uninhibited (compared to longlines) allowing particles to be carried away.

Thus, although oyster aquaculture appears to influence eelgrass recruitment, these impacts are not consistent across culture types. We found that while dredged ground culture can enhance or at least maintain seed density and seed germination, longline aquaculture significantly reduces eelgrass recruitment. Greater recruitment in dredged beds is likely due to both enhanced seed densities as well as removal of neighboring adult plants.

Off-bottom shellfish aquaculture was recommended by the Pew Commission because of a perceived reduction in negative impacts to community structure compared to those of ground culture techniques, which were perceived to create greater disturbance (Kennedy et al. 2002). However, off-bottom culture techniques can also negatively impact eelgrass (Tallis et al. in review), and hinder recruitment as our study shows. Although there are likely many other contributing factors (e.g., site conditions,

differences in dredging equipment and technique, timing of dredging, etc.), banning all on-bottom culture techniques may not be the best strategy. Furthermore, disturbance effects will vary according to the temporal, spatial, and intensity scales over which they occur (Simenstad and Fresh 1995, Neckles et al. 2005).

Recovery periods for eelgrass in oyster beds following oyster harvest will vary as they likely depend on a combination of factors including the type of oyster culture, duration of culture, spatial configuration of culture operation and nearby meadows, and the frequency of oyster harvest events (Waddell 1964, Orth 2002a,). Our recruitment data suggest that recovery via seed germination is possible and may occur over a relatively short time frame. Enhanced reproductive effort and rapid recovery have also been reported in *Zostera noltii* meadows subject to intense recreational clam harvest southern Portugal (Alexandre et al. 2005, Cabaço et al. 2005). Other studies, however, have reported little or no recovery following aquaculture activities (Waddell 1964, Peterson et al. 1987, Necles et al. 2005). For example, Peterson et al. (1987) reported slow recovery time for eelgrass following disturbance from mechanical clam harvesting. Recovery took seven years, nearly four years longer than the normal rotation period of oyster culture in the Pacific Northwest (Peterson et al. 1987, Simenstad and Fresh 1995). Neckles et al. (2005) report an average recovery time of ten years following mussel harvest. These differences could be maintained by larger scale variables such as water quality. For example, the lack of development surrounding Willapa Bay may mean recovery is faster than in more developed estuaries facing water quality problems that could hinder recovery.

Individual life history characteristics can play a role in determining what the impact of disturbance will be on species distribution. Moloney and Levin (1996) found that the response of *Plantago* sp. to disturbance was largely explained by the plant's poor dispersal ability. However, *Plantago* sp. was able to persist in the presence of very high disturbance rates when at least a few sites that served as seed sources were protected from disturbance (Moloney and Levin 1996). A similar response would likely occur with *Z. marina*. Dredge harvest operations on a given bed occur generally every three years, and large contiguous eelgrass meadows surrounding the impacted areas provide a seed source that could allow for recovery of the disturbed beds. Although eelgrass in a dredged bed may be reduced following harvest, seed production and germination are relatively high within the bed and if adjacent beds are unharvested during that dredging event, a substantial seed source should persist.

While our data may have important management implications suggesting that eelgrass recovery post-dredging is rapid and that harvest dredge sites may act as sources of eelgrass seed, we know little about how these results vary among sites (either within or among estuaries). Tidelands used for aquaculture in Willapa Bay comprise a mosaic of disturbance and habitat types. Beds vary in size and use. Some beds are left unmanipulated for the three years it takes the oysters to reach maturity while other beds are subject to frequent disturbance as oysters are moved on and off-site depending on conditions. Thus some beds may have little to no eelgrass cover due to frequent harvest and management activities while other beds are left unmanipulated for long periods enabling dense stands of eelgrass to form and persist (Tallis et al. in review). Further, little is known about the potential for seeds to travel from one bed to another and how the

mosaic of different aquaculture types affects the longer-term sustainability of eelgrass populations. Future studies should examine the site-dependent and landscape-scale effects of oyster aquaculture on eelgrass population dynamics. These issues must be addressed in order to address whether, and at what scale, no net loss policies should be applied to eelgrass habitat and aquaculture operations.

Acknowledgements

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Table 1. The density of adult *Zostera marina* shoots and the percent cover of the oyster *Crassostrea gigas* and macroalgae in the experimental seed addition plots, April 2005. Adult neighbors were removed from the surrounding $0.25m^2$ in the removal plots while controls were left with adult neighbors present.

	no. *Z. marina* adults (per $0.25m^{2)}$)		% *Crassostrea gigas* (per $0.25m^{2)}$)		% Macroalgae (per $0.25m^{2)}$)	
	Control	Removal	Control	Removal	Control	Removal
Longlines	7.6 (1.1)	0.5 (0.3)	1.2 (.7)	1.2 (0.6)	7.9 (2.7)	6.1 (1.5)
Dredged	1.4 (0.8)	0.5 (0.2)	25 (5)	33 (7.5)	11.5 (2.8)	15.5 (2.7)
Reference	6.5 (1.2)	3 (1.0)	0 (0)	0 (0)	5.7 (1.5)	5 (1.5)

Figure 1. Mean number of (A) *Zostera marina* seedlings, (B) *Z. marina* adult shoots and the mean percent cover of (C) macroalgae and (D) *Crassostrea gigas* oysters per 0.25m^2 in different aquaculture types at Stony Point, Willapa Bay, WA. Values represent means ± 1 SE. Letters indicate statistically significant differences as indicated by Kruskal-Wallis multiple comparison Z-value tests.
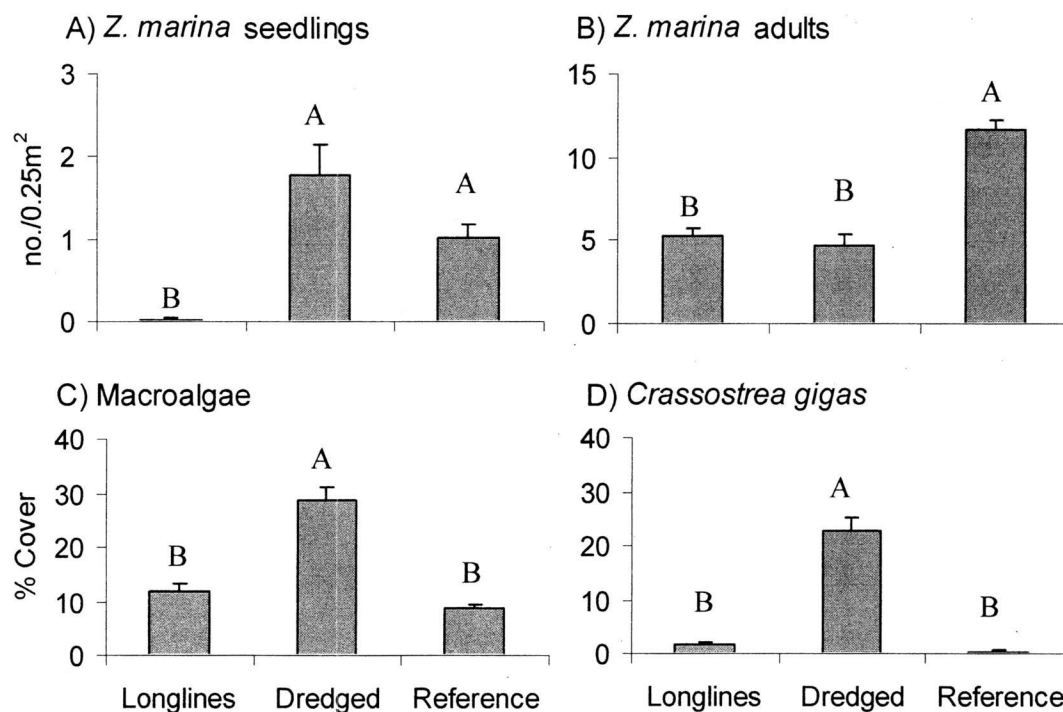
Figure 2. Relationship between density of naturally occurring *Zostera marina* seedlings and percent cover of *Crassostrea gigas* in different aquaculture types. Dashed line indicates target *C. gigas* cover in aquaculture beds. Equation for best fit line of dredged is y=-0.0396x + 2.6839, R^2 = 0.0346.
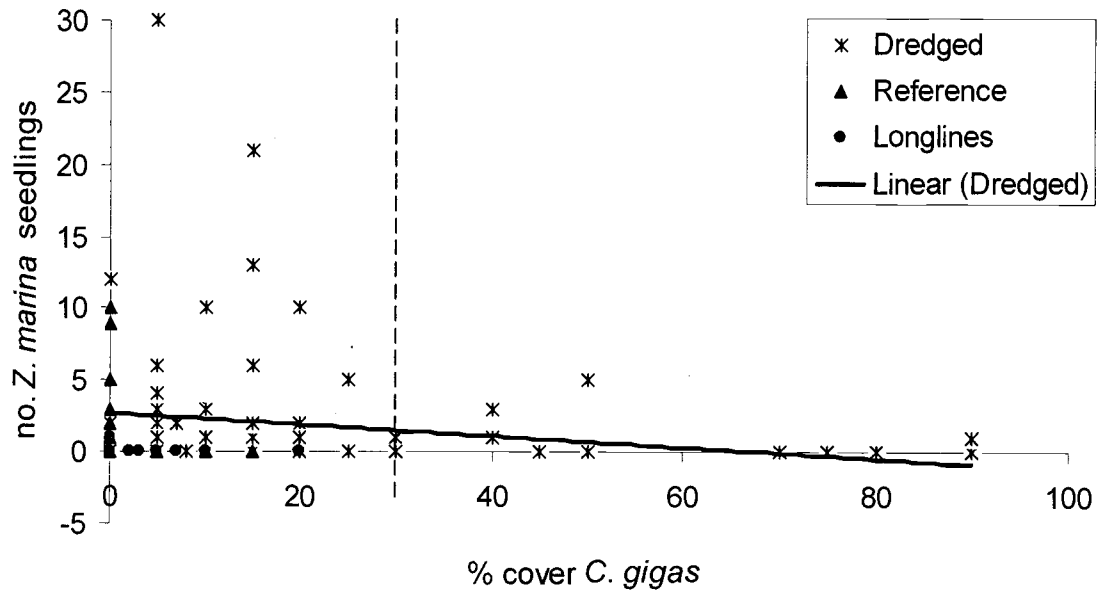
Figure 3. Mean number of (A) *Zostera marina* seeds produced per 0.25 m^2, and (B) *Z. marina* seeds in sediment cores (10 x 15cm) in different aquaculture types at Stony Point, Willapa Bay, WA. Seeds in sediment include both filled seeds as well as empty seed coats; mean values of filled seeds are 0.07 ± 0.07 (longlines), 2.73 ± 0.73 (dredged) and 0.2 ± 0.11 (reference). Values represent means ± 1 SE. Letters indicate statistically significant differences as indicated by Kruskal-Wallis multiple comparison Z-value test (A) and Scheffe's multiple comparison test (B).

A) *Z. marina* seed production

A) *Z. marina* seed production
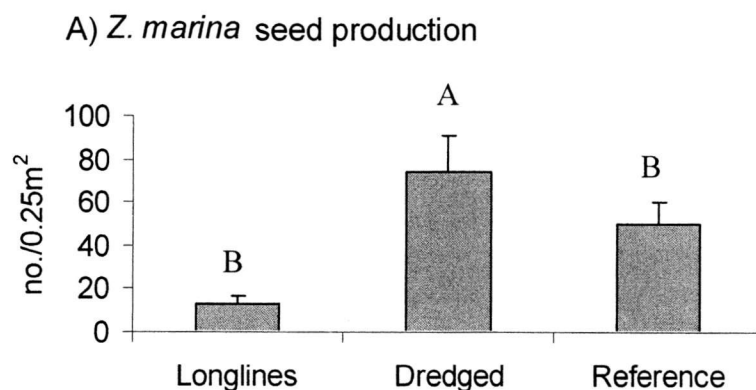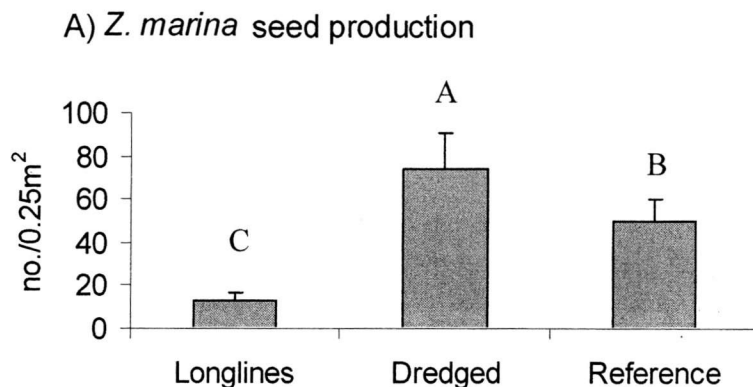
Figure 4. Mean number of *Zostera marina* seedlings in (A) March, (B) April and (C) August of 2005 under control and eelgrass removal treatments in different aquaculture types at Stony Point, Willapa Bay, WA. Values represent means ± 1 SE. Letters represent significant differences between aquaculture type.
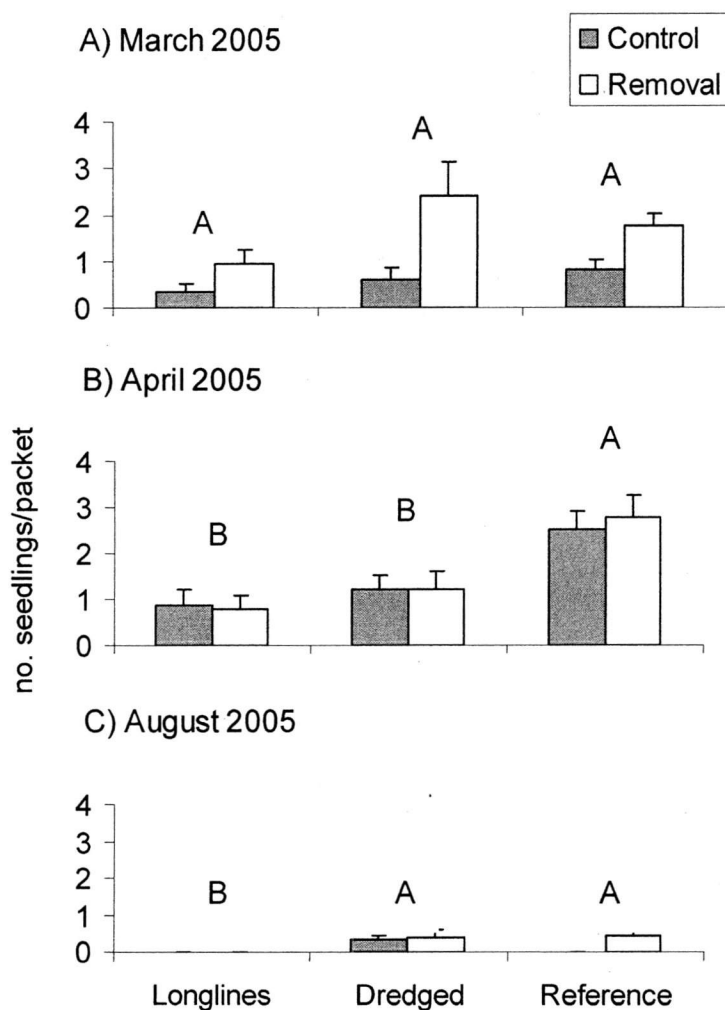
Figure 5. Mean *Zostera marina* seedling (A) length and (B) biomass for control and eelgrass removal treatments in different aquaculture types at the end of a seed addition experiment in August 2005. Letters indicate significant differences (Kruskal-Wallis multiple comparison Z-value test). Values represent means ± 1 SE. The relationship between adult shoot density and seedling biomass is given in (C). Equation for best-fit line is $y = -0.0154x + 0.3661$, $R^2 = 0.2679$.

A) Seedling length

B) Seedling biomass

C) *Z. marina* biomass vs. adult density
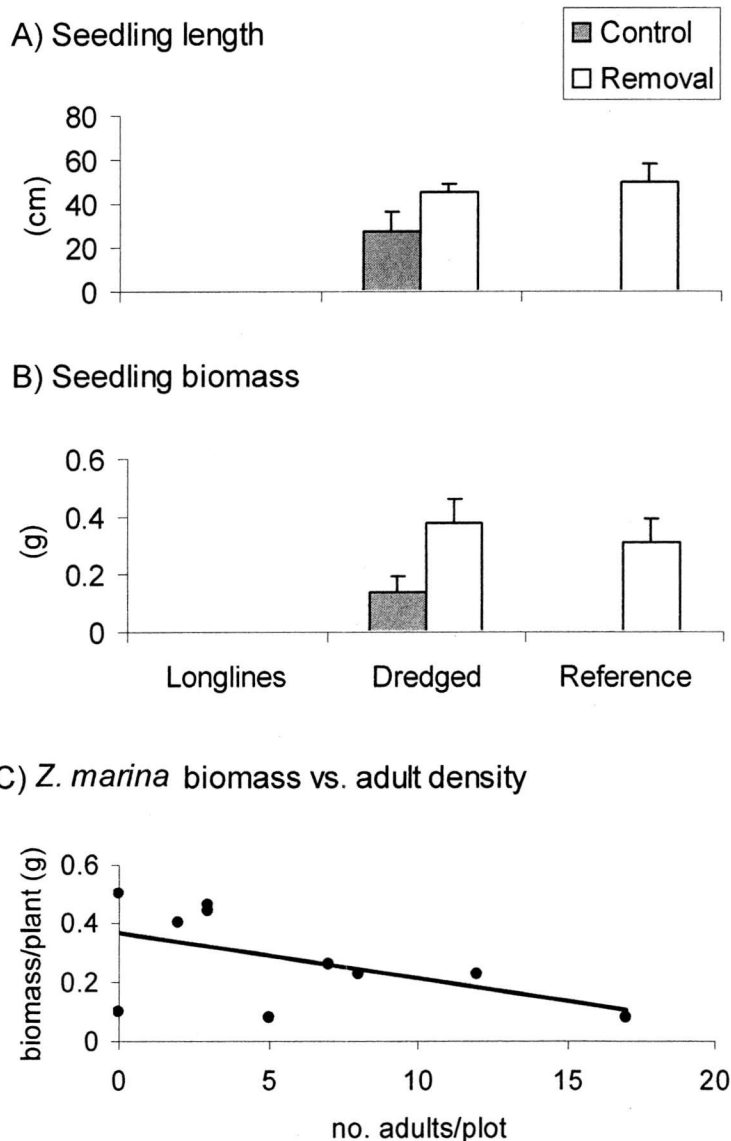
Figure 6. Mean (A) sediment redox potential, (B) light intensity, (C) sediment silt to sand ratio and (D) sediment accretion for different aquaculture types. Values represent means ± 1 SE. Letters indicate statistically significant differences (Scheffe's multiple comparison test).
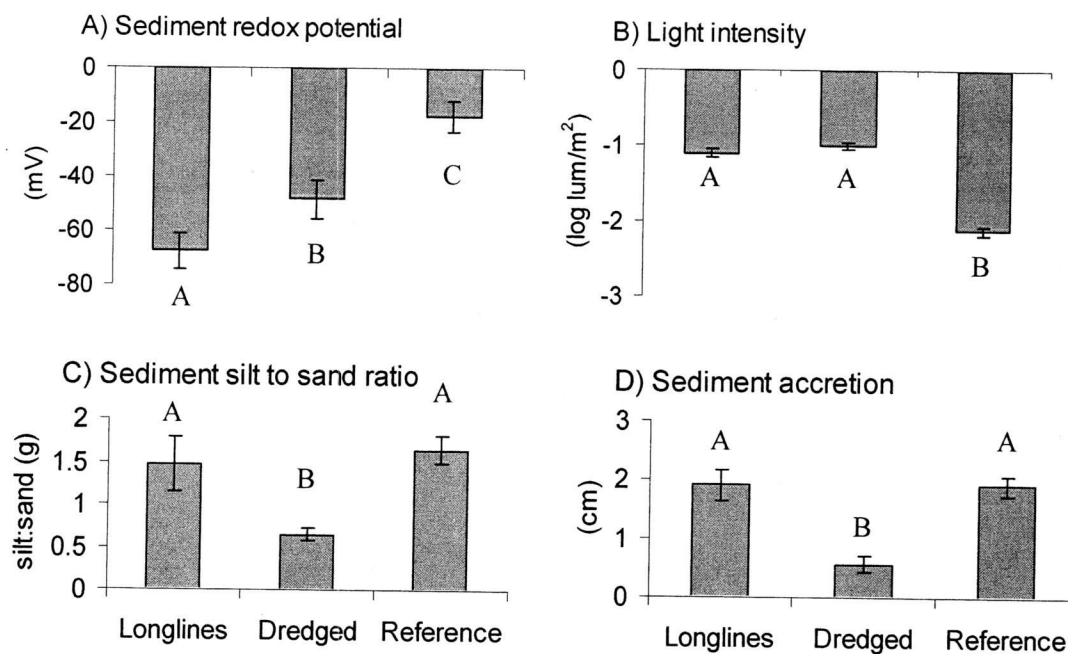
Chapter 3. SPATIAL VARIATION IN EELGRASS RECRUITMENT FROM SEED: INFLUENCE OF SEED PRODUCTION, PHYSICAL FACTORS, AND ADULT NEIGHBORS

Abstract

Zostera marina seed production and germination were examined across five sites in Willapa Bay, WA. Oservational surveys showed that natural seedling densities varied significantly with site, with some sites having more seedlings than others (0.36 – 4.8 seedlings m^{-2}). The objective of our study was to understand whether the variation was due to seed supply, physical factors, and/or biological factors. Some of the variation in natural recruitment could be explained by patterns of flowering and seed production with some sites that had higher seedling numbers producing significantly more propagules. Recruitment was highest under colder water temperatures, while other physical factors (redox potential, sediment grain size, and sediment accretion) were less important. To understand what may be influencing seedling density, we conducted a seed addition experiment that showed differences in germination and early seedling survival were controlled in part by number of adult neighbors, although adult densities at a larger scale did not predict germination success. The results of this study suggest that *Z. marina* recruitment patterns in Willapa Bay are driven primarily by seed abundance, but differences in germination success were also important.

Introduction

The ecological importance of seagrasses, combined with their recent worldwide declines, has led researchers and managers to focus on developing effective conservation and restoration strategies (Fonseca et al. 1998). Initial restoration approaches have involved

harvesting shoots from donor beds and planting at sites where new beds are desired. However, the cost and labor is substantial with limited success (Fonseca et al. 1998). As a result, alternative restoration methods, including the use of seeds, are being explored (Granger et al. 2002). Recent attempts have included the use of transplanted seedlings as well as broadcasting seeds by hand (Balestri et al. 1998, deJonge et al. 2000, Orth et al., 2006c).

Restoration via seeding may prove to be a viable alternative to transplantation of adult shoots, particularly since harvesting seeds may have less of an impact on donor beds than extraction of adult plants (Orth et al. 2006a). A further advantage to using seeds and or/seedlings for restoration purposes is that many areas that could support eelgrass remain unvegetated, thus no donor bed is available for extraction of mature plants. Seeds produced in abundance elsewhere can be easily harvested, stored, and then broadcast by hand onto the sediment surface (Granger et al. 2002). Restoration via seed sowing is likely to result in substantial reductions in cost and labor, especially given that current estimates for adult transplant restoration average $37,000 per acre (Fonseca et al. 1998). As with vegetative transplants, restoration using seeds will require careful attention to genetic diversity, contamination, and disease.

Restoration via seeding and seedling planting has historically been given little attention because sexual reproduction has been viewed as less important than vegetative reproduction in seagrass bed population dynamics. Although meadows are largely maintained by vegetative growth, it is becoming increasingly more evident that sexual reproduction also has an important role in the maintenance of seagrass beds, especially in recolonizing disturbed areas (Whitfield et al. 2004, Wisehart et al. *in prep*). Seed

production is high in many species and is thought to be important for colonizing new areas and increasing genetic diversity within beds (Orth et al. 1994, Ruckelshaus 1996). Seedling recruitment is believed to be a regular and reliable source of recovery following disturbance, especially in areas where vegetative regrowth from the perimeter is limited (Whitfield et al. 2004).

Eelgrass recruitment is dependent on seed supply, physical factors, and biological interactions. The influence of these three factors on successful seedling establishment is not well understood. Past research has focused on mature vegetative plants and the few experimental studies that have addressed early life history stages generally have not been conducted in the field under natural conditions. However, laboratory experiments and observational studies do provide some insight into the factors driving patterns of seed production, seed germination, and seedling survival.

Seed supply at a site is dependent on seed production and dispersal. Seed production has been shown to vary with environmental factors that include light (DeCock 1981a), temperature (DeCock 1981b, Phillips et al. 1983), nutrients (Churchill et al. 1978), salinity, (Phillips et al. 1983) and water depth (Jacobs et al. 1981). Following successful fertilization, mature seeds are released from the shoot, sink to the sediment surface, and then are quickly buried through physical and/or biological processes (Luckenbach and Orth 1999) and begin the germination process. Seedling density is often low suggesting that seed loss is great in many seagrass systems (Phillips and McRoy 1990) and may be due to dispersal, predation, or microtopographical features of the sediment which can influence seed settlement (Churchill et al. 1985, Fishman and Orth 1996, Luckenbach and Orth 1999, Harwell and Orth 2002a,b, Nakaoka 2002).

Once seeds arrive at a site, the physical environment is important to germination success and seedling survival. Germination is influenced by physical factors that include salinity, light, tidal height and sediment type (Churchill 1983, Orth and Moore 1983, Phillips et al. 1983, Hootsmans et al. 1987). In many cases, it is the interactions between these different factors that are most important. For example, Harrison (1991) reported no effect of light on germination in a lab experiment. More recently though, Moore and colleagues (1993) suggested that under natural conditions, light has an effect that varies with oxygen availability. Effects of temperature on germination have also been shown to be strongly dependent on presence or absence of oxygen (Probert and Brenchley 1999, Orth et al. 2000). Oxygen or other sediment conditions are likely the primary drivers but other factors such as light and water temperature need to be examined with more rigorous experimentation in the field. Additionally, it has been suggested that the relative importance of these different physical factors varies with geographical origin of the population, especially latitude (Probert and Brenchley 1999). For this reason, it is essential that studies address the habitat requirements for successful germination and establishment in a number of locations; this is particularly true on the west coast of North America where very little is know about eelgrass seed dynamics compared to elsewhere.

If physical conditions are sufficient for germination, biological interactions may then become important. The influence of adult neighbors on seed germination and seedling survival are not well understood because few studies have addressed these interactions. Adult plants may negatively impact seedlings through competition for light and nutrients (Orth et al. 2006a). For example, adult *Posidonia australis* plants negatively affect recruitment via increases in seed predation compared to adjacent unvegetated plots

(Orth et al. 2002b, Orth et al. 2006b). Alternatively, adult neighbors may positively affect seedlings by providing shelter, anchorage, and an increase in organic content (Orth et al. 2006a).

Our overall objective was to understand the relative role of seed supply, physical factors, and biological interactions on early life history stage patterns. We conducted an observational study to describe the pattern of natural seedling recruitment in Willapa Bay, WA, and how this pattern relates to physical differences and possible interaction with adult plants. We then implemented a seed sowing experiment to determine whether once a seed arrives at a site, its germination and survival are dependent on local factors such as sediment type and/or biological interactions. Through the use of these two methods, we were able to determine the eelgrass recruitment dynamics within the bay.

Methods

Study Site

This study was conducted at Willapa Bay, a coastal estuary located in southwest Washington state, that is connected to the Pacific Ocean by only a shallow narrow entrance (10km across). Of the nearly 35,000 ha which make up the bay, approximately half are exposed during extreme low tides (Borde et al. 2003). Mooring and water sample data provided by the Washington Department of Ecology (1999-2000) suggest strong gradients in temperature and salinity exist within the bay (Ruesink et al. 2003). Salinity spans a gradient from oceanic (34 ppt) near the mouth to less than 25 ppt in parts of the bay more than 20 km from the mouth (Ruesink et al. 2003, Thom et al. 2003). The eastern arm of the estuary has relatively high freshwater input from the Willapa and

North Rivers, while the southern region of the bay is similar to a coastal lagoon (Ruesink et al. 2003).

Five sites encompassing a range of salinities and sediment types were selected for this study: Stackpole (ST; 46.6039°N, 124.0317 °W), Oysterville (OY; 46.5514°N, 124.0164°W), Stony Point (SP; 46.68046° N, 123.92047° W), Long Island (LI; 46.5110°N, 123.9967°W) and Peterson (PE; 46.4267°N, 123.9917°W).

Two species of seagrass are present in the bay, *Z. marina* (native) and *Z. japonica* (introduced). *Zostera marina* typically occurs at and below mean lower low water throughout the bay (Thom et al. 2003) while *Z. japonica* is most abundant in the high intertidal where *Z. marina* is restricted to pools (LW personal observation).

Patterns of Natural Eelgrass Seedling Recruitment and Seed Supply

To understand the pattern of eelgrass seedling recruitment across different sites in the bay, we surveyed eelgrass seedlings in May 2005. Thirty quadrats (0.25 m^2) were haphazardly tossed in three spatially separate areas ($\approx$ 30 m apart) at each site (n = 90) and measurements were recorded for *Z. marina* seedlings (no. 0.25 m^{-2}), vegetative *Z. marina* adult shoots (no. 0.25 m^{-2}), *Z. japonica* (% cover), and benthic macroalgae (% cover).

Flowering shoot densities were surveyed in July 2005 to estimate potential seed production. At each site we recorded the number of flowering shoots present in 50 haphazardly tossed quadrats (0.25 m^2) in three separate areas (n = 150). Thirty flowering shoots were haphazardly collected from each site and brought to the lab where the number of seeds per spathe [seeds included total number of female flowers (ovaries) and

fruits (fertilized ovaries)] and the number of spathes per plant were recorded. We

estimated average seed production per flowering shoot per site by multiplying the number

of seeds per spathe by the number of spathes per shoot. We then estimated the number of

seeds produced per 0.25 m^2 by multiplying the number of flowering shoots per 0.25 m^2

quadrat by the mean number of seeds produced per plant for each site.

We surveyed eelgrass seeds in the sediment in August 2005, after seed rain but

prior to germination. Ten cores (10 cm diameter x 15 cm depth) were haphazardly

collected from each of three areas at each site (n = 30). The samples were first sieved in

the field using a 0.5 mm sieve then bagged individually and taken to the lab where they

were visually inspected for the number of fully developed seeds and empty seed coats of

both Z. japonica and Z. marina. We report seed bank densities as number of seeds per

core following Inglis (2000) and Harwell and Orth (2002).

Experimental Manipulations of Eelgrass Recruitment

To understand whether germination would vary at different sites if seed supply

were equivalent, we conducted an eelgrass seed addition experiment. We collected seed-

bearing shoots from several sites throughout Willapa Bay in August 2004 and transferred

them to large outdoor tanks partially filled with seawater. As the seeds fell from the

parent plants they were collected and then stored in seawater at 4 deg C in the dark. In

September 2004, mesh packets were constructed and filled with 30 seeds each. The

packets were then planted in paired control and eelgrass removal plots (0.25 m^2) in each

of three areas at each of the five sites and were secured at the sediment surface using wire

stakes.

Seed germination and survival in the packets was assessed in April and August of 2005. The number of *Z. marina* shoots in the 0.25 m^2 plot surrounding each packet was also recorded. Seedling emergence and survival were monitored with platforms constructed using PVC frames and clear plexiglass tops. Transparent sheets were used to map seedlings and record approximate dates of seedling emergence and disappearance. Seedlings were harvested from the plots in August 2005, and taken to the lab, where length (cm) and dry biomass (g) were recorded.

Physical factors

Sediment characteristics at the five sites were measured in April, 2005. Sediment redox potential was measured adjacent to each of the seed addition plots (n = 30 per site) using a redox meter fitted with a platinum redox electrode and filled with AgCl solution (Thermo Orion Company, Beverly, MA, USA). Sediment grain size (silt to sand ratio) was determined by collecting three sediment samples (~ 50 g) in three locations at each of the sites (n = 9), weighing them before and after drying, and then wet sieving them through a 63 μm sieve (0 - 63 μm = silt) followed by a 2 mm sieve (63 μm ≥ 2mm = sand). In addition, sediment accretion at each site was approximated in June, 2005, by measuring the depth of sediment deposition on the seed packets (n = 3 per packet). Water temperature was recorded at each site over a two week period in July 2005 using iButton® temperature loggers (Dallas Semiconductor Corp., Dallas, TX, USA) anchored at the sediment surface with PVC pipe. Light intensity (log Lum m^{-2}) was measured in April 2005 using HOBO® light intensity loggers (Onset Computer Corp., Pocasset, MA, USA). The loggers were vacuumed sealed and fixed to the sediment surface by attaching

them to PVC pipes that were dug into the sediment. At each site, one logger was placed

on bare substrate and one on substrate with adult eelgrass present. The loggers recorded

light intensity every three minutes for 24 hours.

Statistical Analyses

To test for differences in natural seedling density and percent cover of *Z. japonica*

and macroalgae among sites, a non-parametric Kruskal-Wallis test was used since the

data did not meet assumptions of ANOVA. Adult shoot densities were compared using

single-factor ANOVA. The single factor ANOVA was also used to test for differences in

eelgrass seed production (per $0.25m^2$) between sites while a Kruskal-Wallis single factor

ANOVA was used to test for differences in seed density in sediments. Two factor

ANOVAs were used to test for the effects of eelgrass treatment, site, and possible

interaction, on seedling density in the seed addition experiments. When the interaction

was significant, single factor ANOVA tests were run to determine differences within

levels. A two-way ANOVA was used to test for differences in seedling length between

sites and treatments in June. However, by August, significant seedling mortality

prevented accurate statistical analysis for site and treatment effects for some of the plots.

Where at least three seedlings survived, one-way ANOVAs were used to test for

significant site and treatment effects. Physical factors including redox potential,

sediment grain size, sedimentation, and water temperature were analyzed for differences

between sites with single factor ANOVAs. Differences in light intensity among sites and

treatments were analyzed with a 2-factor ANOVA. Scheffe's Multiple Comparison Test

was used as a post hoc test for all significant factors ($p < 0.05$). Data were square-root or

log transformed when necessary although figures represent untransformed values (Gotelli

and Ellison 2004). Statistical tests were run using NCSS 2001 software (NCSS,

Kaysville, UT, USA).

Results

Natural recruitment

     Zostera marina seedling density, *Z. marina* adult shoot density, *Z. japonica* shoot

density, and percent cover of macroalgae all differed among sites (seedlings: Kruskal-

wallis single factor ANOVA; df = 4, H = 54.2, p < 0.01; adult shoots: single factor

ANOVA; $F_{4,445}$ = 29.5, P < 0.01; *Z. japonica*: Kruskal-wallis single factor ANOVA; df =

4, H = 54.2, p < 0.01; macroalgae: Kruskal-wallis single factor ANOVA df = 4, H =

136.9, p < 0.01). Post hoc tests showed that *Z. marina* seedlings were similarly abundant

at Stackpole, Stony Point and Long Island while fewer were observed at Peterson and the

fewest at Oysterville (Figure 7A). The highest number of *Z. marina* adult shoots were

found at Stackpole and Oysterville while lowest densities occurred at Stony Point (Figure

7B). Adult shoot densities at Long Island and Peterson were similar. Coverage of the

non-native seagrass, *Z. japonica*, was greatest at Stackpole and Oysterville, negligible at

Stony Point and Long Island, and absent at Peterson (Figure 7C). Benthic macroalgae

was significantly more abundant at Peterson and Stony Point although it was present at

all sites surveyed (Figure 7D).

     Flowering shoot densities, seed production per plant, seed production per 0.25 m^2,

and seed numbers in the sediment differed significantly among sites (flowering shoots:

single factor ANOVA; $F_{4,750}$ = 93.64, p < 0.01; seed production/plant: single factor

ANOVA, $F_{4,157} = 30.88$, $p < 0.01$; seed production/0.25 m^2: single factor ANOVA, $F_{4,749} = 51.8$, $p < 0.01$; seeds in sediment: single factor ANOVA; $F_{4,145} = 3.59$ $p = 0.01$). Post hoc tests showed the highest flowering shoot densities were at Stackpole and Oysterville, while densities were significantly lower at Stony Point and Long Island compared to the other sites (Figure 8A). The number of seeds produced per plant was greatest at Stony Point, Long Island and Peterson (Figure 8B) although significantly more seeds were produced per 0.25m^2 at Stackpole compared to all other sites (e.g. five times more than at Stony Point) (Figure 8C). Seed production (per 0.25m^2) was similar at Peterson and Oysterville and also between Stony Point and Long Island. The number of seeds found in the sediment was also highest at Stackpole (Figure 8D). Significantly fewer seeds were found at Oysterville but seed bank numbers were similar among other sites.

Seed addition experiment

In April, seedling numbers varied significantly with removal treatment and site but there was no interaction (Figure 9A; two factor ANOVA; treatment: $F_{1,137} = 3.8$, $p = 0.05$, site: $F_{4,137} = 7.6$, $p < 0.01$, interaction: $F_{4,137} = 0.4$, $p = 0.81$). Post hoc tests showed plots without adult neighbors had better germination than those with adult neighbors. Greatest germination occurred at Stackpole and Stony Point, while the other sites had lower but no difference in numbers. In June, there was a significant site effect but seedling numbers were similar between control and removal plots (two factor ANOVA; treatment: $F_{1,137} = 3.6$, $p = 0.06$, site: $F_{4,137} = 2.6$, $p = 0.04$, interaction: $F_{4,137} = 1.6$, $p = 0.19$). At the termination of the experiment in August, neither treatment nor site were significant (Figure 9B; two factor ANOVA; treatment: $F_{1,137} = 0.17$, $p = 0.68$, site: $F_{4,137}$

$= 2.1$, $p = 0.08$). However, there was a significant interaction between site and treatment and there were no surviving seedlings in the Stony Point control plots (interaction: $F_{4,137} = 3.1$, $p = 0.02$). In August, the eelgrass removal treatment had a significant effect at Stony Point (single factor ANOVA; $F_{1,28} = 7.98$, $p = 0.01$) but no significant differences were detected in seedling densities between control and eelgrass removal treatments at the other sites (single factor ANOVA; Long Island: $F_{1,28} = 0$, $p = 1$, Oysterville: $F_{1,26} = 0.91$, $p = 0.35$, Peterson: $F_{1,28} = 3.57$, $p = 0.07$, Stackpole: $F_{1,27} = 0.69$, $p = 0.42$).

In addition, in April, adult shoot density differed significantly between control and removal plots at all sites in April (Figure 9C; single factor ANOVA; Stackpole: $F_{1,27} = 8.41$, $p < 0.01$, Oysterville: $F_{1,26} = 54.73$, $p < 0.01$, Stony Point: $F_{1,27} = 42.3$, $p < 0.01$, Long Island: $F_{1,28} = 14.92$, $p < 0.01$, Peterson: $F_{1,28} = 21.98$, $p < 0.01$), however, by August, densities between control and removal plots were similar at all sites except Oysterville and Peterson (Figure 9D; single factor ANOVA; Stackpole: $F_{1,27} = 0.8$, $p = 3.8$, Oysterville: $F_{1,26} = 20.15$, $p < 0.01$, Stony Point: $F_{1,28} = 1.92$, $p = 0.18$, Long Island: $F_{1,28} = 0.46$, $p = 0.5$, Peterson: $F_{1,28} = 7.36$, $p = 0.01$). In April, there was significant variation of adult density within control plots among sites (single factor ANOVA; $F_{4,69} = 4.0$, $p < 0.01$) although adult densities within removal plots were similar (single factor ANOVA; $F_{4,68} = 0.81$, $p = 0.53$). In August there was significant variation between sites among both the control and eelgrass removal plots (single factor ANOVAs; removal: $F_{4,68} = 6.94$, $p < 0.01$, control: $F_{4,69} = 7.03$, $p < 0.01$)

In June, seedling lengths were similar between control and eelgrass removal treatments, although there was a significant site effect (Figure 10A; two factor ANOVA; Treatment: $F_{1,64} = 2.03$, $p = 0.16$, Site: $F_{4,64} = 4.60$, $p < 0.01$, Interaction: $F_{4,64} = 0.57$, $p =$

0.69). Post hoc tests showed that seedlings were significantly longer at Long Island, compared to Peterson and Stony Point, where seedlings were shorter. By August, seedling lengths were not significantly different between control and eelgrass removal treatments, however, there was a trend of longer seedlings in removal plots (Figure 10B). Seedling length continued to vary significantly between sites in the removal treatments but not in the controls (single factor ANOVAs; Removal: $F_{3,17} = 4.31$, $p = 0.02$, Control: $F_{1,10} = 2.16$, $p = 0.17$). Post hoc tests showed that eelgrass removal seedlings were significantly longer at Peterson compared to Stackpole.

In August, eelgrass removal had a significant effect on seedling biomass at one site (Figure 10C; Oysterville: single factor ANOVA; $F_{1,10} = 6.2$, $p = 0.03$), although the treatment effect was not significant at Stackpole ($p > 0.05$). At Long Island, where only two seedlings survived in each of the control and eelgrass removal treatments, seedling biomass in the eelgrass removal plots was more than three times that of the control plots. Seedling biomass was also great in the Stony Point and Peterson eelgrass removal plots, although they could not be compared to control seedlings due to high mortality in those plots. Seedling biomass was similar between sites in both the control and eelgrass removal treatments ($p > 0.05$).

Physical factors

Redox potential, silt to sand ratios, sediment accretion, and water temperature all showed significant variation between sites (single factor ANOVAs; redox potential: $F_{4,139} = 10.05$, $p < 0.01$, silt to sand: $F_{4,39} = 69.5$, $p < 0.01$, sediment accretion: $F_{4,126} = 36.58$, $p < 0.01$, water temperature: $F_{4,10239} = 202.4$, $p < 0.01$). Post hoc tests showed that redox

potential was low at Stony Point compared to Stackpole, Oysterville and Long Island where redox potential was significantly higher (Figure 11A). The ratio of silt to sand was greater at Stony Point, intermediate at Long Island and Peterson, and lowest at Stackpole and Oysterville (Figure 11B). Sediment accretion was greatest at Stony Point (~ nine times higher than other sites) while levels were similar at all other sites (Figure 11C). Water temperatures were warmest at Peterson and Oysterville while Long Island temperatures were significantly lower than all other sites (Figure 11D). A two factor ANOVA for light intensity revealed a site effect and although there was a trend of higher light in bare areas compared to areas with adult plants present the difference was not statistically significant (two factor ANOVA; treatment: $F_{1,3245} = 0.9$, p = 0.34, site: $F_{3,3245} = 2.49$, p = 0.059, interaction: $F_{3,3245} = 0.1$, p = 0.96). Post hoc tests showed that light intensity was greater at Oysterville compared to Peterson (Figure 11E).

Discussion

The goal of our study was to understand the processes driving *Z. marina* recruitment patterns in a large west coast estuary. Observational surveys of seedlings at five sites in Willapa Bay showed that natural seedling densities varied significantly with location. At some sites, high seedling numbers were observed despite low seed production estimates and vice versa. These patterns are likely due to a combination of recruitment limitation and reduced germination that appear to be influenced by both physical setting as well as presence of neighboring adult eelgrass plants.

Seed production

We did not find a clear relationship between seed production and natural seedling abundance at different sites. We found that even though flowering was much more prolific at Stackpole and Oysterville, more seeds were produced per plant at Stony Point, Long Island, and Peterson (Figure 7A,B). Although plants at these sites were larger, the frequency of flowering was so much greater at Stackpole and Oysterville, that seed production was also greater for these two sites (Figure 7C). Olesen (1999) and Harwell and Orth (2002b) also reported significant differences in seed production among populations which were attributed to differences in flowering shoot densities rather than variation in the number of seeds produced by individual plants.

Seed loss, however, may be a critical factor. At Oysterville and Peterson seed production was high, but seed bank densities and seedling densities were the lowest of all sites surveyed (Figure 8C, D) suggesting disappearence of seeds. Seeds may be lost before they get to the seed bank (transport via rafting or gas bubbles; DeCock 1980, Churchill et al. 1985), or upon entry to seed bank through senescence, failed germination and physical or biotic factors (Chambers and MacMahon, 1994). These factors may include non-viability, damage, disease burial, and predation (Wigand and Churchill 1988, Phillips and McRoy 1990, Orth et al. 1994, Fishman et al. 1996, Nakaoka 2002).

Physical factors

We found conflicting results for the relationship between sediment characteristics and recruitment success in Willapa Bay. At Stony Point, where high natural seedling recruitment and high seed germination were observed, sediments were significantly more

anoxic with greater silt to sand ratio and greater sediment accretion (Figure 11A, B, C). In contrast, at Stackpole where recruitment was also high, sediments were sandier and more oxygenated (Figure 11B, A). Previous studies have reported enhanced germination in anoxic conditions (Phillips 1983, Moore et al. 1993, Probert and Brenchley 1999) and such oxygen-deprived sediments are characteristic of muddy areas (Koch et al. 2000, van Katwijk and Wijgerangs 2004). However anoxic conditions can also cause elevated sulfide levels, which have been demonstrated to be toxic to eelgrass seedlings (Thayer et al. 1984, Goodman et al. 1995, Fonseca et al. 1998, Koch et al. 2000). This study did not address sulfide concentrations in the sediment, although it might explain some of the variation in recruitment.

We observed little variation in light intensity and found no obvious correlation between light intensity and eelgrass recruitment. For example, light intensity was low at Peterson but high at Oysterville, even though both sites experienced low natural recruitment. Light has been studied by other authors as well, who also determined it was not an important factor for germination (Hootsmans et al. 1987, Harrison 1991, Moore et al. 1993). However, although Bintz and Nixon (2001) reported no effect of light on germination they did report lower biomass, lateral shoot formation, leaf length, and leaf width of seedlings subjected to lower light levels. We found a similar pattern in our seed addition plots with smaller seedlings in eelgrass control plots where light levels were likely to be reduced compared to adjacent removal plots.

We did find a relationship between water temperature and natural recruitment. At Peterson and Oysterville, where natural recruitment as well as germination were low,

water temperatures were highest, suggesting there may be a temperature threshold above which germination and seedling survival are reduced.

Finally, we did not measure salinity directly, but when compared to known salinity trends in the bay, spatial patterns of recruitment do not appear to be correlated with salinity. Although laboratory experiments have typically suggested that salinity promotes germination (e.g. Phillips et al. 1983) more recent experiments using natural conditions have also suggested salinity has little to no effect (Probert and Brenchley 1999).

Biological factors

Recruitment patterns may also be influenced by biological interactions. We found better germination in plots where adult plants were removed (Figure 9A). However, this trend weakened with time. The cause of this declining association is likely due to both increasing light availability with longer day lengths as well as rapid regrowth of adult plants such that by August, adult densities were similar between control and eelgrass removal treatments. Robertson and Mann (1984) reported a similar trend with increased seed germination in areas where ice scour removed above ground plant biomass but no effect of canopy shading on seedling survival.

There was a strong relationship between seedling size and adult shoot density. Seedlings were larger in plots where adults were removed and in areas where adult densities were generally fewer (Figure 10B, C). Other studies have shown that seedlings within meadows experience reduced growth compared to seedlings in unvegetated areas (Robertson and Mann 1984), or die because of self-shading (Olesen 1999). Competitive

interactions between conspecific adults and seedlings have been observed in other species as well. Past studies have shown that resource exploitation by neighboring plants severely constrains seedling establishment, growth, and survival (Louda et al. 1990, Aguilera et al. 1993, Aguilera et al. 1995, Foster et al. 1997).

Interestingly, experimental germination and natural recruitment of eelgrass were not correlated with adult shoot densities at the scale of sites. This may be due to the fact that density is not necessarily the best indicator of biomass, as low-density areas have characteristically larger plants (LW unpublished data), making it difficult to interpret where seedlings may be facing greatest competitive pressure from adults. Recruitment patterns also appear unrelated to cover of other macrophytes (e.g. *Z. japonica* and benthic macroalgae; Figure 7C, D). *Zostera japonica* was most abundant at Stackpole while little to no cover was observed at Stony Point, Long Island and Peterson. Similar algal cover was observed between Stony Point and Peterson but the highest densities of natural seedlings were observed at Stony Point and significantly fewer were observed at Peterson. The two seagrass species generally occur in separate habitats, however, they do overlap in some areas and potential competitive and/or facilitative interactions between them need to be further explored. Although at least one study has addressed the negative effect of macroalgal cover on vegetative shoot densities in the Pacific Northwest (Nelson and Lee 2001) there are no data available on the impacts to early life history stages.

Conclusions

There is significant spatial variation of eelgrass recruitment within Willapa Bay. This variation is likely the result of both seed limitation (seed loss) as well as differences

in germination. Both of these factors appear to be influenced by physical setting and interactions with adult plants, however, how these factors interact, and the scales at which they are important are not clear. Despite this uncertainty, our data provide much needed understanding of habitat requirements of eelgrass seedlings in Pacific Northwest estuaries.

Although sexual reproduction is often regarded as unimportant for maintenance of viable seagrass populations, our study showed significant seed production and high seedling densities at some sites. These data are encouraging for future seed-based eelgrass restoration projects in the Pacific Northwest, as they suggest that there is an abundant seed source and that a sufficiently large amount of seeds will likely germinate successfully.

There are complex interactions between physical and biological factors that affect seed germination and seedling survival (Moore 1993, Probert and Brenchley 1999, this study). Our findings indicate that careful selection of seagrass donor and transplantation sites is important, not only at a regional scale, but also at a local scale. It appears that small-scale locally varying factors like water temperature, sediment type, and adult density, are important for germination and seedling survival (van Katwijk and Wijgergangs 2004, this study). Our study shows that sites will vary in their potential for germination success, seedling survival, and seedling growth. Although some sites will produce high numbers of seeds, they are not necessarily suitable habitats for germination. Therefore, prior to seeding an area, surveys should be completed to ensure the environment will be conducive to successful germination and seedling survival.

As more energy is devoted to understanding seagrass conservation and restoration ecology, seed ecology research will likely become increasingly more important in landscape level issues including reserve design, habitat fragmentation and basic population biology (Montalvo 1997, Orth et al. 2000).

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Figure 7. Density of *Zostera marina* (A) seedlings and (B) adults, percent cover of (C) *Z. japonica*, and (D) macroalgae at five sites in Willapa Bay, WA (Stackpole = ST, Oysterville = OY, Stony Point = SP, Long Island = LI, Peterson = PE). Error bars represent standard error. Letters above bars represent significant difference between sites (Scheffe's multiple comparison test p < 0.05).
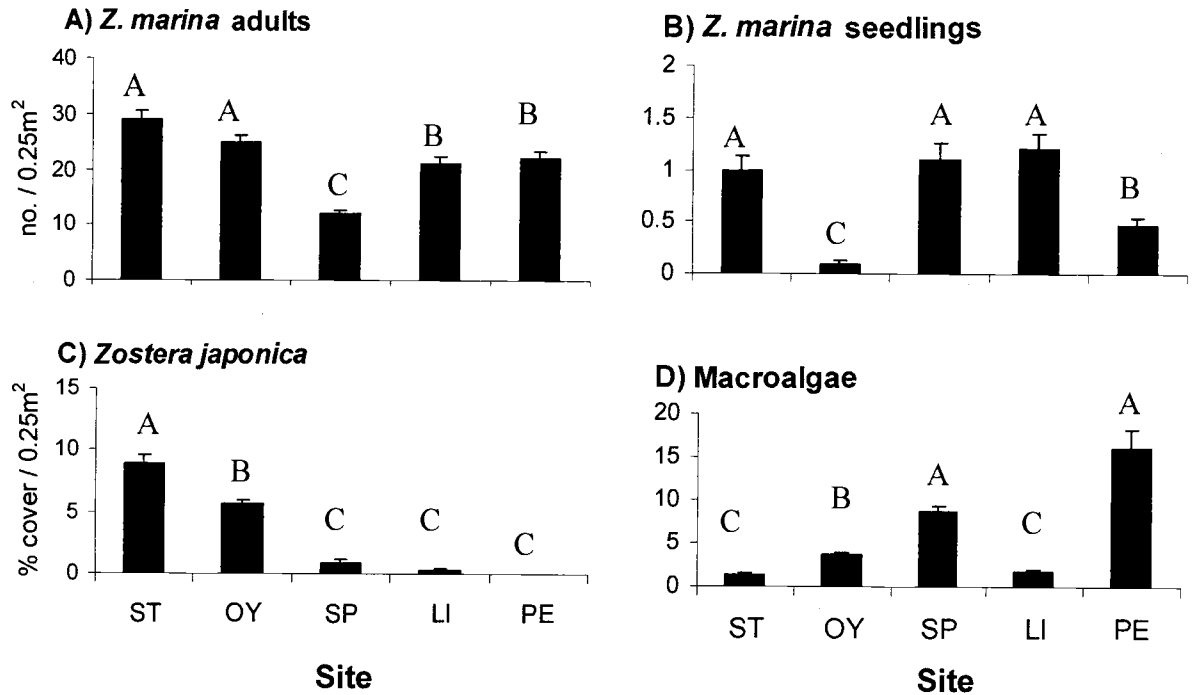
Figure 8. Zostera marina (A) flowering shoots, (B) seeds produced per shoot, (C) estimated seed production per 0.25m2 and (D) seeds in sediment at five sites (site abbreviations in Figure 1). Error bars represent standard error. Letters above bars represent significant difference between sites (Scheffe's multiple comparison test p<0.05).
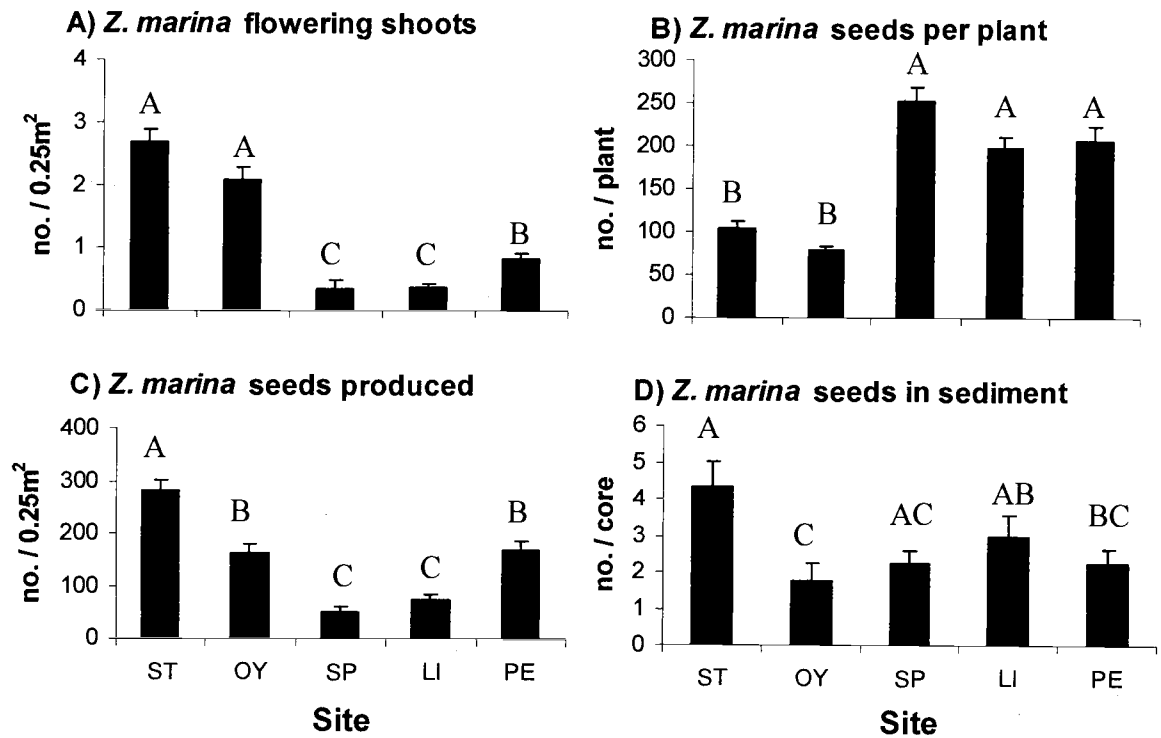
Figure 9. *Zostera marina* seed germination experiment for (A) April, and (B) August in control and eelgrass removal plots at five sites in Willapa Bay, WA (site abbreviations in Figure 1). The number of adult plants in the 0.25m^2 plots surrounding each packet for (C) April and (D) August. Error bars represent standard error (no error reported when n < 3).
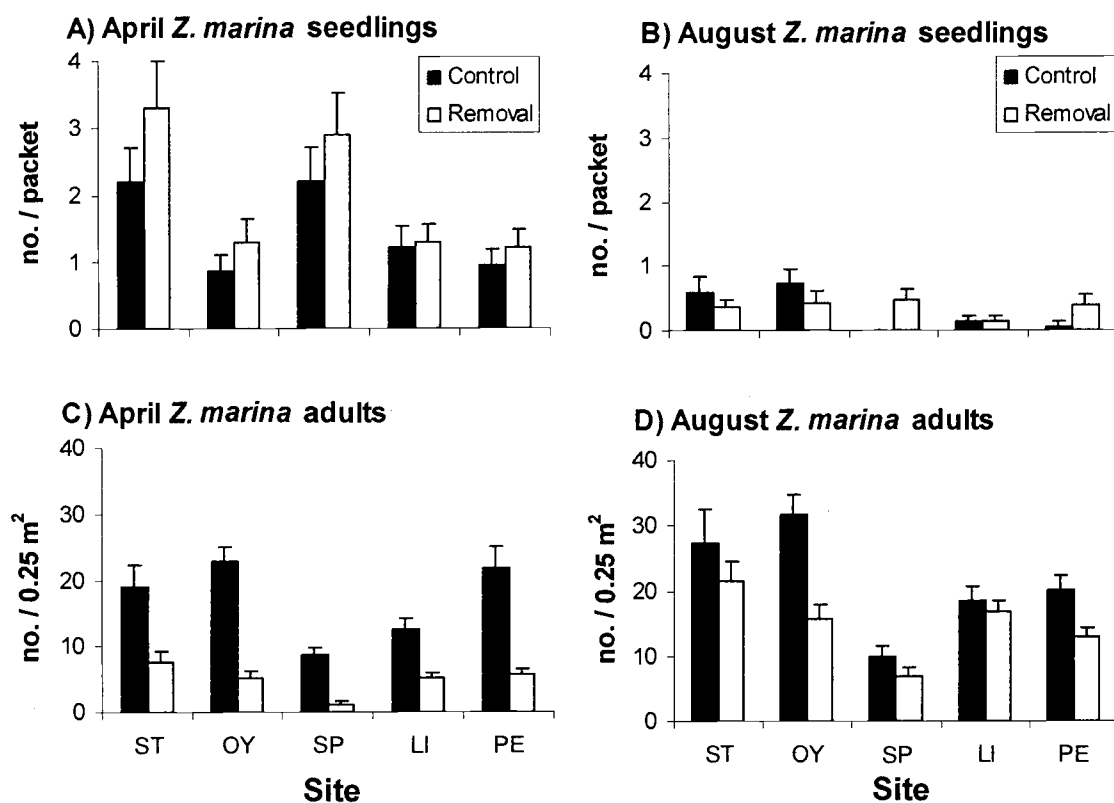
Figure 10. *Zostera marina* seedling length in (A) June, 2005 and (B) August, 2005 and (C) seedling biomass in August 2005 for seedlings in control and eelgrass removal plots (site abbreviations in Figure 1). Numbers above bars represent sample size. Error bars represent standard error (no error reported when n < 3).
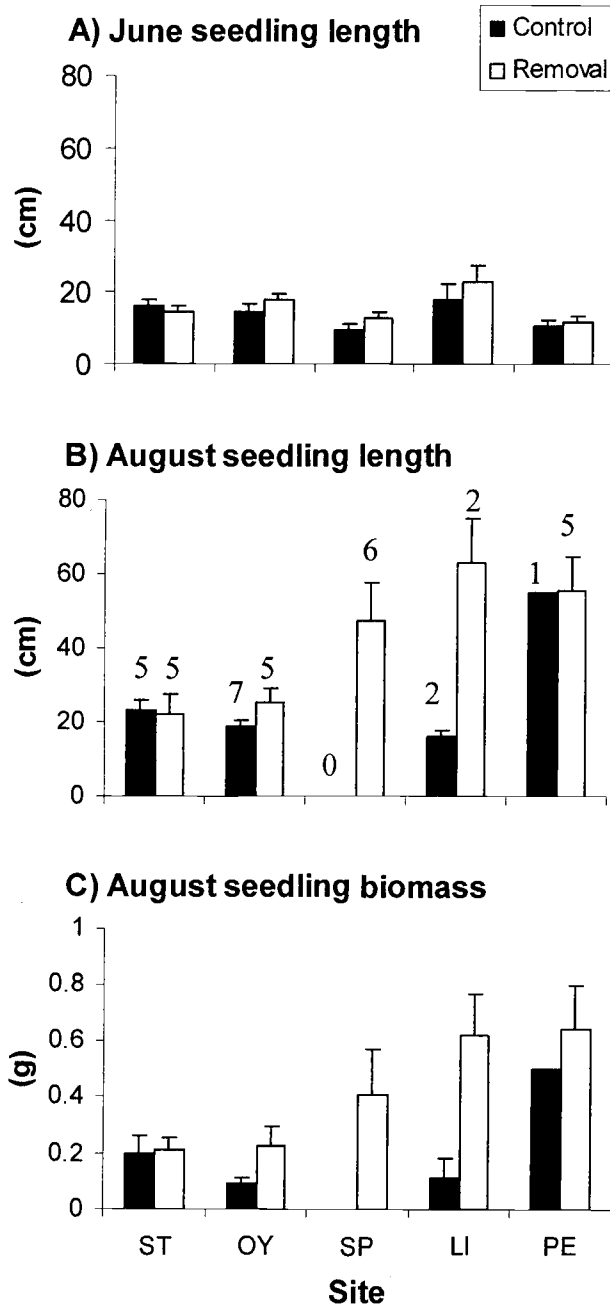
Figure 11. Physical factors at five sites in Willapa Bay, WA, (site abbreviations in Figure 1) (A) sediment redox potential, (B) sediment accretion, (C) sediment grain size, (D) water temperautre, and (E) light intensity. Light was measured in bare areas and adjacent areas with adult plants present. No data (ND) for LI eelgrass. Error bars represent standard error. Letters above bars represent differences between sites (Scheffe's multiple comparison test p < 0.05).
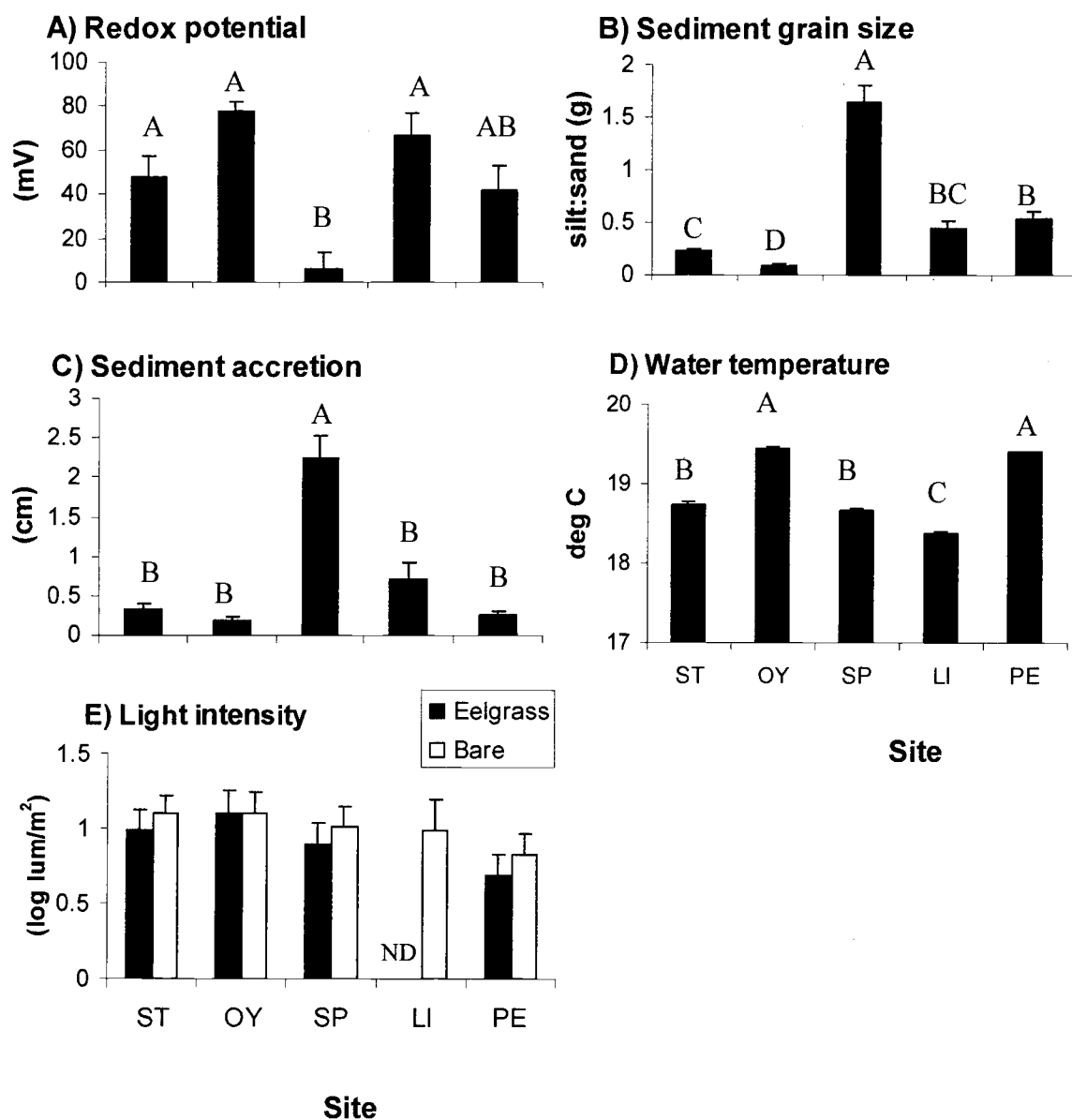
CHAPTER 4. GENERAL CONCLUSION

The importance of seagrasses to estuarine communities is widely accepted and, this combined with their continued decline throughout the world, have resulted in a need to better understand the factors affecting their growth and reproduction. Conservation and restoration of seagrasses will require an understanding of their population biology including the role of seeds in maintaining populations following disturbance. This thesis addresses the impacts of oyster aquaculture as a disturbance on these early life history stages.

In Chapter Two I defined patterns of seed production, germination, and seedling growth and survival of eelgrass under different oyster aquaculture practices: dredging and off-bottom longline culture. Natural seedling recruitment and seed production were high in dredged beds compared to longline beds and reference areas. A seed addition experiment showed that germination was most successful in dredged areas, where adult shoot densities were lowest, and that seedlings survived better and were bigger when adult neighbors were removed. Based on these studies, I proposed that high recruitment in dredged beds was due to both enhanced seed densities and removal of adult plants while low success in longlines was likely due to a combination of physical factors including increases in sediment accretion and significantly lower redox values. These results suggest that oyster aquaculture has a significant influence on eelgrass recruitment and that the impacts may be positive or negative depending on the aquaculture technique. While dredged ground culture can enhance or at least maintain seed density and seed

germination, I found that longline aquaculture can significantly reduce eelgrass recruitment.

Although eelgrass in a dredged bed may be reduced following harvest, this study suggests that recovery via seed germination is possible and may occur over a relatively short time frame. Seed production and germination are relatively high within dredged beds and, if adjacent beds are unharvested during that dredging event, a substantial seed source should persist. These data may have important management implications as they suggest that eelgrass recovery post-dredging may be rapid and that dredge sites may act as sources of eelgrass seed. However, aquaculture impacts will likely vary at different sites within and among estuaries.

In Chapter Three I identified significant spatial variation of eelgrass recruitment within Willapa Bay. This variation is likely the result of both recruitment limitation and germination success. Both of these factors appear to be influenced by physical setting and interactions with adult plants, however, the ways in which these factors interact, and the scales at which they are important are not clear. The interactions between physical and biological factors that affect seed germination and seedling survival are complex and deserve further study.

Although sexual reproduction is often regarded as unimportant for maintenance of viable eelgrass populations, I observed high seed production and high seedling densities throughout Willapa Bay. These data are encouraging for eelgrass conservation and restoration, as they suggest that seeds may contribute to initiation of new beds and that there is an abundant seed source for restoration use.

Preservation of existing seagrass beds and implementation of sustainable practices affecting seagrasses are critical first priorities in stemming further seagrass declines. This thesis provides knowledge about eelgrass recruitment that will be important for managers and shellfish farmers as they develop best management practices to ensure ecologically sound shellfish farming and management practices.

Replacement of lost eelgrass may also be possible if restoration is done using the correct techniques. While some transplant techniques have been successful in the Pacific Northwest (see review by Thom 1990), restoration methods involving the use of seeds have largely been ignored in the region. Many researchers now encourage the use of seeds and/or seedlings in restoration projects because seeds are often abundant and the impacts to existing beds are decreased (Orth et al. 2006a). In addition, the labor and cost involved can be significantly reduced. However, as chapter two suggests, careful selection of seagrass donor and transplantation sites is important, not only at a regional scale, but also at a local scale because small-scale locally varying factors like water temperature, sediment type, and adult density, are important for germination and seedling survival.

This thesis provides important information about the impacts of longline and dredged oyster culture on eelgrass recruitment as well as the relative importance of seed supply, physical factors, and germination for recruitment success, yet some questions remain. Although it appears longlines may decrease eelgrass recruitment, the mechanism responsible for this is unknown. Increased sedimentation resulting in seed burial is one factor that should be studied. The potential build-up of toxic sulfides in longline areas should also be tested as a potential source of seed and/or seedling mortality. More

generally, mechanisms responsible for seed loss should be addressed. Despite high seed production in some areas, seed bank densities and seedling densities were relatively low. Future research should attempt to determine the cause of this seed loss so that reproductive success can be maximized.

Future studies should also address landscape-scale issues including the potential for seeds to travel from one bed to another and how the mosaic of different aquaculture types within a bay may affect the longer-term sustainability of eelgrass populations. Understanding these issues will be critical for the determination of no net loss policies applied to eelgrass habitat. Further, as more energy is devoted to understanding seagrass conservation and restoration ecology, seed ecology research will likely become increasingly more important in other landscape level issues including reserve design, habitat fragmentation, and basic population biology (Montalvo 1997, Orth et al. 2000). Continued research on sexual reproduction in eelgrass and other seagrass species will lead to the development of more effective management strategies for sustaining seagrass populations worldwide.

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