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Eelgrass (*Zostera marina*) Restoration Techniques

by

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Abstract

Eelgrass (*Zostera marina*) Restoration Techniques

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Five environmental variables, including oxidation/reduction potentials (redox; Eh_7), and three vegetative characteristics were measured at 14 northern Puget Sound sites to better describe the variability of factors influencing the existence of *Zostera marina* (eelgrass) populations. Redox in marine sediments were found to be negatively correlated with leaf size for potentials less than -150 mV.

Redox potentials were manipulated in both a closed, seawater system (in lab and greenhouse) and *in situ* for determination of growth impacts and transplantation success of eelgrass. Correlation was found between marine sediment redox potentials and new growth characteristics in lab, and between *in situ* marine sediment redox potentials and transplantation survival/success. Transplant success rates above 80% were found when *in situ* redox potentials were artificially raised by 250 mV for a period of no more than four days.

In addition, a case study involving a blend of low-cost restoration techniques was completed beneath and adjacent to a highly utilized commercial dock in order to restore the submarine environment to a condition that would promote the natural recolonization of *Z. marina*. Effective techniques included submarine debris removal, installation of reflective material beneath the dock, and minimal transplantation of donor *Z. marina* stock.

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CHAPTER I: DISSERTATION INTRODUCTION

“All my life through, the new sights of Nature made me rejoice like a child.”
Marie Curie

In the wake of our recent and horrific national tragedy (11 September 2001), I cannot help but think how different individuals and cultures are around the globe; they are comprised of differing religious passions, financial goals, and environmental concerns alike. In a world where our currently accepted laws of physics indicate a trend towards entropy, directed effort is required if this trend is to be reverse for most situations. Effort can be defined as the conscious exertion of power, a serious attempt to achieve a particular end. It may include physical energy, financial support, scientific investigation, human passion, ideals acted upon, or purely the lack of consumption or contribution of debris within the gravitational pull of our planet. This definition is not exhaustive.

As I and so many others see and experience the destructive capabilities by humans to each other and our planet, my own passion continues to drive me to aid in some fashion the reversal of the anthropogenically enhanced trend towards greater entropy, experienced as the ever-growing effects of commercial development. In conducting my research, I place priority in many of the items listed above in the definition of effort. Examples of this include not leaving scientific/sampling debris within the studied environments, reducing as much as possible methodologies that involve destructive sampling, or utilizing methods that do not require the continual use of non-

renewable natural resources as an element of restoration. If cost-effective and applicable methods are found to be successful, hopefully they will more likely be utilized or implemented by others.

Coastal marine ecosystems, such as the seagrasses, kelp forests, mangrove forests, and coral reefs, are some of the most productive ecosystems in the world, yet we are losing them at alarming rates (Crooks and Turner 1999). This is largely due to the fact that approximately 40% of the world's population lives within 100 kilometers of a coastline, an area that accounts for only about 20 percent of the Earth's land mass. Human population increase and development are reducing global populations of mangrove forests, coastal wetlands, seagrass populations, kelp forests, and coral reefs at ever increasing rates. Fish and shellfish, most of which are found in these coastal ecosystems and depend upon them for their survival, provide about a sixth of the animal protein consumed by people worldwide. Over one billion people, mostly in developing countries, depend on fish for their prime source of protein. Coastal ecosystems have already lost much of their capacity to sustain fish populations because of overfishing, destructive trawling techniques, and destruction of nursery habitats (World Resources 2000).

As the human population of our planet continues to grow above six billion, continued stresses will be placed on our coastal marine ecosystems, their available habitats, and

overall health. As demonstrated by our most recent national tragedy, humans are capable of achieving great amounts of destruction. Whether it comes by way of large-scale, single events or by the cumulative effect of smaller, often unrecognized sources, the systematic elimination of our planet's natural resources requires attention.

The research described in this dissertation focuses on restoration techniques for eelgrass, *Zostera marina* L., focusing largely on the redoximorphic characteristics of marine sediments (micro-scale) and its effects on *Z. marina*, as well as submarine habitat condition (macro-scale). The specific issues addressed are aimed at increasing the success rate of restoration for *Z. marina* through better understanding the environmental conditions in which it exists and through scientific experimentation of new, cost-effective restoration techniques.

Chapter II introduces the initial investigation whereby the redoximorphic conditions and other environmental parameters associated with the marine sediments were correlated to growth variables of *Z. marina*. Even though the oxidation/reduction potentials of marine sediments were highly variable, a trend was found indicating that stronger growth parameters were associated with higher redox conditions.

Chapter III describes the multi-stage experimentation that includes the initial laboratory experiments defining the range of redox (in mV) in Puget Sound marine

sediments. Following this investigation, greenhouse experiments were conducted to determine whether higher redox potentials (imposed) stimulated the growth rate of *Z. marina* significantly more than a naturally occurring, low redox potentials. After showing that a higher redox environment positively affected the growth of *Z. marina*, a field experiment was conducted to determine whether *in situ* marine sediments could be altered in such a way to raise the redox potentials and to contribute to a higher transplantation success rate. Ultimately, a higher transplantation success rate was found in areas that had their redox potentials artificially raised during the period of transplantation.

Chapter IV describes a case study where inter- and subtidal conditions were altered in ways to allow natural recolonization of *Z. marina*. Low-technology, cost-effective methods were accomplished in hopes that following their success, they would be able to be easily implemented by others. Largely, the methods were aimed at returning the submarine environment to pre-disturbance conditions; removing submarine debris and enhancing the light conditions of the previously shaded submarine environments. These methods were also successful and consequently are being included in dock modification plans in the Puget Sound area.

CHAPTER II: ECOLOGICAL ANALYSIS OF ZOSTERA MARINA IN PUGET SOUND, WASHINGTON; A LATITUDINAL STUDY OF SELECTED VEGETATION AND SEDIMENT CHARACTERISTICS

“What I see in nature is a magnificent structure that we can comprehend only very imperfectly, and that must fill a thinking person with a feeling of humility.”

Albert Einstein

ABSTRACT

Relationships between various sediment characteristics and submerged aquatic vegetation were studied across fourteen eelgrass (*Zostera marina* L.) meadows in northern Puget Sound, Washington, U.S.A.. A sampling scheme comprised of 15, 0.25 m² quadrats was established at each site to adequately sample the existing population. Information was collected on sediment oxidation/reduction potential (*Eh*), sediment pH, sediment organic matter, and sediment grain size as well as vegetation variables including species density, blade height, and blade width. In addition, fetch and shoreline aspect were determined. Additive stepwise regression and analysis of variance was used to determine relative influences on the populations. The majority of samples consisted of sand percentages greater than 85%. A linear relationship was found between latitude plus silt percentage and density of *Z. marina* ($R = 0.499$, $p\text{-value} < 0.001$). Cubic relationships were found between Eh_7 and leaf area ($R = 0.351$, $p\text{-value} < 0.001$) and between depth and density ($R = 0.286$, $p\text{-value} = 0.0007$) in the shallower $\frac{1}{3}$ of depths where *Z. marina* is commonly found in the Pacific Northwest. When considered for the purpose of restoration of *Z. marina* by transplantation of whole plants, marine sediments with Eh_7 values less than -150 mV

appear to negatively effect blade growth and overall plant health, thus contributing to transplant shock.

INTRODUCTION

An inter- and subtidal group of plants, seagrasses are found globally with varied canopy architecture providing habitat for many coastal marine species. Due in part to their proximity to coastal zone development, dredging of navigable waters, and anthropogenic and stochastic disturbances, seagrass populations are susceptible to damage from these types of disturbances and have been on the decline globally (Costa 1988, Giesen *et al.* 1990, Kemp 1984, Hemminga and Duarte 2000, Nichols and Pamatmat 1988, Rasmussen 1977, Short and Wyllie-Echeverria 1996, Thom and Hallum 1991). Understanding the characteristics of the environment in which they exist helps us predict how our controllable actions affect seagrass populations. Appreciation of this knowledge can reduce the rate at which we are losing global seagrass populations and increase the rate of success with seagrass restoration efforts.

Eelgrass (*Zostera marina* L.), a seagrass in the family *Zosteraceae*, is common among temperate shorelines in the northern hemisphere (den Hartog 1970). It is typically found in coastal waters where temperatures are between 0-30°C and where shoreline wave energy is minimal (Koch and Beer 1996, Phillips 1984). In addition, *Z. marina* is most common in bays, inlets, or sounds where currents can be substantial (e.g., 20-

40 cm sec⁻¹) (Fonseca *et al.* 1998, Phillips 1972). *Zostera marina* is shade intolerant, and therefore responds dramatically to either natural or anthropogenic reduction of available light (Backman and Barilotti 1976, Dennison and Alberte 1985, Dennison *et al.* 1993, Duarte 1991, Moore *et al.* 1997, Zimmerman *et al.* 1991).

Of the variables found to have an impact on *Z. marina* establishment and growth, light is one of the most researched. As submarine light levels decrease, physiological responses have been seen in *Z. marina* as well as the limitation of areal extent of populations (Abal and Dennison 1996, Alcoverro *et al.* 1999, Dennison 1987a, Duarte 1991, Zimmerman and Alberte 1996). Light, however, is not the only variable affecting the physiology and distribution of *Z. marina*. Little research has focused on sediment characteristics, and even less regarding the oxidation/reduction (redox) potentials of the sediments. This research demonstrates how redox plays an important role in the establishment and existence of *Z. marina*.

The redox potential within the rooting zone of a plant is indicative of the available O₂ to the roots (Armstrong *et al.* 1991). Redox potential measurements are used to indicate the electro-chemical state of elemental substances in soils or sediments (Beckman 1977). The measurement is a quantification of the ion activity associated with the substances. In many chemical reactions, electrons are transferred from one substance to another, and it is this tendency which is measured; whether substances

gain (relative reduction) or lose (relative oxidation) electrons (Gambrell and Patrick 1978, Langmuir 1997). In natural systems, redox potentials are often interpreted as equilibrium potentials, where there is no neutral redox point as in pH (Bohn 1971, Ponnampereuma 1972).

Redox can be viewed as an intensity factor indicating reducing or oxidizing ability of the soil/sediment. It is largely driven by the aerobic/anaerobic respiration of microorganisms. In oxygenated sediments, aerobic respiration can occur. In sediments where oxygen has been depleted, microorganisms oxidize organic matter to utilize NO_3^- , Mn^{4+} , Fe^{3+} , and SO_4^{4-} as alternative electron acceptors to O_2 in the respiration process (Faulkner *et al.* 1989, Mitsch and Gosselink 1993). This condition leads to low redox potentials which may result in the increased availability of toxic soluble forms of Fe and Mn, as well as the production of H_2S and methane gas.

Knowledge about seagrass populations along shorelines and the relationships with their environmental conditions (e.g., sediment characteristics, latitude) may be critical to better understanding what influences their existence. *Zostera marina* populations are known to be annually variable in distribution and density (Hemminga and Duarte 2000). This study measured stem density, leaf size, sediment pH, sediment oxidation/reduction potential, sediment temperature, and latitude of site. Stepwise regression and analysis of variance techniques were performed on the data. The

nature of the relationships between *Z. marina* populations and marine sediment characteristics in northern Puget Sound was investigated.

STUDY AREA

Northern Puget Sound, Washington, U.S.A., was chosen as the study area due to the existence of *Z. marina* and its minimally urbanized character as compared to other reaches of the sound. While *Z. marina* does exist in south Puget Sound and parts of the Hood Canal, variability in the marine environment due to freshwater input, water temperature fluctuations, and shoreline armoring made it less than suitable for inclusion in the study area.

In early 1998, twenty nine sites were identified as historically supporting *Z. marina* populations in northern Puget Sound (Washington State Department of Natural Resources 1995); 14 of those sites were found to be accessible and still maintained a *Z. marina* population (Figure II.1). They were found in the north Puget Sound between latitudes 49°59'30 and 48°28'49. In July 1998, these 14 sites were sampled on foot during the daily low tide (two sites per day) for vegetation and environmental characteristics (Figure II.2).

METHODS AND SAMPLING DESIGN

Sampling Design

Prior to sampling of the 14 identified sites, an initial sampling effort was conducted in August of 1997 (Anacortes, Washington) to determine a sample size minimum.

Equation II.1 was used to estimate the number of individual sample plots necessary at each site.

$$P (| [(\hat{\theta} - \theta) / \theta] < \varepsilon) = 1 - \alpha \quad \text{Equation II.1.}$$

where

$\hat{\theta}$ = estimated density of *Zostera marina*,

θ = true density of *Zostera marina*,

ε = acceptable error,

$1 - \alpha$ = how often sampling effort should achieve precision goals.

A minimum sampling size of 14.1 was determined where $\varepsilon = 0.10$ and $1 - \alpha = 0.90$.

Consequently, a sample size of 15 was used for each site (Sfriso and Ghetti 1998).

Two hundred and ten samples were measured across 14 sites. Samples were measured on foot and with mask/snorkel during a summer low tide series. Field sampling of the 14 sites was carried out during a summer low tide series, between August 18-25 1998. At each site, the center of the *Z. marina* population was subjectively determined and

marked along the shoreline. From this point, a transect was measured beginning from the shallowest point which supported *Z. marina* to the deepest. A point randomly located along this transect was selected. This point became the first of 15 sampling points. Each successive sampling point was selected by randomly choosing a heading within the half-compass hemisphere in the direction of tidal movement, and proceeding 15 meters. In the case where a sampling point fell outside the extent of the *Z. marina* population, a 180° heading from the last randomly selected heading was used from the preceding sampling point (Figure II.2).

Vegetation and Environmental Measurements

At each sampling point, seagrass vegetation and sediment characteristics were sampled using a 0.25 m² quadrat (Backman 1990, Dennison 1990). Stem density of *Z. marina* was measured within each quadrat, as well as blade height and width of four *Z. marina* individuals, selected 10 cm towards the center of the quadrat from each corner. Stem density combined with stem height and width offers a rough estimate of productivity. From the center of the quadrat, a sediment sample was removed and placed in a resealable plastic bag for transport to the lab to determine grain size. *In situ* oxidation/reduction potentials (Eh_7 ; mv) and pH values were measured at each of the four selected *Z. marina* individuals using a hand-held Digi-Sense[®] pH/mV/Eh/Temperature Meter and a personally designed plexiglass chamber in which the meter was placed. A waterproof bushing attached to one end of the chamber

allowed access to the probe cable. The distal end of the each probe (pH, Eh, Temp.) was inserted into the marine sediments at a 45° angle to a depth of 15 centimeters (within the rooting zone of *Z. marina*). Care was taken not to disturb the probe following insertion. Eh measurements were recorded upon initial insertion, and each minute thereafter for a period no longer than 10 minutes. pH and temperature measurements were recorded upon measurement stabilization. Aspect was measured on-site from the shallow endpoint of the measured transect, and fetch was determined using USGS quadrangles.

Grain size analysis conducted in the lab was consistent with Gee and Bauder's (1986) hydrometer method. Equation II.2 was used to calculate simplified clay fractions.

$$P_{2\mu\text{m}} = m \ln (2/X_{24}) + P_{24} \quad \text{Equation II.2}$$

where

X_{24} = mean particle diameter in suspension at 24 h (see Equation II.3),

P_{24} = summation percentage at 24 h (see Equation II.4),

$m = (P_{1.5} - P_{24}) / \ln (X_{1.5} / X_{24})$; slope of the summation percentage curve between X at 1.5 h and X at 24 h,

$X_{1.5}$ = mean particle diameter in suspension at 1.5 h, and

$X_{1.5}$ = summation percentage at 1.5 h.

$$X = \theta t^{-0.5} \quad \text{Equation II.3}$$

where

θ = pre-tabulated sedimentation parameter as a function of hydrometer readings, R , for ASTM 152 hydrometer (Day 1965),

t = time from initial mixing; minutes.

$$P = (C/C_0) * 100 \quad \text{Equation II.4}$$

where

C = (concentration of soil in suspension in g/L) = $R - R_L$; R is the uncorrected hydrometer reading in g/L, and R_L is the hydrometer reading of a blank solution,

C_0 = oven-dry weight of the soil sample.

The sand fraction was calculated using the same procedure as for $P_{2\mu\text{m}}$, but using the 30-sec and 60-sec hydrometer readings rather than the 1.5 h and 24 h readings, respectively. The $P_{50\mu\text{m}}$ value was then subtracted from 100 to obtain sand percentage.

The silt fraction was calculated using Equation II.5.

$$\% \text{ silt} = 100 - (\% \text{ sand} + \% \text{ clay}) \quad \text{Equation II.5}$$

Statistical Analyses

Descriptive statistics were calculated for each measured variable by site. A forward stepwise regression search algorithm was used to determine the best model that would describe density and leaf area of *Z. marina*. Simple linear regression, polynomial regression, and analysis of variance (ANOVA) were used to draw inferences regarding environmental and vegetation variables (Kent and Coker 1997, Neter *et al.* 1990, Zar 1984).

RESULTS

Sampling depths ranged from -0.21 m MLLW at the shallowest to -2.12 m MLLW at the deepest (Figures II.3 and II.4). These sampling depths represent the shallower $\frac{1}{3}$ of depths where *Z. marina* is commonly found in the Pacific Northwest; 0.0 to -6.6 m MLLW (Phillips 1984, Thom 1990).

Vegetation Characteristics

Across all 14 sites, the mean density of *Z. marina* was 93 m^{-2} (s.e. = 5.4 m^{-2}), with a maximum density of 400 m^{-2} . *Zostera japonica* was found at five sites with a mean density of 9 m^{-2} (s.e. = 2.4) and a maximum density of 280 m^{-2} . No significant effect on density was found between these two species (p-value = 0.144).

Blade widths for *Z. marina* were found between 1.0 and 5.5 mm with a mean of 2.63 mm (s.d. = 0.92). Blade heights were found at a mean of 41 cm (s.d. = 29.3 cm) and a maximum of 130 cm. Combining these data, an estimate of leaf area ranged between 27 and 433 cm² (Table II.1).

Environmental Characteristics

The redox potential, corrected to pH 7.0 (Eh_7), of marine sediment are highly variable due to the many contributing factors; complex combination of physical, chemical, and biological factors. Redox potential is not a direct measurement of the amount of oxygen found *in situ*, however does indicate at relative values which sediments are likely lacking in oxygen and contributing to the reduction of elements into possibly toxic forms to plants (Hemminga and Duarte 2000). By definition, redox potential is a measurement of intensity, not capacity, of the chemical status of marine sediments with regard to the propensity for which chemical elements receive or supply electrons (Armstrong 1976). It is largely because of this that Eh_7 potentials are continually shifting on an hourly basis depending upon the environmental and biological conditions present; existence of aerobes and/or anaerobes, temperature, organic matter, current movement, benthic disturbances, wave activity, and even local disturbances caused by sediment sampling techniques.

Higher redox potentials (e.g., > 250 mV) indicate the prevalence of O_2 in the system, thus affecting the oxidized states of such chemical elements as ferric iron (Fe^{3+}), manganic manganese (Mn^{4+}), nitrate (NO_3^-), and sulfate (SO_4^{2-}). In sediments of low redox potentials (e.g., $250 - -400$ mV), the reduced forms of these elements are prevalent; ferrous iron (Fe^{2+}), manganous manganese (Mn^{2+}), ammonium (NH_4^+), and sulfides (S^{2-}) (Faulkner *et al.* 1989, Gambrell and Patrick 1978, Langmuir 1997, Mitsch and Gosselink 1993, Ponnampereuma 1972). Across all 14 sites, the lowest Eh_7 was -429 mV and the highest was 390 mV, with a mean of -4 mV (s.d. = 176) (Table II.1). Because Eh is affected by pH (i.e., -59 mV Eh per unit change from pH 7.0), it was also measured at each site (Gambrell and Patrick 1978). Across all 14 sites, the mean pH was 6.27 (s.d. = 1.63).

The particle size analysis showed a majority of sediment samples were found to consist of sand (0.05 mm $> X > 2.0$ mm) percentages between 80-100% (Figure II.5). A second group of sediment samples consisted of sand percentages between 50-60%. In all cases, as the percentage of sand increased, the silt and clay percentages decreased; clay percentage changed the least (Figure II.6). Sand and organic matter were highly and significantly negatively correlated ($R = -0.729$; p-value < 0.001) (Figure II.7). Silt (0.05 mm $> X > 0.002$ mm) and Eh were significantly negatively correlated ($R = -0.265$, p-value < 0.001) (Figure II.8).

In fewer than five samples was sand found in lesser quantity than silt. Silt is the majority particle size when sand is found below 45% by weight; the maximum silt percentage was nearly 60%. The lowest percentage of sand measured was 27%, while the lowest silt and clay percentages were found between 0-3%. Clay content increased slightly as silt increased and sand decreased, and was not found higher than approximately 15%.

The mean organic matter content for all samples was 1.7 % (s.e. = 0.99%), with a maximum of 4.5%. The mean surface water salinity for all sites was 28.7 ppt (s.d. = 1.9), with a maximum of 34 ppt and a minimum of 25 ppt. Fetch for all 14 sites ranged between approximately 1-17 km. All major compass headings were represented (Table II.1).

Statistical Analyses

An attempt to predict density of *Z. marina* from the measured environmental variables (Eh_7 , pH, grain size, O.M., latitude, depth, fetch) was accomplished through a forward stepwise regression search algorithm. An R of 0.550 was found when all variables were included (p-value < 0.001). Latitude and silt were selected as the variables which each individually and coincidentally contributed significantly to the model predictive ability (R = .499, p-value < 0.001) (Equation II.6, Table II.2).

$$(\text{Density; m}^{-2}) = -23.933 + (174.44)L - (0.869)S \quad \text{Equation II.6}$$

where:

L = latitude of site (between northern and southern U.S. boundary of Puget Sound, converted to 0.0-1.0 scale)

S = percentage of silt (1.0 -100.0 scale)

The same algorithmic method was applied to the environmental variables as they pertained to leaf area per plant. An R of 0.676 was found when all variables were included (p-value < 0.001). The redox potential of marine sediments was found to be the single most contributing variable in predicting leaf area of *Z. marina* (R = 0.351, p-value < 0.001) (Equation II.7, Table II.3).

$$(\text{Leaf Area; cm}^2) = 152.7 + (0.217) Eh_7 \quad \text{Equation II.7}$$

where:

Eh_7 = redox potential (mV), adjusted to pH 7.0

Additional analysis further refined the predictive capability of Eh_7 to leaf area of *Z. marina*. Regression analysis identified a third-order polynomial model that describes a curvilinear response of leaf area to Eh_7 (R = 0.439, p-value < 0.001) (Equation II.8, Table II.4, Figure II.9).

$$(\text{Leaf Area; cm}^2) = 128.64 - (0.139)Eh_7 + (0.001)Eh_7^2 - (0.0000025)Eh_7^3 \quad \text{Equation II.8}$$

where:

Eh_7 = redox potential (mV), adjusted to pH 7.0

A third-order polynomial relationship between depth and density was identified for this data set; shallower $\frac{1}{3}$ of depths ($R = 0.286$, $p\text{-value} = 0.0007$) (Equation II.9, Table II.5, Figure II.10).

$$(\text{Density; m}^{-2}) = 237.70 - (455.45)X + (378.90)X^2 - (87.47)X^3 \quad \text{Equation II.9}$$

where:

X = depth, m (MLLW)

A second-order polynomial relationship between depth and Eh_7 was identified ($R = 0.286$, $p\text{-value} = 0.0002$) (Equation II.10, Table II.6, Figure II.11).

$$(Eh_7; \text{mV}) = -211.89 - (439.81)X - (169.31)X^2 \quad \text{Equation II.10}$$

where:

X = depth, m (MLLW)

DISCUSSION

Vegetation and Environmental Characteristics

Sediment characteristics play a significant role in the establishment, distribution, and success of seagrasses (Bulthuis and Woelkerling 1981, Dennison 1987b, Goodman *et*

al. 1995, Pregnall *et al.* 1984, Short 1987, Terrados *et al.* 1999, Udy and Dennison 1997). In Puget Sound, Washington, *Z. marina* is typically found between 0.0 and -6.6 m MLLW (Phillips 1984, Thom 1990). *Zostera japonica* is typically found in depths shallower than *Z. marina*, with a swath of overlapping habitat at the lower intertidal/upper subtidal margin. The data show no effect on density in areas where they co-exist.

The majority of sampled sites populated by *Z. marina* in the northern Puget Sound have sandy sediment profiles, with sand percentages between 80-100%, as indicated by sediment profile results. These sites consist of protected coves or bays and exposed channel boundaries. What remains consistent between these sites is the exposure to strong currents able to transport larger sand particles to and from the site (Downing 1983). Often, the familiar sediment condition for *Z. marina* in the Puget Sound is thought of as unconsolidated, soft, or rich in silt. This type of sediment profile was found, but in a minority of sites. These sites, however, tend to be ones that actively attract visitors (e.g., coastal marine research and interpretive centers or mudflats adjacent to commercialized/vacation destinations) thus the public perception that eelgrass meadows are found in muddy sediments.

Edaphic characteristics, such as Eh_7 and pH are also affected by sediment grain size (Alongi 1998). Redox measurements were found to be between approximately

400 mV and -450 mV. A non-significant negative relationship was found between the amount of silt present and redox, however because of the natural variability of redox (Bohn 1971, de la Cruz *et al.* 1989, Faulkner *et al.* 1989, Langmuir 1997, Ponnampereuma 1972), a comparable R value was calculated ($R = 0.265$). The trend was only moderately negative. This is expected as soil permeability is related to grain and pore size; small-grain sediments have smaller pore sizes and less opportunity for permeation by gas or liquid. Silt can minimally seal the benthic surface from O₂ exchange with the water column. This relationship between redox and silt was stronger than between redox and sand because of the prevalent characteristic of sand particles in all samples.

Statistical Models and Analyses

Density

In northern Puget Sound, the prevailing wind direction is from the south (Downing 1983, Kruckeberg 1991). In winter months, marine air enters the Puget lowlands through Chehalis gap south of the Olympic Peninsula and produces a southerly wind over most of Puget Sound. In summer months, marine air predominantly enters the Strait of Juan de Fuca and flows both north and south from where it first encounters Puget Sound. All sampled sites lie north of this intersection and therefore principally receive southerly winds year-round. Sites further from the eastern end of the Strait of Juan de Fuca (northeast of the strait and in the rainshadow of Vancouver Island)

tended to have eelgrass populations with slightly larger leaves than those sites closer to the strait. This may have been the result of lesser wave intensities at higher latitudes of the sound due to diminished wind intensities, contributing to fewer highly disturbing natural storm events. *Zostera marina* individuals with larger leaves, as those in the more northerly sites, may become more susceptible to being pulled from the sediment due to the greater amount of leaf surface area in the water column. This may lead to the scouring of larger-leaved individuals following rare but intense storm events in these areas.

Silt also significantly contributed to the linear model. The relationship between *Z. marina* density and silt was negative. In areas of rapid currents, smaller particles sizes are less common because the particles are held in suspension and transported off-site. In Puget Sound, Phillips (1972) found that the most productive *Z. marina* populations were in nearshore habitat where currents reached 3.5 knots (180 cm/sec). Conover (1964) also found that rapid currents (20-40 cm/sec) helped to reduce the diffusion gradient across the leaf surface making more available CO₂ and nutrients, and helped to control epiphytic growth.

In general, density increased as depth decreased (became more shallow). This relationship, however, was not linear. Between -2.0 and -0.5 m depth, the relationship between density and depth is negative. This effect is likely caused by a complex set of

variables, which may include both physical and chemical processes. This zone may represent the depth at which the longshore currents are most rapid (Downing 1983), thus transporting more particulate matter and thereby decreasing the available submarine light to *Z. marina*. It may also represent the zone which undergoes the greatest amount of change between summer and winter bathymetric profiles; summer sediment nourishment and winter erosion. This annual disturbance regime may be more representative of depths just above MLLW, which also succumb to similar disturbances due to daily wave energetics.

Leaf Area vs. Eh

Many studies have been conducted on the light requirements of seagrasses, and others on the effects of water quality. Some studies have focused on below-ground processes. Of those very few have investigated the effects of Eh on the growth characteristics of *Z. marina* (Goodman *et al.* 1995, Hamminga 1998, Terrados *et al.* 1999). Of all the variables measured in this study, Eh_7 was found to be the single most important variable in predicting leaf area per plant of *Z. marina*. Kemp *et al.* (1990) described how the transport mechanism of lacunae in *Z. marina* produces only a 5-25 minute lag between the initial production of O_2 in photosynthesis and its release through the roots. This rapid mechanism allows *Z. marina* individuals to tolerate the continually changing and often very low redox potentials of the marine sediments in which they are rooted. As Eh of marine sediments decreases, there is a need for a greater input of O_2 in order to reverse the cascading effect of sediment reduction, thus

minimizing the amount of potentially toxic elements made available by those conditions (Armstrong *et al.* 1991). *Zostera marina* plants are able to increase the volume of O₂ in the rhizosphere by increasing their effective photosynthesizing surfaces; their leaves.

Ultimately, a cubic model was found to best predict the response of leaf area to Eh_7 . This model, and the associated regression, indicates that Eh_7 values below -150 mV play an important role in affecting leaf size. Below this value, reduced forms of iron and manganese are more soluble and potentially toxic (Lambers *et al.* 1998, Mitsch and Gosselink 1993).

I suspect that a complex relationship exists between the *Z. marina* individuals, the submarine light environment and the redox potential of marine sediments in which they are growing. The inclusion of redox into the study of seagrass growth limitations may produce results that show their distribution, especially at their deeper boundary, is controlled by edaphic characteristics to a greater degree than currently understood. This has implications to restoration activities as well. Common transplant failures of *Z. marina* may be partially due to an individual's inability to produce and transport enough O₂ into the newly transplanted rhizosphere to counter the detrimental effects of a low redox environment.

When Eh_7 was compared to depth, a quadratic model best described the relationship. A maximum Eh_7 value was found in the middle of the depth measurements, which corresponds with the depth zone in which the relationship between *Z. marina* density and depth was negative. Eh_7 values may be highest in this zone for a similar argument as for the density relationship. If a greater amount of bathymetric change occurs in this depth zone on an annual basis than in the depths just above and below, then it is understandable that those sediments are being affected to some degree by more turbulent water. This water may not transmit as much light, however it may introduce more oxygen into the pore spaces of the sediments, thus raising the redox potential.

The effects of both light (as a result of depth and water clarity) and redox potentials may control the lower depth limits of *Z. marina*. Currently, submarine light availability is accepted as the limiting environmental condition affecting *Z. marina*'s ability to survive at depth or to be restored at an identified site (Abal and Dennison 1996, Alcoverro *et al.* 1999, Dennison and Alberte 1985, Dennison 1987a, Duarte 1991, Koch and Beer 1996, Longstaff and Dennison 1999, Olesen 1996, Zimmerman *et al.* 1995). This environmental limit should be investigated further in combination with the redox potential of the sediments.

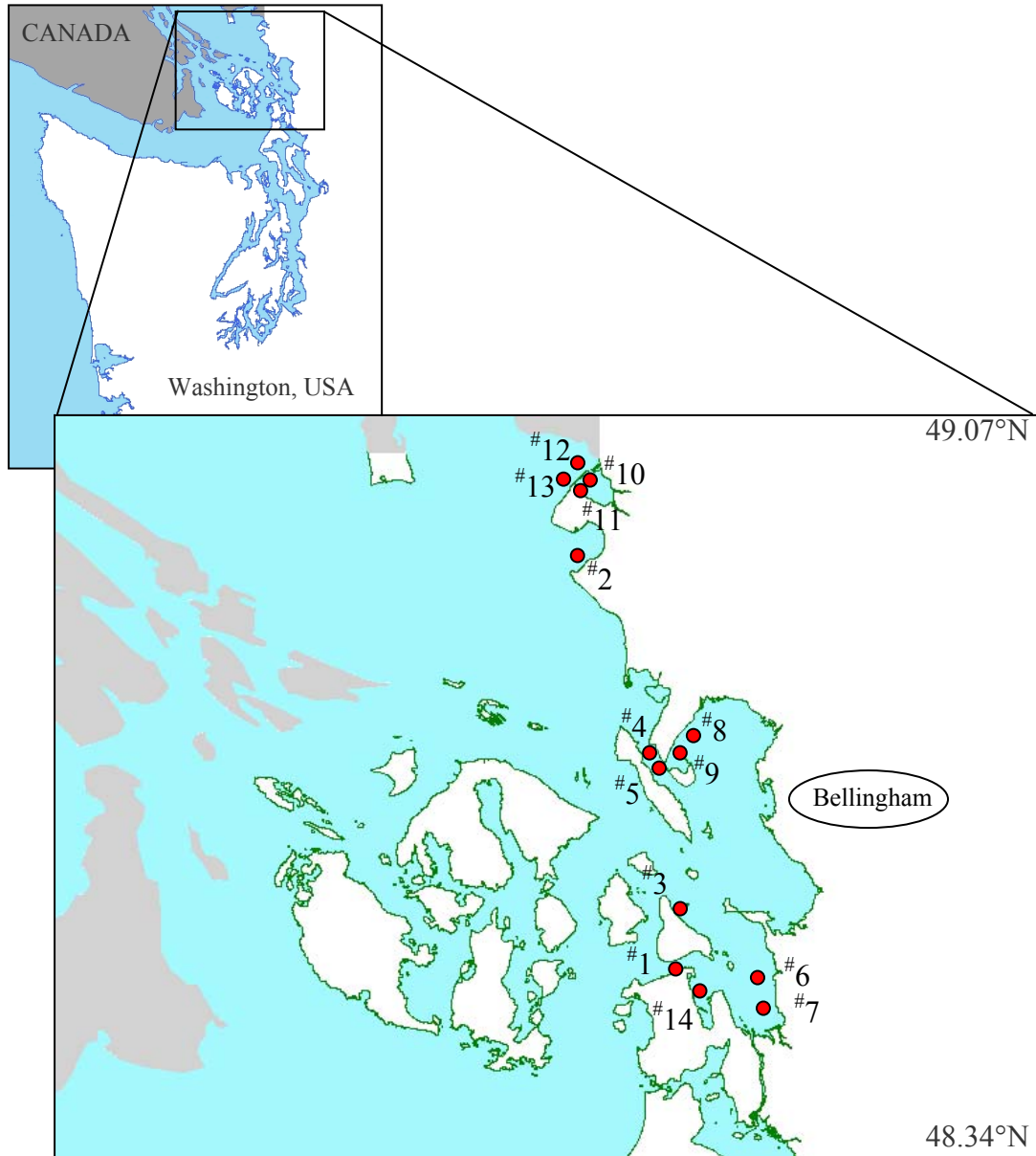


Figure II.1. Field Sites.

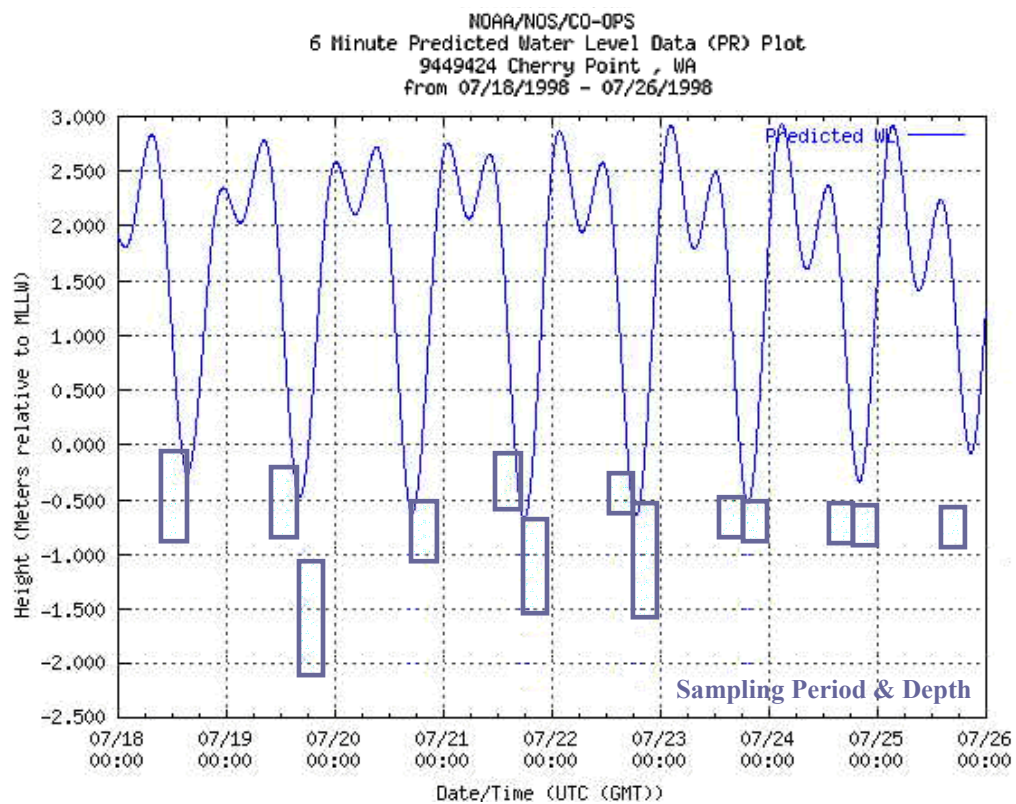


Figure II.3. Tidal elevations during sampling effort, 1998.

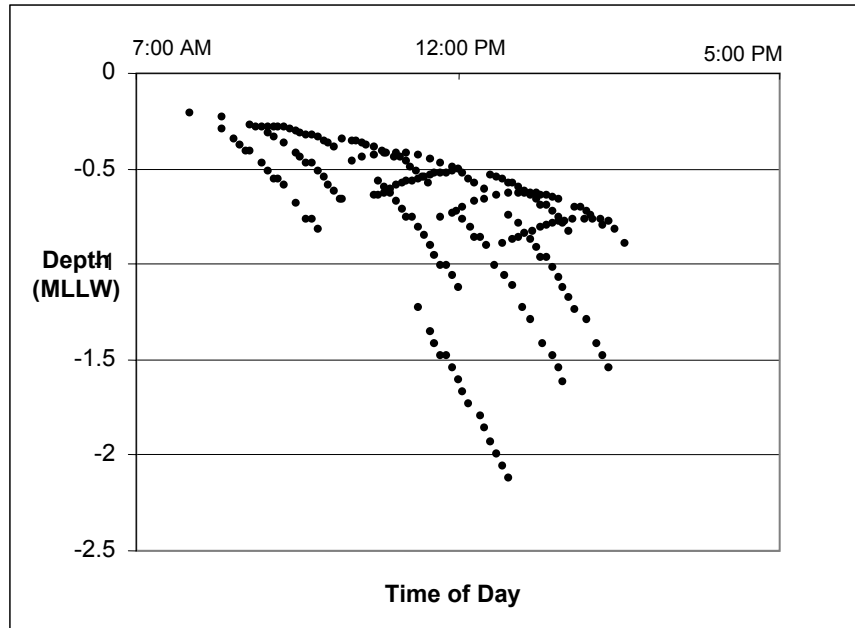


Figure II.4. Sampling Depths.

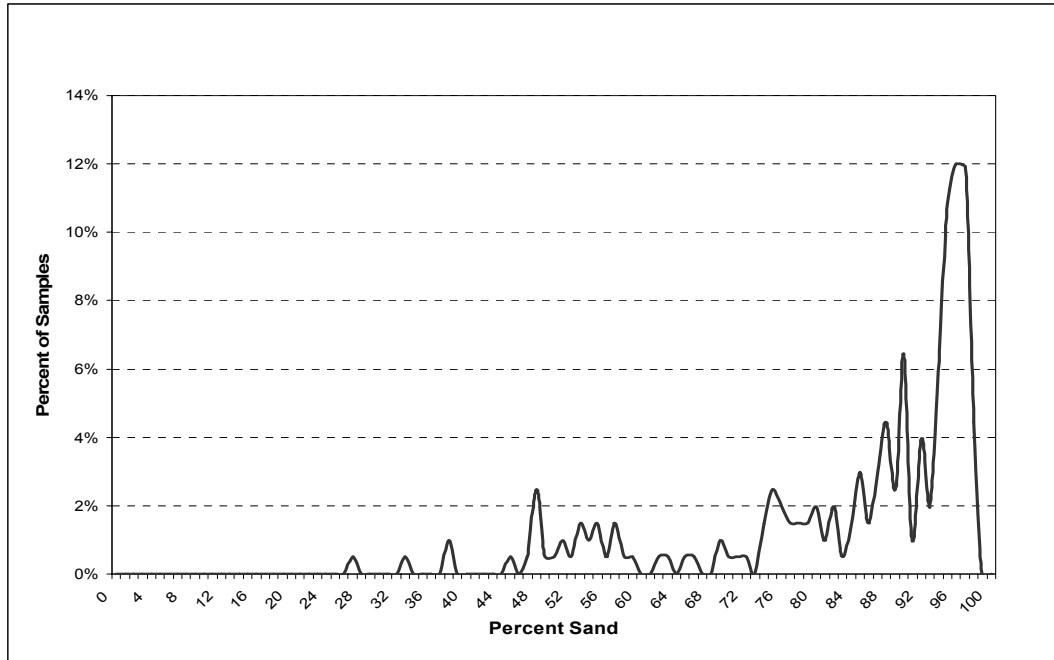


Figure II.5. Sand Percentage in Samples.

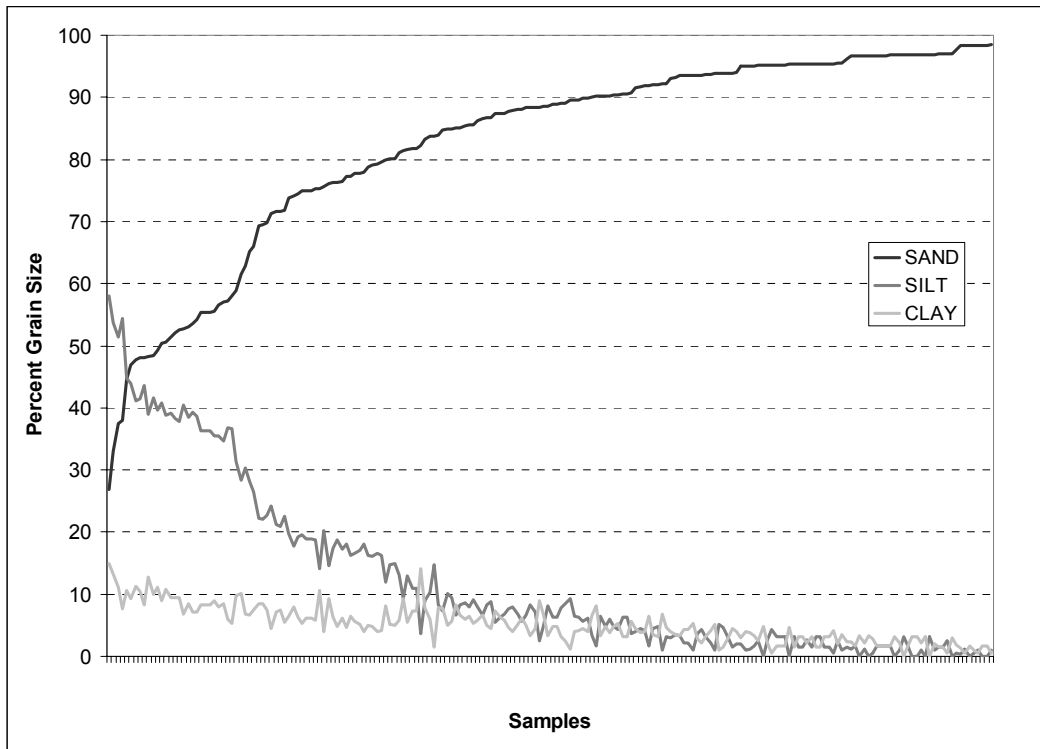


Figure II.6. Grain Sizes in Samples.

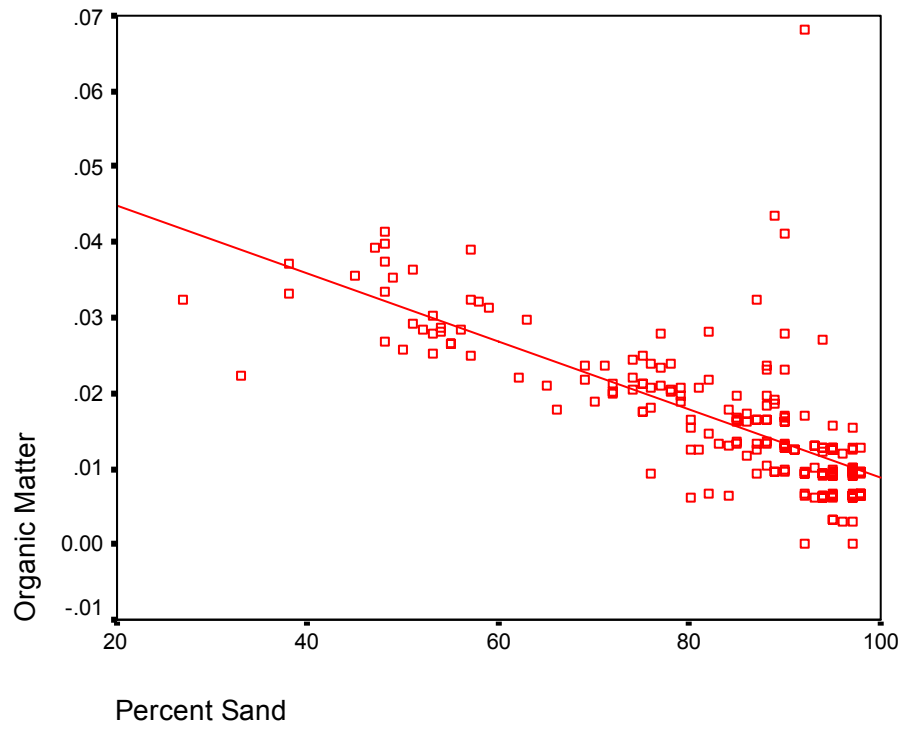


Figure II.7. Percent Sand vs. Organic Matter.

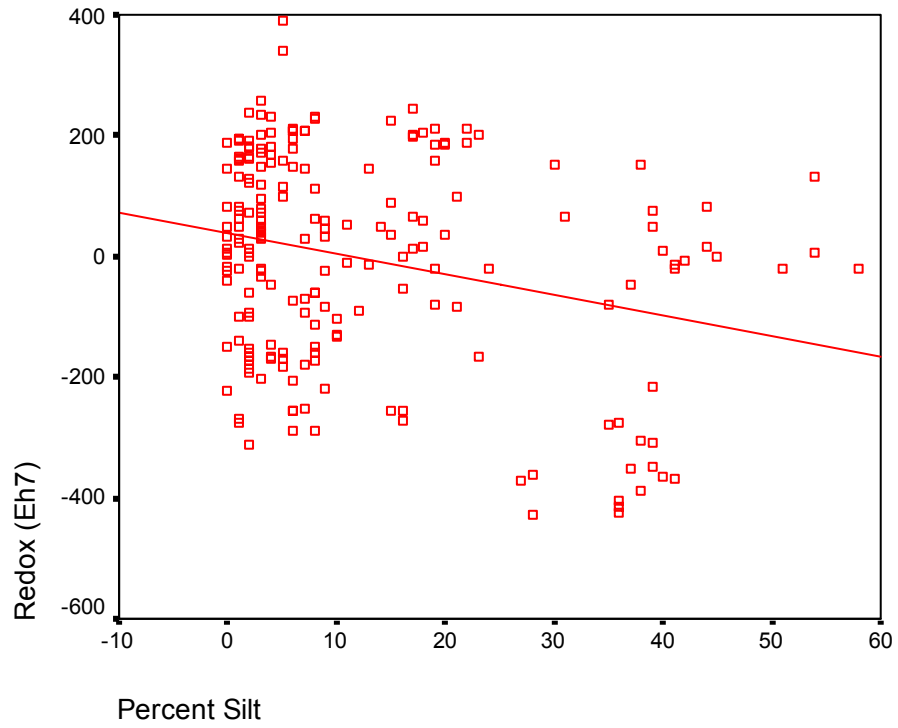


Figure II.8. Percent Silt vs. Redox (Eh_7).

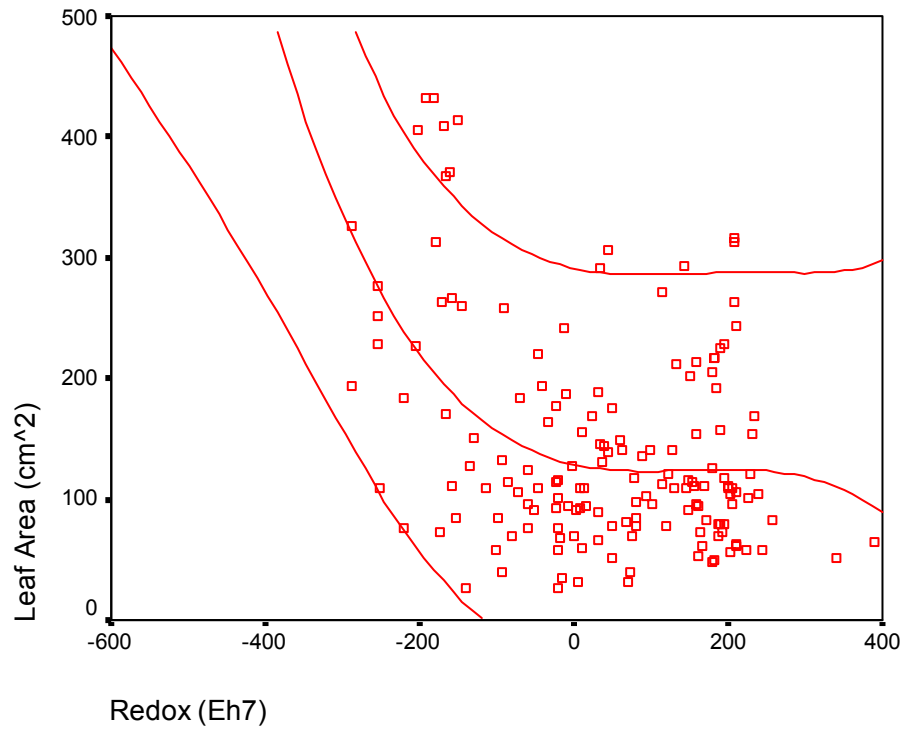


Figure II.9. Redox (Eh_7) vs. Leaf Area (cm^2). 95% Confidence Interval shown for Leaf Area Measurements.

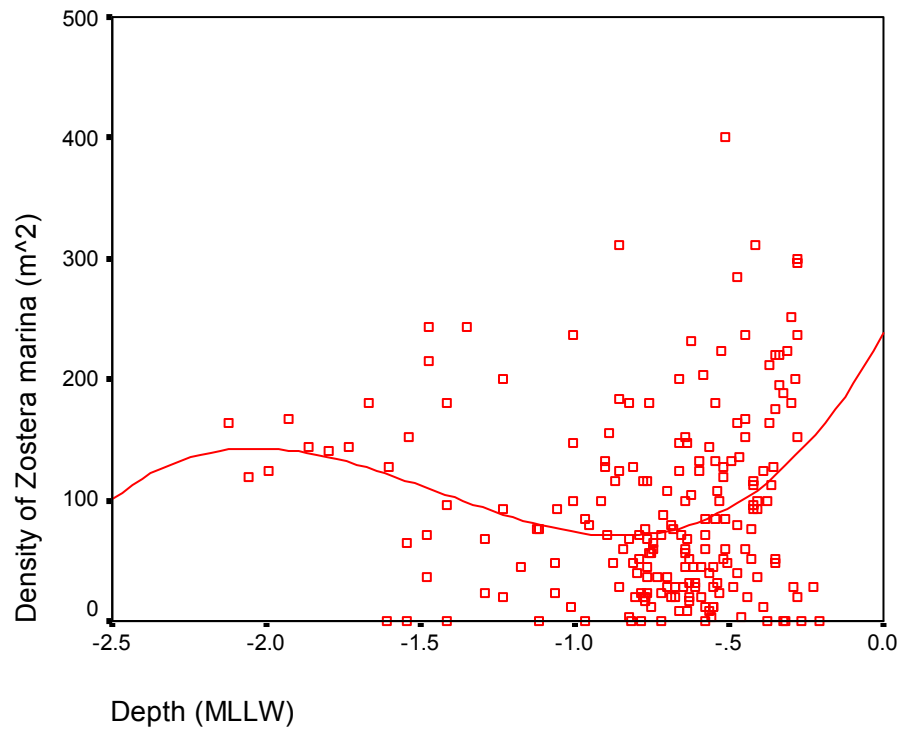


Figure II.10. Depth (MLLW) vs. Density of *Zostera marina* (m^2).

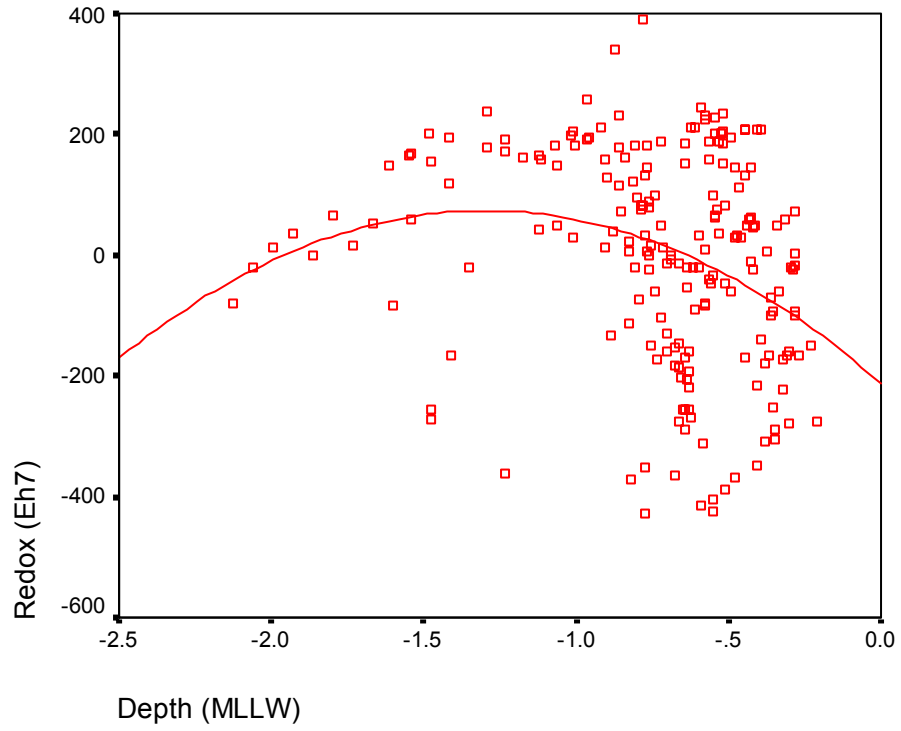


Figure II.11. Depth (MLLW) vs. Redox (Eh_7).

Table II.1. Data Summary by Site.

SITE	Density (m ⁻²)	Width (mm)	Height (m)	pH	Eh ₇ (mV)	O.M. (%)	Clay (%)	Sand (%)	Silt (%)	Fetch (km)	Latitude	Longitude	Aspect (°)
1	178			7.8	-76.9	1.1	2.0	96.8	1.2	11.0	48°59'30	122°46'18	284
2	170			5.9	-67.7	2.1	6.1	75.0	18.9	4.0	48°59'17	122°46'00	152
3	53			7.3	-350.1	2.7	8.3	55.3	36.4	3.1	48°58'45	122°46'30	88
4	166	2.21	0.51	6.7	-79.1	0.9	3.6	94.6	1.8	11.7	48°58'42	122°47'02	250
5	117	2.44	0.61	4.6	30.3	0.9	2.8	93.6	3.6	4.0	48°54'30	122°45'30	282
6	131	3.33	1.01	4.6	67.7	1.5	5.4	88.1	6.6	1.4	48°44'00	122°40'28	193
7	44	1.95	0.57	5.4	199.3	1.0	3.5	92.0	4.5	12.0	48°43'55	122°38'52	86
8	92	2.50	0.87	2.9	183.0	1.0	4.0	93.4	2.6	1.6	48°43'35	122°39'58	230
9	49	1.25	1.31	6.6	82.1	0.9	2.1	95.3	2.5	17.0	48°34'00	122°37'55	25
10	54	3.07	0.88	7.5	6.7	1.8	8.1	79.9	12.0	2.3	48°33'35	122°39'00	54
11	79	2.70	0.70	6.5	-89.7	1.5	4.9	85.7	9.4	1.1	48°31'08	122°37'48	310
12	55	4.41	1.06	7.4	-200.8	2.2	4.0	89.6	6.4	9.0	48°30'18	122°36'41	104
13	63	2.54	0.58	7.4	187.3	2.2	6.1	72.6	21.3	4.6	48°29'50	122°29'10	226
14	42	2.57	0.47	7.4	6.2	3.5	10.0	47.2	42.8	4.6	48°28'49	122°29'12	244
Mean:	92	2.6	0.8	6.3	-7.3	1.7	5.1	82.8	12.1	6.2			

Table II.2. Analysis of Variance for Linear Model of Latitude & Silt vs. Density.

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Regression	298939.210	2	149469.605	33.062	.000
Residual	899645.186	199	4520.830		
Total	1198584.396	201			

Table II.3. Analysis of Variance for Linear Model of Eh₇ vs. Leaf Area.

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Regression	156204.324	1	156204.324	21.784	.000
Residual	1111424.924	155	7170.483		
Total	1267629.248	156			

Table II.4. Analysis of Variance for Cubic Model of Eh_7 vs. Leaf Area.

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Regression	244744.6	3	81581.54	12.203	.000
Residual	1022884.6	153	6685.52		
Total	1267629.2	156			

Table II.5. Analysis of Variance for Cubic Model of Depth vs. Density.

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Regression	97946.2	3	32648.74	5.8734	.000
Residual	1100638.2	198	5558.78		
Total	1198584.4	202			

Table II.6. Analysis of Variance for Quadratic Model of Depth vs. Eh_7 .

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Regression	508975.9	2	254487.96	8.879	.000
Residual	5703913.3	154	28662.88		
Total	6212889.2	156			

CHAPTER III: REDUCTION/OXIDATION (REDOX) MANIPULATION FOR THE PURPOSE OF EELGRASS (*ZOSTERA MARINA*) RESTORATION

“Pluralitas non est ponenda sine necessitate” also interpreted as the scientific principle that, all things being equal, the simplest answer is usually the right one.
William of Ockham

ABSTRACT

Oxidation/reduction potentials (redox) of marine sediments are highly variable, yet typically low. The anoxic condition of marine sediments was examined to determine whether it had an inhibitory affect on the growth of newly transplanted *Zostera marina*. Both in controlled laboratory experiments consisting of closed-seawater systems and *in situ*, the typical redox range was found to be between 300 mV and – 500 mV. Correlation was found between redox conditions and leaf growth of *Z. marina*, as well as the number of roots per node and root condition. Laboratory experiments predicted higher successful transplantation rates in the field for those sites that were artificially altered to raise the redox of the marine sediments into which *Z. marina* individuals would be planted.

INTRODUCTION

Restoration ecologists are continually searching to identify and understand the variables that influence the successful transplantation of individual plants, whether terrestrial, aquatic, or marine (Harrison 1990, Phillips and Lewis 1984). ‘Transplant shock’ is a term informally used to describe the trauma individual plants experience

when moved from one habitat into a new location (Cope *et al.* 1986, Nilsen and Orcutt 1996). Shock is no more than a plant's inability to adapt to a quickly changing environment; shock may also be induced through loss of fine roots if transplanted poorly or disturbed. Because below-ground conditions are more stable (slowly changing) than above-ground (Alongi 1998, Brady 1990, Northington and Goodin 1984), it makes sense that roots of plants do not adapt to rapidly changing environments as above-ground organs do. Because of this, those conditions that are rapidly changed during restoration activities should be investigated and evaluated for their effect on plants. One such variable whose condition can rapidly be altered during restoration activities is the oxidation/reduction (redox) potential of a rooting medium.

Redox potentials have been measured in terrestrial, limnological, and marine systems because electrons and their electromotive tendencies are essential to inorganic, organic, and biochemical reactions (Bohn 1971, Faulkner *et al.* 1989). Redox is a chemical reaction in which electrons are transferred from a donor (oxidant) to an acceptor (reductant). The source of electrons for biological reductions is organic matter (Armstrong 1976, Ponnampuruma 1972). The electrochemical state of an element will dictate its biological behavior, including toxicity of that element as well as its mobility in the environment (Goodman *et al.* 1995, Langmuir 1997).

During periods of prolonged flooding and/or limited oxygen exchange, root and microbial respiration as well as chemical oxidation of reduced organic and inorganic components can lower the redox potential of soils (Armstrong 1976, Gambrell and Patrick 1978). Because the pore spaces of marine sediments are occupied by water, oxygen exchange is entirely limited to diffusion through solution. Also because the diffusion coefficient of oxygen in water ($0.26 \times 10^{-4} \text{ cm}^2\text{s}^{-1}$) is nearly 10,000 times less than that of air ($0.23 \text{ cm}^2\text{s}^{-1}$), microorganisms can reduce the oxygen concentration of marine sediments to zero within a few hours of being flooded (e.g., during a tidal change) (Armstrong 1976). When the oxygen supply is limited, as is the case with marine sediments or flooded soils, a proportion of benthic microorganisms utilize electron acceptors other than oxygen for their respiratory requirements. This results in a semi-predictable reduction cascade of compounds (Table III.1) (Mitsch and Gosselink 1993, Ponnampereuma 1972).

Redox potentials of soils or marine sediments can be indicative of plant growth success (Goodman *et al.* 1995, Hemminga 1998, Ponnampereuma 1972, Terrados *et al.* 1999). As redox decreases, elements essential to plant growth may become unavailable for root uptake (e.g., having changed its form or having been leached from the site), may become toxic, or may make it difficult for a plant to maintain an oxidized rhizosphere since reduced compounds react with oxygen (Armstrong 1976, de la Cruz *et al.* 1989, Stepniewski *et al.* 1991).

Zostera marina L. is found to grow most commonly in very sandy marine sediments (80-100%), with many populations also found in growing silty sediments (sand \approx 50%, silt \approx 45%) (den Hartog 1970, Thayer *et al.* 1984). In all reported cases, *Z. marina* creates an oxidized zone within its rhizosphere (Hemminga 1998, Pregnall *et al.* 1984, Smith *et al.* 1984, Terrados *et al.* 1999). This adaptation for rhizospheric oxygenation allows aerobic respiration in the roots and of aerobic bacteria in the oxic zone around the roots. The bacteria are able to render toxic amounts of ferrous iron or other heavy metals insoluble near the aerobic root surface (Nilsen and Orcutt 1996).

When *Z. marina* individuals are removed from a growth medium and transplanted, the oxic zone is immediately removed. It is the abrupt change in rhizospheric condition from higher redox (oxic) to lower redox (hypoxic or anoxic) that I am suggesting is a contributing factor to 'transplant shock'. One physiological response by *Z. marina* seedlings to hypoxic or anoxic rooting media is the inhibition of carbon translocation from shoots to roots (Zimmerman and Alberte 1996). Without a carbon pool from which to draw, extended periods of low redox conditions may lead to impaired root functioning and possibly plant death. By effectively minimizing the redoximorphic difference between the original rhizospheric conditions and that of the transplant site, the amount of shock may be effectively reduced, thus contributing to more successful establishment.

METHODS AND SAMPLING DESIGNS

A series of experiments was performed to determine the impact of redox potential on *Z. marina* transplant success. Initial experiments defined the maximum rate of redoximorphic change within marine sediments and the upper/lower limits of those changes. The second experiment identified growth rate differences of *Z. marina* in high versus low redox environments within a controlled, closed- seawater system. The final experiment applied a unique technique for raising the redox potential of *in situ* marine sediments for the purpose of reducing transplant shock of *Z. marina*.

Experiment 1

In Autumn 1998, intertidal marine sediment (>95% sand, common for Puget Sound *Z. marina*; see Chapter II) and water from Puget Sound were collected and placed in 30, 2.5-gallon buckets at room temperature (18 °C); $\frac{1}{3}$ sediment, $\frac{2}{3}$ water. Fifteen buckets were left undisturbed for 10 days, while the water of fifteen buckets was continually stirred using an edge-mounted aquarium water pump at a rate of 2.1 gpm (AquaClear Powerhead 201) in order to simulate currents of the Puget Sound. Following the 10 day equilibration period, sediment redox potentials were measured for all buckets at a depth of 5 cm. Next, six sugar cubes ($C_{12}H_{22}O_{11}$; common table sugar) were placed in three undisturbed and three stirred buckets and allowed to dissolve. In three undisturbed and three stirred buckets, atmospheric O_2 was pumped

into the water at a rate of 0.5 gpm. In three undisturbed and three stirred buckets, lab-bench CH_4 was pumped into the water at a rate of approximately 0.5 gpm. In three undisturbed and three stirred buckets, one O_2 -effervescing tablet ('OTABS'; diameter = 31.75 mm, height = 19.05 mm) was placed 7 cm deep in the center of the bucket (Pemble-Halverson Inc. 2000) (Figure III.1). The sugar treatment encouraged microbial activity within the marine sediments to identify the lowest redox potential value of the sediment (NCARS 1996). The CH_4 treatment identified the equilibrated redox potential upon removal of all atmospheric O_2 . The atmospheric O_2 treatment identified the highest redox potential value of the sediment.

The four treatments and one control were then applied for a period of time long enough (approximately one week) for each treatment to reach their maximum, minimum, or equilibrated redox potentials, and to allow the effect from the O_2 -effervescing tablets to be fully realized.

Experiment 2

Between May and August 1999, two 38-day experiments were conducted in the Douglas Research Conservancy Greenhouse, University of Washington, Seattle. Six 55-gallon drums were placed on two greenhouse tables. They were first filled with 17.27 cm of intertidal marine sediment (from the same location as used in the 2.5-gallon experiments). The drums were then filled to capacity with water from the

Puget Sound. All six drums were equipped with the edge-mounted aquarium water pumps used in the 2.5-gallon experiments. Each of the six drums had a halogen spotlight (GE[®] 120W 38-Spot) illuminating the water in the drum from three feet above the surface. The spotlights were timed to turn on for 18 hours per day. Three of the six drums had atmospheric O₂ pumped into the water at a rate of 5.0 gpm (Figure III.2). All six drums were allowed to equilibrate for a 10-day period. After the equilibration period, daily redox potential measurements were made in the sediments of each drum. In addition, submarine photosynthetically active radiation (PAR) was continually measured using underwater spherical quantum sensors (LiCor LI-193SA) at the benthic surface (approximately 60 cm from the water surface, 9 cm from the benthic surface) for three drums; two drums were receiving atmospheric O₂ and one was not receiving this treatment (Kirk 1994) (Figure III.3). Daily water temperatures were also measured for all drums. Due to evaporative processes, deionized tapwater was added to each drum in order to maintain salinity.

The greenhouse environment was programmed (computerized) for the lowest possible summer air temperature (18 °C with swamp coolers) and maximum sunlight. High pressure sodium greenhouse lights were used when outside PAR values fell below 500 $\mu\text{mol m}^{-2} \text{sec}^{-1}$, and 50% shade cloth closed when air temperatures were greater than 25°C.

Following the 10-day equilibration period, 60 adult *Z. marina* individuals were harvested from one site along the same depth and transported in a cooler with seawater within three hours to the greenhouse. The same day, all 60 individuals were replanted, bare root, 10 individuals per drum. All plants were of similar blade length, blade width, and had no less than five internodes each with intact roots. Plants were allowed to equilibrate with their environment for 14 days. After this equilibration period, each plant was punctured with a pin just above the meristematic sheath, through each of five leaves, in order to establish a reference point for leaf growth (Dennison 1990). The distance above the sediment surface was also recorded for each puncture. Following another 14-day period, all plants were severed at their sediment interface and subsequently measured for the distance between the severed end of the leaf and the previously punctured tissue (Olesen and Sand-Jensen 1994). Also, the length of the longest leaf as well as the leaf area (LiCor 3100 Leaf Area Meter) for all leaves of each plant was measured (Bulthuis 1990). In addition, each rhizome was excavated and measured for root count per rhizomal node and subjectively categorized for root condition (i.e., healthy, weak, or dead) based on turgidity (i.e., with or without full turgor pressure) and color (i.e., white/grey or black).

Following this experiment, all atmospheric O₂ pumping, floodlights, and edge-mounted fish tank pumps were stopped until all sediment redox potentials were

approximately equal. A second 38-day experiment was then conducted; same conditions as before.

Experiment 3

In June 2000, 20 subtidal experimental plots were established in Fidalgo Bay, Washington (48.508°N, 122.600°W) using S.C.U.B.A. (Figure III.3). Four replicates of three treatments and two controls were conducted for a period of 10 weeks. Each replicate consisted of transplanting four *Z. marina* planting units (PU) per 0.5 m² plot. Each PU for Treatment #1 consisted of a sediment/plant core (core diameter = 15.24 cm) containing three adult *Z. marina* plants (Fonseca *et al.* 1998). Cores were removed from adjacent *Z. marina* populations and set in similarly-sized, excavated holes within treatment boundary. Each PU for treatment #2 consisted of three adult, bare root *Z. marina* individuals, also removed from adjacent populations. Each PU was placed in excavated holes within treatment boundary. *Zostera marina* individuals were replanted with their rhizomes parallel to the sediment surface and crossed in the center of the PU. Each PU for treatment #3 consisted of three adult, bare root *Z. marina* individuals as in Treatment #2, however included two O₂-effervescing tablets (Pemble-Halverson, Inc. 2000) placed in the center of the excavated hole prior to placement of the PU. Control plots consisted of two conditions. Both controls consisted of plots which initially maintained *Z. marina* individuals. One control had

all *Z. marina* individuals removed (consequently used in PU's of experimental treatments) while the second control was left undisturbed (Figure III.4).

In each of the 20 experimental plots (three treatments and two controls, each with four replicates), sediment redox potential was measured in the center of each planting unit at a depth of 5 cm at the time of installation, 6 hours, 26 hours and 14 days after installation. These sampling times were defined by the results of Experiment 1 to capture initial, maximum and equilibrated redox conditions of each treatment. *Zostera marina* individuals were also counted to determine success of transplantation.

Statistical Analyses

One-way analysis of variance (ANOVA) was conducted between redox measurements within the stirred 2.5 gallon buckets and the unstirred to determine whether the movement of the water had an affect on the sediment redox. Similarly, ANOVA was used to determine significant differences between O₂ and no-O₂ treatments within the 55 gallon drums for longest leaf lengths, leaf widths, leaf growth, leaf area, roots per node, and root condition. Descriptive statistics were used to describe water temperature and redox conditions within the 55 gallon drums receiving O₂ and those not receiving O₂, as well as to demonstrate the relative effectiveness of each transplantation treatment in the field.

RESULTS

Experiment 1

No significant difference was found between the 2.5 gallon buckets which were stirred versus those which were not (Table III.2). Upon the completion of the initial 10 day equilibration period, the mean redox (Eh_7) measurement, standardized to pH 7.0, for all 30 buckets was -297.20 mV (s.d. = 46.47). This value constituted the baseline redox condition. The four treatments and one control were then conducted for a period of 10 days, allowing each treatment to reach their maximum, minimum, or equilibrated redox potentials.

The first treatment which involved the injection of atmospheric O_2 into the seawater of six 2.5 gallon buckets raised Eh_7 to $+209$ mV (s.d. 19.3) after 24 hours. For the remaining nine days, Eh_7 was $+288$ (s.d. = 10.3) (Figure III.5). The second treatment which involved the placement of O_2 -effervescent tablets into the sediment of six buckets raised the redox to -67 mV (s.d. = 13.2) after 24 hours, then dropped to -286 mV during the following 48 hours and averaged -284 mV (s.d. = 22.6) for the remainder of the experiment. The third treatment which involved the injection of CH_4 into the seawater of six buckets had very little effect on their redox condition (mean $Eh_7 = -293$ mV, s.d. = 14.8). The fourth treatment which involved the dissolving of sucrose into each of six buckets eventually lowered Eh_7 to a mean of -503 mV (s.d. = 4.1) after four days.

Experiment 2

The greenhouse experiment showed a significant difference in leaf growth rate and root condition for *Z. marina* between oxic (high E_h) and anoxic (low E_h) environments. The mean water temperature for all six drums was 23.8°C while the E_{h7} of the oxygenated drums averaged +193 mV and the anoxic drums averaged -392 mV (Table III.3). The maximum daily PAR levels measured for the three drums were 300, 326, and 411 $\mu\text{mol m}^{-2} \text{sec}^{-1}$ (drums #1, #2, and #4 respectively) (Figure III.6).

In comparing the 120 *Z. marina* individuals planted in the 55-gallon drums, the leaf growth rate of those planted in the oxygenated environment (2.85 cm day^{-1}) were significantly greater than those planted in the anoxic environment (1.63 cm day^{-1}) (p-value = 0.0132) (Table III.4). There were positive trends associated with redox environment regarding the longest leaf length, mean leaf width and mean leaf area of the *Z. marina* individuals, however no significant differences were found between the two oxidation treatments. The number of roots per rhizomal node was significantly greater for those plants growing in an oxygenated environment (8.7) versus those in an anoxic environment (6.2) (p-value = 0.0021). The analysis of root condition per plant indicated that the roots of plants grown in the oxygenated environment were significantly more healthy than those grown in an anoxic environment (p-value = 0.0112). The analysis criteria for root condition consisted of turgidity and color.

Together, a subjective value of ‘dead’, ‘weak’, or ‘healthy’ was determined for roots of each node.

Experiment 3

The redox measurements of the 20 experimental plots in Fidalgo Bay showed a maximum increase in Eh_7 of 278 mV for those plots installed with the O₂-effervescing tablets (Figure III.7). There was no significant difference in Eh_7 between all experimental plots with *Z. marina* at 14 days (p-value = 0.5656), however the plots which had all plant material removed maintained a significantly lower Eh_7 than the all other plots (p-value = 0.0279) at 14 days.

The highest survival percentage among transplantation techniques was with the bare root technique which included the use of the O₂-effervescing tablets (Figure III.8). Transplantation of *Z. marina* with the sediment core technique was more successful than bare root without O₂-effervescing tablets. During the first 14 days, there was a loss of approximately one *Z. marina* individual per experimental plot in the plots that were left undisturbed. In addition, one newly established *Z. marina* individual was found in the plots which previously had all of the vegetation removed.

DISCUSSION

Experiment 1

The effective ability of the O₂-effervescing tablets to raise the redox potential of sediments directly above them appeared to last no more than four days, with a peak at approximately 30 hours. The slope of decline from peak Eh_7 values was constant ($m = -3.32$). Similar rates of decline were found in flooded agricultural soils and soil suspensions using laboratory rhizotrons (Faulkner and Richardson 1989, Sposito 1989, Stepniewski *et al.* 1991). After 96 hours, there was no significant effect of the tablets on the redox potential of the sediments.

It was the intention to force O₂ out of the sediments by the injection of CH₄ to determine whether there were any aerobic microorganisms within the treatment sediments which were actively respiring. Methane is a typical end product of anaerobic decomposition of organic matter by obligate anaerobes found in reduced conditions (Ponnamperuma 1972). Because there was no significant difference between the methane treatment and the control, little or no oxygen appeared to be present in the equilibrated sediments, leading to the assumption that anaerobic microorganisms must dominate the medium under untreated conditions.

The equilibrated values of Eh_7 found in the lab (~ -300 mV) were in within the range of those found in many seagrass communities of Puget Sound (Chapter II). The highest Eh_7 values found in the lab were also within the range of those found in Puget Sound seagrass communities. The lower Eh_7 values found in the lab, however, were

below the range of observed values found in Puget Sound seagrass communities. The buckets that had their redox potentials driven abnormally low (Patrick and Mahapatra 1968) through the addition of sugar developed a micro-algal bloom within their water column. Because of this, Experiment 2 did not involve the use of sugar to forcibly lower the redox potentials. It was expected that a micro-algal bloom in the 55-gallon drums would have significantly reduced the available submarine light to the *Z. marina* individuals as compared to those which would not have had sugar added.

Two days after the addition of sugar, a spike in Eh_7 values was recorded. The raise in redox potential occurred between 24 and 48 hours and consisted of 50 mV mean increase. This increase coincided with the algal-bloom and may have been a result of the O_2 production by the bloom. By 72 hours, the redox potentials were again at pre-bloom levels and continued to fall until reaching a low equilibrium of approximately -500 mV. The volume of living micro-algae is suspected to have decreased from peak bloom levels to such a volume as could be sustained by O_2 levels found at the surface of the water.

Experiment 2

Following the laboratory experiments, a larger scale experiment was engaged with *Z. marina* individuals to determine whether higher redox potentials would positively affect *Z. marina* individuals in terms of growth rates and relative health. For those drums that received atmospheric O_2 pumped into the water column, peak PAR

measurements were approximately $100 \mu\text{mol m}^{-2} \text{sec}^{-1}$ lower than those that did not receive atmospheric O_2 . This difference was most likely due to the surface disturbance caused by the bubbling/foaming of the O_2 . Even with comparatively lower PAR levels, the *Z. marina* individuals in these drums had higher growth rates and healthier root characteristics than those individuals in the drums that received more light but maintained anoxic sediment characteristics (Filskov 1990, Nienhuis 1984).

In a 14-day period, leaf growth rates of *Z. marina* individuals were affected by the redox potential of their growth medium. Whereas their leaf lengths, widths, and area were not significantly different, those individuals growing in higher redox environments had higher mean values than those in low redox environments. In this experiment, fourteen days was a period long enough to detect an acute response by *Z. marina* individuals (Terrados *et al.* 1999). However, a longer period of time under these conditions would elicit a chronic response to which the plants may alter their physiology or morphology.

Upon inspection of individual roots, many of those found on plants harvested from the low redox treatment were dead. Without the influences of an *in situ* field environment such as current, predation, fluctuating available light, and wave activity, I suspect that low redox potentials of sediments is an important contributing factor to the failure of

many *Z. marina* transplantation/restoration efforts due to the evidence of root weakness and/or root death found in this experiment. Because *Z. marina* habitat is typically highly anoxic, Eh_7 levels may be too low to support cell division and growth in the roots of newly planted individuals; individuals may not be able to diffuse enough O_2 to the roots to counteract the effect of the low redox potential of the sediment. Armstrong *et al.* (1991) and Drew (1983) found similar results while conducting flooding experiments with agricultural species. Morphological or physiological adaptations by many species, such as seagrasses, that grow in low redox environments help them to avoid or minimize anoxic conditions by passively transporting photosynthetically created O_2 from their leaves to their roots, and eventually into the rooting medium (Smith *et al.* 1984). Having O_2 artificially forced into the system may relieve the low- O_2 stress caused by transplantation and allow those *Z. marina* individuals to grow more rapidly and develop a greater volume of and more healthy roots per rhizomal node.

Experiment 3

After identifying the effect of the O_2 -effervescent tablets on Eh_7 (in lab) and high/low redox potentials on the growth rates and root health of *Z. marina* individuals (in greenhouse), I attempted to apply these results to a field experiment to determine the feasibility for increasing transplant success rates of *Z. marina* individuals in a restoration setting.

When O₂-effervescing tablets were placed within the holes into which *Z. marina* individuals were planted, the Eh₇ was raised for several hours following transplantation. Normally, *Z. marina* individuals exude O₂ from their roots during normal hours of photosynthesis (de la Cruz *et al.* 1989, Filskov 1990, Smith *et al.* 1984). Because of this, the immediate area surrounding the roots and rhizomes of *Z. marina* individuals has a higher redox potential than that of adjacent marine sediments. When individuals are removed from a site as donor plants to be transplanted into another site, they are moved from an established higher redox environment to a much lower one. The O₂-effervescing tablets raise the redox potentials of the marine sediments into which *Z. marina* plants are transplanted and keep it raised for a few days, allowing the newly transplanted roots to survive. Eventually, the newly planted individuals begin to exude O₂ into the adjacent sediment as they photosynthesize, thus raising oxic conditions on their own.

It appears that with this extra boost in Eh₇ during the first few days following the transplantation of *Z. marina* individuals, the transplant success rate was increased. When the effervescing tablets were used with bare root transplantation of *Z. marina*, an initial transplant success of 86% after two weeks was reached. When bare root *Z. marina* individuals were transplanted alone, the initial transplant success after two weeks was only 33%. When sediment cores were used as a transplant method, an initial transplant success of 47% was reached. The sediment core method may

contribute to a higher initial transplant success than the bare root method because the *in situ* redox condition of the donor site may be transferred somewhat intact within the core. However, as the roots and rhizomes of the *Z. marina* individuals are disturbed or moved within the core, this condition may be altered too much and reduce the potential transplant success.

Common survival percentages for seagrass transplantation projects carried out in the U.S.A. are below 50% (Fonseca *et al.* 1998), as demonstrated by these bare root and core transplant results. Transplant methods which include such treatments as O₂-effervescing tablets may help to raise overall transplantation success within seagrass communities.

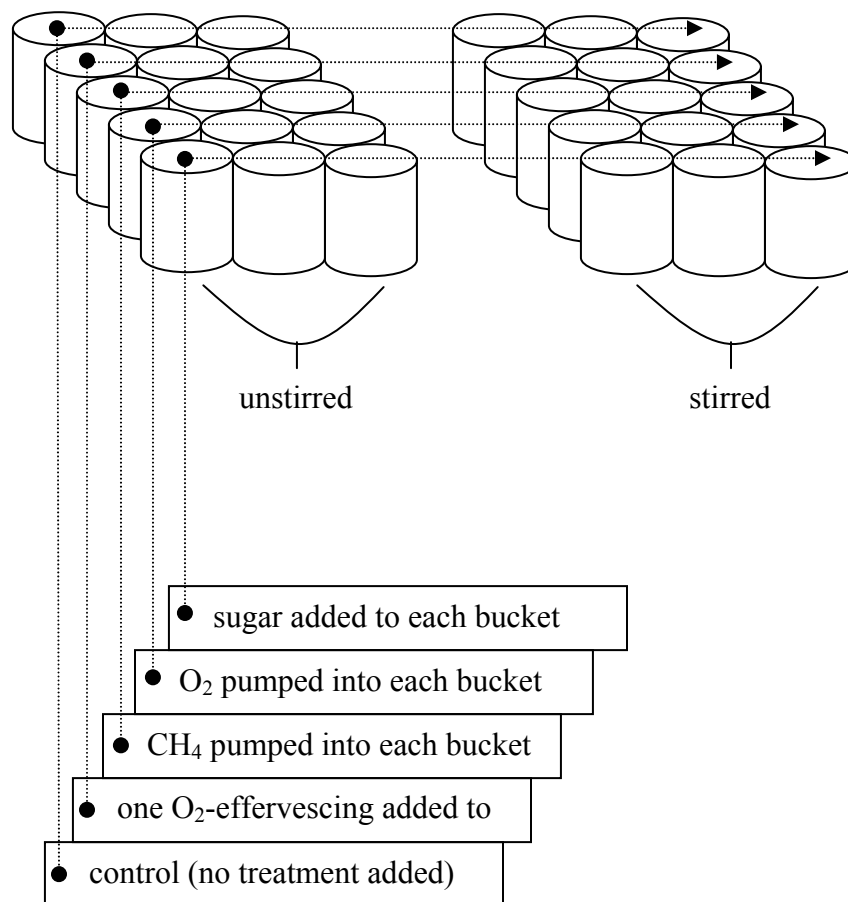


Figure III.1. Experimental design for lab experiment; 30, 2.5-gallon buckets.

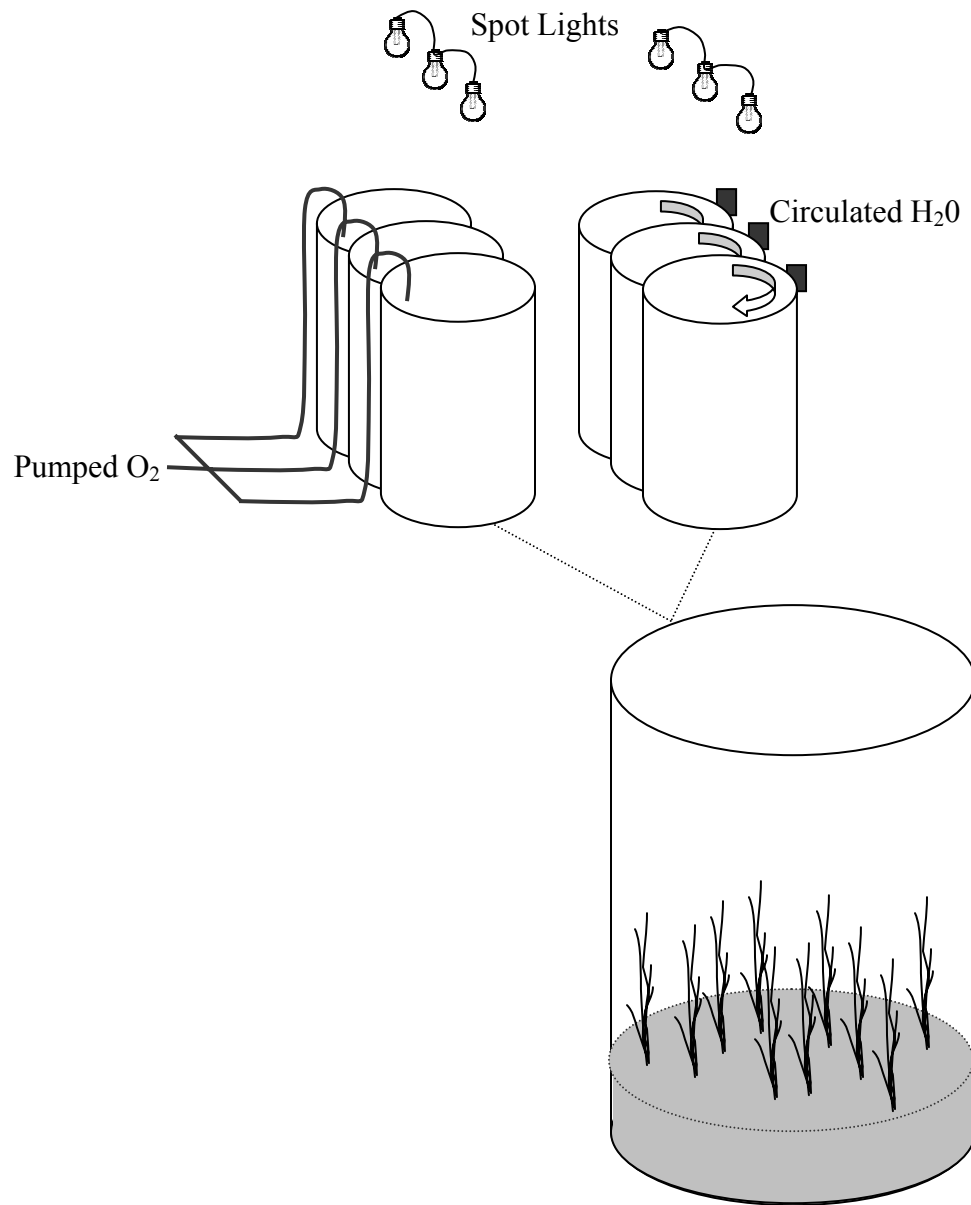


Figure III.2. Experimental design for greenhouse experiment; 60 *Zostera marina* plants in six, 55-gallon drums.

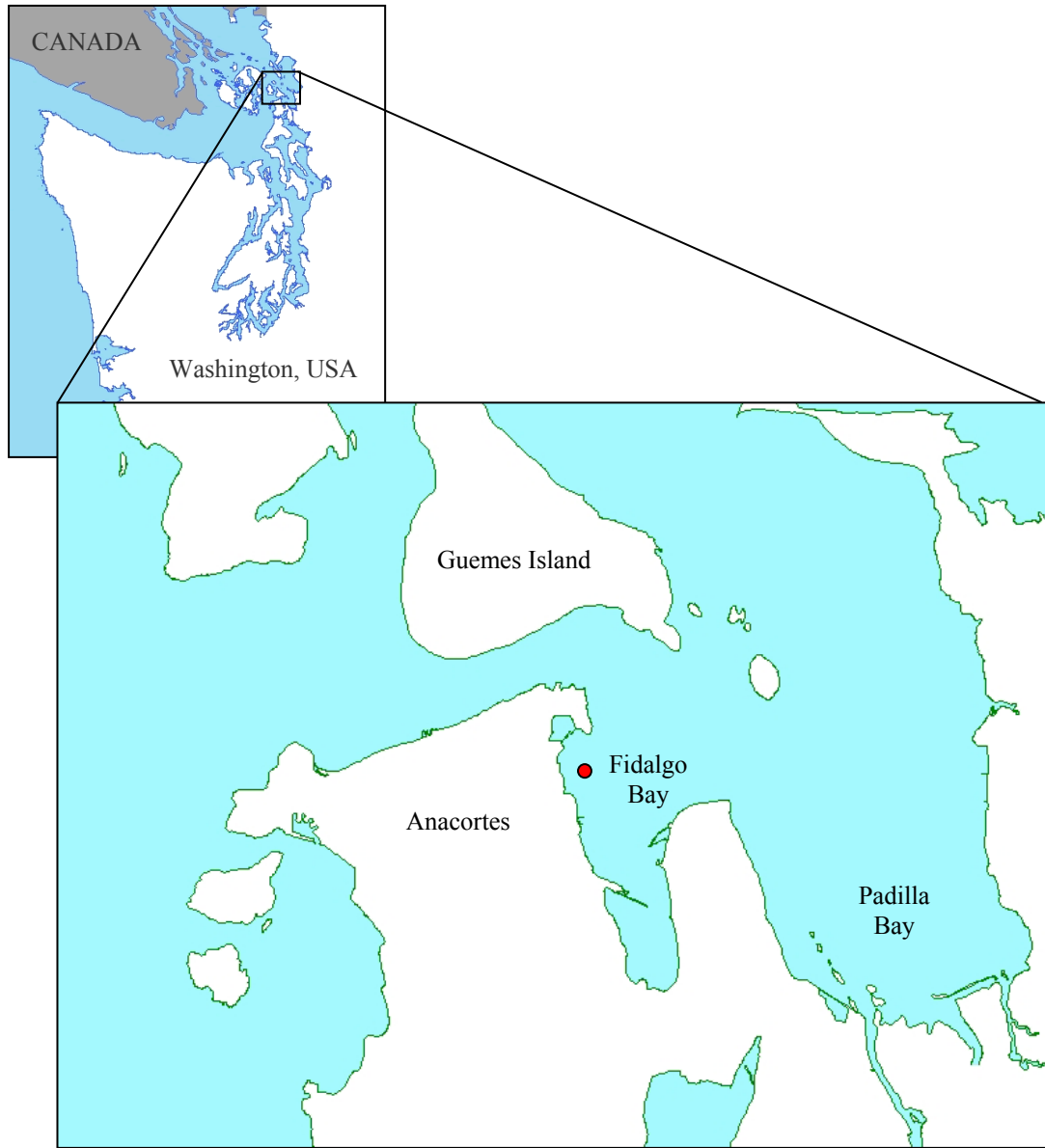


Figure III.3. Site Map.

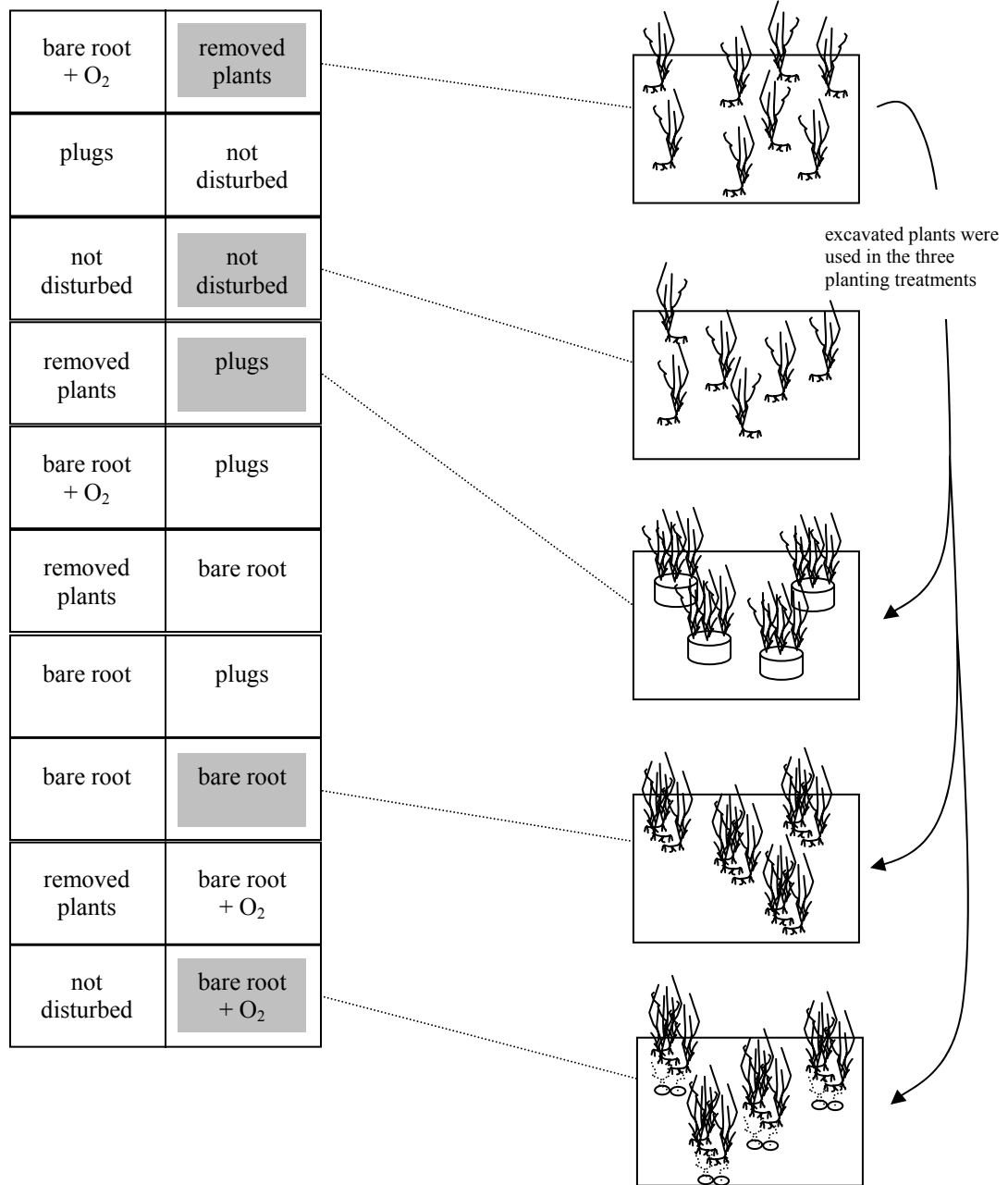


Figure III.4. Field design for *in situ* transplantation treatments and controls.

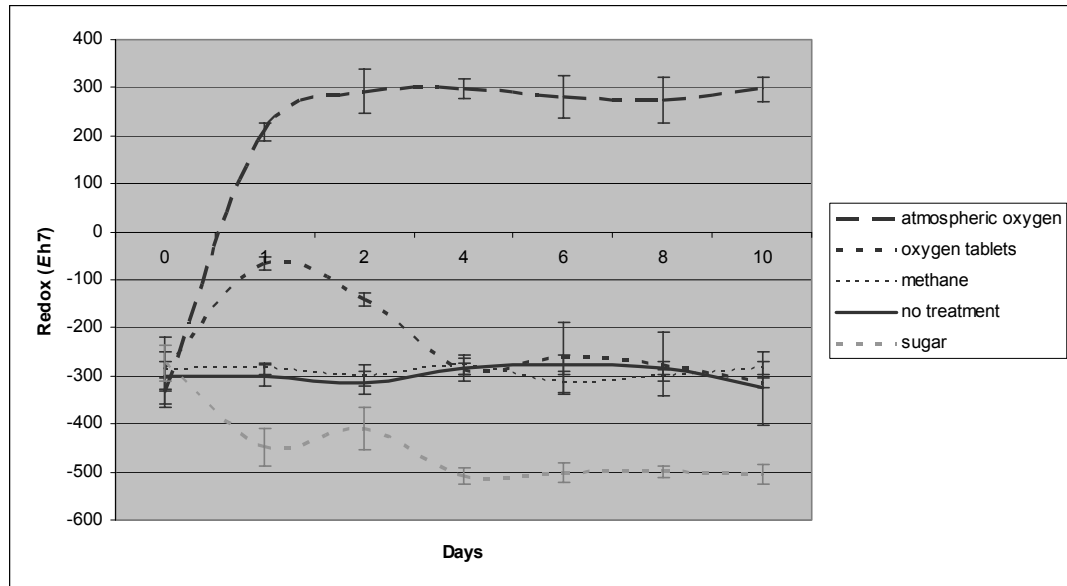


Figure III.5. Mean redox (Eh_7) potentials of four treatments and one control in 2.5-gallon buckets.

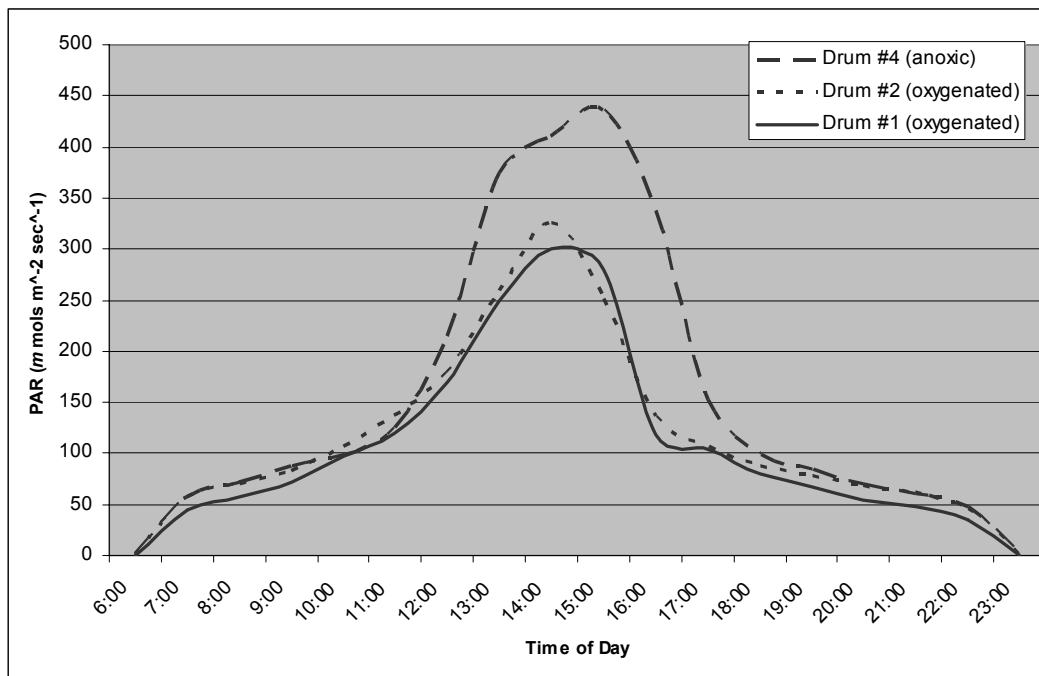


Figure III.6. Daily PAR values for oxygenated and anoxic treatments in three 55-gallon drums.

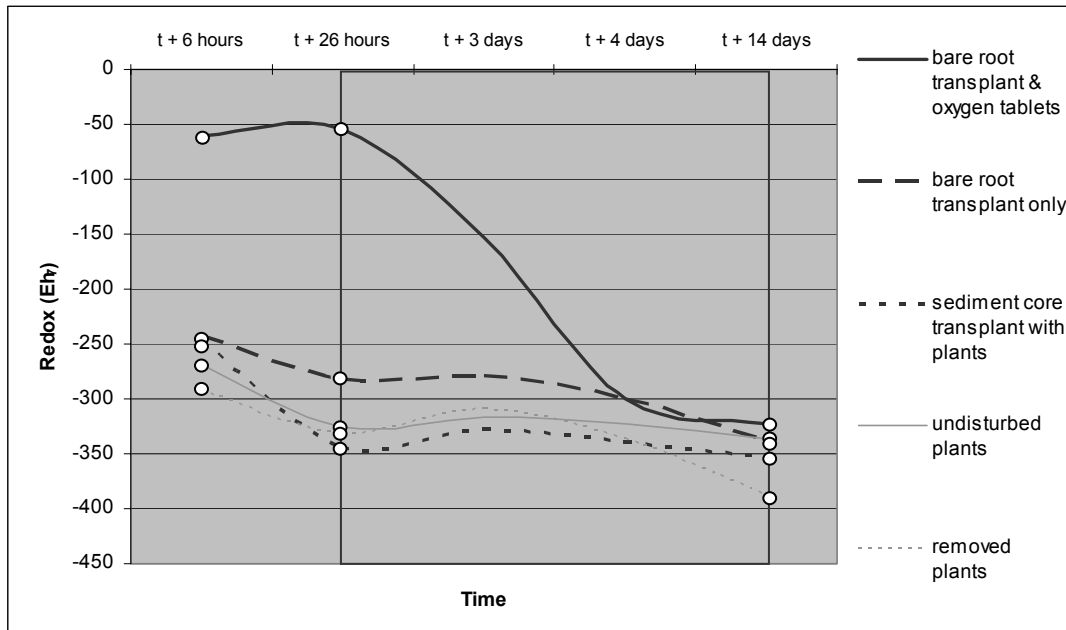


Figure III.7. *In situ* redox (Eh_7) potentials for three treatments and two controls. Darkly shaded area represents speculative trend-lines based on slopes identified in Experiment 1.

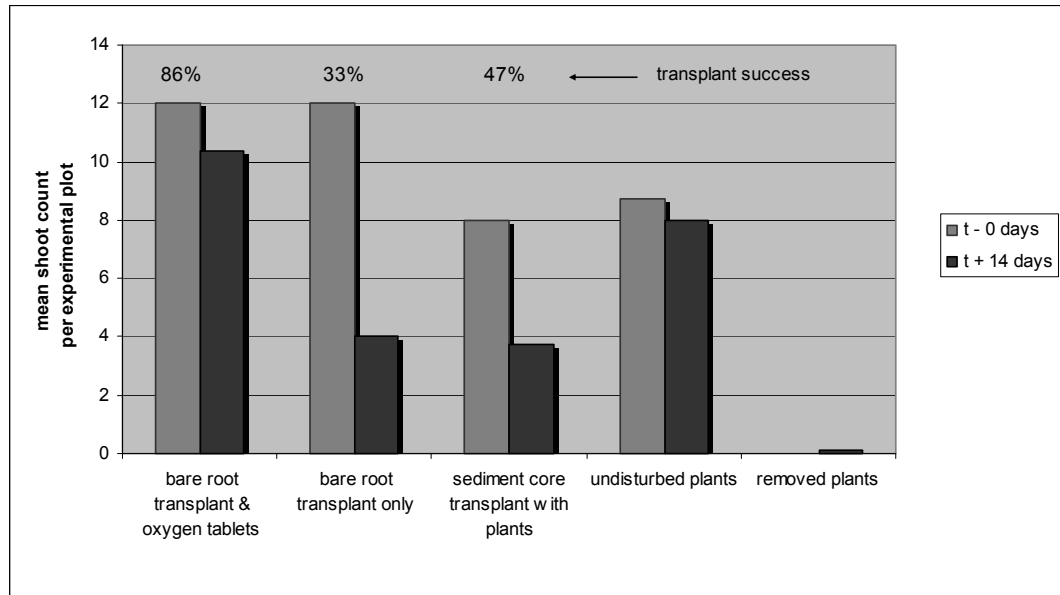


Figure III.8. Transplant successes for three treatments compared to two controls.

Table III.1. Oxidized/reduced forms of several common elements and their approximate redox potentials.

<i>Element</i>	<i>Oxidized Form</i>	<i>Reduced Form</i>	<i>Approximate Redox Potential for Transformation, mV</i>
Nitrogen	NO ₃ ⁻ (Nitrate)	N ₂ O, N ₂ , NH ₄ ⁺	+250
Manganese	MN ⁺⁴ (Manganic)	MN ⁺⁺ (Manganous)	+225
Iron	Fe ⁺⁺⁺ (Ferric)	Fe ⁺⁺ (Ferrous)	+120
Sulfur	SO ₄ ⁻ (Sulfate)	S ⁻ (Sulfide)	-75 to -150
Carbon	CO ₂ (Carbon Dioxide)	CH ₄ (Methane)	-250 to -350

Table III.2. Analysis of variance (*Eh*₇); stirred vs. unstirred 2.5-gallon buckets.

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
stirred vs. unstirred	115.89	1	115.89	0.00	0.97	3.89
days	295879.59	6	49313.27	0.80	0.57	2.15
Interaction	4039.51	6	673.25	0.01	1.00	2.15
Within	12119918.93	196	61836.32			
Total	12419953.92	209				

Table III.3. Mean water temperature and *Eh*₇ (Redox) of 55-gallon drums.

<i>Drums (treatment)</i>	<i>Temp °C (s.e.)</i>	<i>Eh₇ (s.e.)</i>
1,2,6 (with atm O ₂)	23.2 (0.6)	+193.0 (34.8)
3,4,5 (no atm O ₂)	24.4 (0.1)	-391.7 (10.4)

Table III.4. Mean growth characteristics for 55-gallon drums during 14-day experiment; oxygenated vs. anoxic.

	<i>mean longest leaf length (cm)</i>	<i>mean leaf width (mm)</i>	<i>mean leaf growth (cm)</i>	<i>mean leaf area (cm²)</i>	<i>mean number of roots per node</i>	<i>root condition 0=dead 1=weak 2=healthy</i>
with O ₂	55.4	1.9	39.9	38.3	8.7	1.9
(s.e.)	(1.5)	(0.1)	(3.0)	(4.8)	(0.1)	(0.01)
without O ₂	48.9	1.7	22.8	28.6	6.2	1.4
(s.e.)	(5.9)	(0.1)	(3.9)	(3.0)	(0.4)	(0.10)
significant difference (<0.05)	0.2636	0.1052	0.0132	0.1042	0.0021	0.0112

CHAPTER IV: TRANSPLANTATION AND ALTERATION OF SUBMARINE ENVIRONMENT FOR RESTORATION OF *ZOSTERA MARINA* (EELGRASS); A CASE STUDY AT CURTIS WHARF (PORT OF ANACORTES), WASHINGTON

“Unless someone like you cares a whole lot, nothing is going to get better. It's not.”
Dr. Suess, from The Lorax

ABSTRACT

Using a blend of restoration techniques, we have altered the nearshore zone on the southern side of Guemes Channel so that an *in situ* eelgrass (*Zostera marina* L.) population has expanded (rhizomal spread) and new patches are forming (seed dispersal). Our project was initiated to mitigate the effect of dock shading by the joining of two adjacent commercial docks. In response to the requirement by the Washington Department of Fish and Wildlife, we concluded that a blend of small-scale, low-cost, restoration techniques to enhance the eelgrass zone immediately adjacent to the project would meet protection goals. Our plan required that 1) historically deposited rubble and debris (e.g., concrete blocks, wire cables, metal fencing and rubber hose) be removed from the eelgrass zone; 2) a section of dock which shaded the substrate be removed; 3) reflective panels be installed to alter submarine light under existing dock; 4) eelgrass plants from the proposed construction area be transplanted to debris-free site; and 5) changes in eelgrass cover be monitored over three years. In a three-year period, a 59 m² area was naturally repopulated by *Z. marina* (mean density = 73 shoots m⁻²) due to blend of these methods. We recommend an approach similar to ours, aimed at removing growth

limitation factors (e.g., shade, benthic debris), be considered at other sites in the Puget Sound Basin.

INTRODUCTION

A combination of low-cost habitat enhancement treatments and naturally occurring rhizomal expansion and seed dispersal mechanisms were applied to enhance an existing population of eelgrass (*Zostera marina* L.) adjacent to an actively used commercial dock, Guemes Channel, Anacortes, Washington. Guemes Channel, located within northern Puget Sound, is an active route for anadromous fish traveling between the freshwater streams of western Washington and the Pacific Ocean (Kruckeberg, 1991) (Figure IV.1). Chinook salmon, locally listed as endangered, are one of the anadromous fish species found to use vegetated tidal habitat for protective habitat and feeding (Shreffler *et al.* 1992, Sheffler and Thom 1993). In addition, crab and other mollusk and fish species require eelgrass meadows for habitat (Baldwin and Lovvorn 1993, Thom 1987).

Under the appropriate environmental conditions, eelgrass can reproduce sexually (seed dispersal) and asexually (rhizomatously) (Harrison 1993, Harrison and Durance 1992, Phillips *et al.* 1983). Restoration of eelgrass meadows has largely focused upon transplantation of whole plants into areas that either once maintained an eelgrass population or into an area designated by the landowner as a potential mitigation site,

whether or not it historically maintained eelgrass (Fonseca *et al.* 1998). Due to the no-net-loss policy within the state of Washington (Washington Administrative Code 220-110 250 through 330), eelgrass has become a highly visible resource requiring attention. Because Washington State's Department of Fish and Wildlife (WDFW) and the National Oceanic and Atmospheric Administration (NOAA) are mandated by the Endangered Species Act (ESA) of 1973 to protect marine species and their habitats, restoration projects that require permits for nearshore activity are closely monitored to demonstrate successful establishment of marine populations associated with the granting of any permits. In this case, low-cost, site-specific methods that achieve success are gaining the attention of those who must demonstrate restoration success in an ecosystem where success is very limited (Thom 1990).

In 1996, the private owner of the Curtis Wharf and Gravel docks was required by WDFW to move a small population of *Z. marina* from an area proposed to be covered by new dock construction. The proposed dock construction was to join the two existing docks while extending farther towards deep water for eventual use as an active commercial dock capable of off-loading deep draft cargo vessels. Upon site reconnaissance, an area to the south of Gravel dock was identified as having potential for *Z. marina* restoration, which would include the transplantation of the limited population impacted by new construction activities (Figure IV.2).

In negotiations between the private owner and WDFW, mitigation goals were defined. The mitigation effort was to restore the area south of the existing docks in such a way that it would provide a minimum 117 m² (1300 ft.²) of new *Z. marina* habitat, and support a community with an average shoot density similar to the impact site (25 shoots m⁻²). Mitigation success would be determined after the third year of monitoring.

Common anthropogenic disturbances to *Z. marina* that lead to its areal reduction are shading (e.g., dock structures, temporary boat moorage), nutrient overloading (leading to excessive algal growth), physical disturbance (e.g., boat anchors and prop scarring, dredging), and siltation (e.g., shoreline runoff, dredging) (Beal and Schmit 2000, Dusek and Battle 1999, Loflin 1995, Nichols and Pamatmat 1988, Short and Burdick 1996, Short and Wyllie-Echeverria 1996). Recreating the environmental conditions that had once existed prior to disturbance and allowing *Z. marina* to naturally recolonize the area is the restoration approach that we used for this project (e.g., Ewing 1995).

METHODS AND SAMPLING DESIGN

Alterations to Dock and Environment

Debris or rubble discarded within the intertidal and subtidal areas of *Z. marina* habitat limit the potential for expansion. The removal of debris/rubble from marine sediments

was expected to create safe-sites for rhizomal growth and seed establishment of *Z. marina*. On 20 June 1997, the lowest tide of season, previously discarded construction debris/rubble was removed from 70 m² of intertidal and subtidal area south of Gravel dock to facilitate the natural recruitment and transplantation of *Zostera marina* var. *phillipsii* Backman (eelgrass) (Figure IV.3). Debris/rubble was removed using a track-hoe that traversed the exposed beach during the low tide. Where possible, the bucket of the track-hoe scraped the surface of the sediments to remove debris. In areas beneath the existing Gravel dock or beyond the reach of the arm and bucket, debris was collected by hand and loaded into the bucket for removal.

On June 23, 1997, a population of 100 *Z. marina* individuals (approximately 69 m² of sparsely populated subtidal area), to be impacted by the dock expansion project, was relocated to a 2.5 m² area not affected by construction. The transplant site was identified along the south side of Gravel dock where debris/rubble had earlier been removed (Figure IV.3).

The following criteria were used to select the transplant site: 1) the area had to be within the same ownership boundary as site of origin; 2) it had to have an elevation similar to site of origin; 3) sediment characteristics had to be similar to site of origin, and 4) no *Z. marina* could be present within the area to be transplanted. The

conditions for *Z. marina* growth at the selected site appeared only to be limited by the existence of rubble, and therefore a high transplantation success rate was expected.

Individual *Z. marina* plants were collected either by using a 6-inch diameter PVC corer (to collect groups of adjacent individuals) or by excavating individuals by hand. The plants were immediately transported to the transplant site. They were then transplanted by either removing a similarly sized sediment core to be replaced by the donor core, or by inserting a finger/hand into the sediments at approximately the same angle as the individual's root orientation, then inserting the plant's roots/rhizomes. Both methods included the gentle tamping/pressing of the marine sediments to help reduce erosion of the disturbed surface sediments. Plants were planted in a patch-like configuration to mimic the distribution of the donor site. All plants were relocated within two hours of original excavation.

To mitigate the effect of shading by dock construction, the removal of a 36 m² section of the existing Gravel dock occurred in March 1998 following the completion of the new dock construction. The objective of this treatment was to increase the submarine light environment to levels adequate for *Z. marina* growth and recolonization (Zimmerman *et al.* 1994).

The 36 m² section of dock was removed from the southern most area of the existing Gravel dock (Figure IV.3). The removal of this section allowed for unobstructed sunlight to reach the submarine environment in the area where debris/rubble had been removed. The combination of full sunlight and newly exposed marine sediments was expected to improve the environmental conditions that limited *Z. marina* from growing in that area.

In May 1999, reflective panels were installed beneath a portion of the southern edge of Gravel dock in order to reflect sunlight beneath its southern portion into the submarine environment (Figure IV.3). Ten panels were placed beneath the dock (Figure IV.4); five installed against the underside of the dock (parallel to water surface) and five along the dock pilings (perpendicular to water surface and joined to the five panels placed against the underside of the dock). Each panel was constructed of a standard 4'x8' sheet of plywood and covered on its exposed side with 2.0 mm-thick reflective Mylar-type film. The reflective film was attached to the plywood sheet by nailing small wooden slats onto the surface of the film, attaching it to the plywood sheet in many places to minimize exposure between the plywood and the film, which could damage the film in high winds.

Vegetation Characteristics

A baseline survey of existing vegetation was accomplished by a diver using S.C.U.B.A. immediately following the rubble/debris removal activities and transplant.

Subsequent resampling was done during the summers of 1998, 1999, and 2000. Five transects, traversing the existing *Z. marina* population, were established south of Gravel dock. The depths of the transects were randomly chosen between +0.2 m and -1.6 m MLLW (transect A was shallowest; transect E was deepest) (Table IV.1 and Figure IV.5). The existing *Z. marina* population is bounded by these depth contours.

Five 0.25 m² quadrats were measured along each transect; they were 3 m apart, starting at 1.5 m from the southern end of each transect. At each quadrat, shoot density of *Z. marina* individuals were recorded. During the 1998 sampling, three plants from each quadrat were measured for blade height and width. Estimated aboveground biomass was later calculated.

Areal mapping of the on-site *Z. marina* population was accomplished following the initial restoration/transplant efforts of 1997, and in the subsequent summers of 1998, 1999, and 2000. Mapping was accomplished by a diver using S.C.U.B.A.

Measurement of Physical Parameters

Underwater spherical quantum sensors (LiCor LI-193SA) were placed to measure photosynthetically active radiation (PAR) at canopy height in three locations (Figure IV.3) (Carruthers and Walker 1997, Duncan 1990, Kirk 1994). Sensor #1 was placed in existing eelgrass cover south of Gravel dock, in full sunlight. Sensor #2 was

placed beneath the reflective panels. Sensor #3 was positioned beneath the existing dock where no reflective treatments were installed. Light measurements were made during each consecutive season for a period no less than 10 days, beginning with Spring 2000 and ending Winter 2001. For each seasonal set of measurements, PAR measurements were made every 60 seconds for the duration of the period and mean hourly and daily PAR values were compared.

During the Summer of 1998, a grab sample of surface sediments taken adjacent to each sampling quadrat (defined in previous section) was collected and analyzed for grain size and organic matter. The hydrometer method (Gee and Bauder 1986) and loss on ignition techniques (Schollenberger 1927, Schollenberger 1945) were used. These measurements were made to identify any anomalous sediment characteristics at the site that might influence the *Z. marina* population.

RESULTS

Alterations to Dock and Environment

A variety of discarded building materials were removed from the intertidal and subtidal areas adjacent to the sound end of Gravel dock. The material consisted of broken concrete slabs, discarded chainlink fencing, discarded tires, metal conduit pipe, rubber hosing, and various pieces of partially buried rope and cable. In total,

approximately 20 m³ of debris was removed from 70 m² of substrate (Figures IV.6 and IV.7).

In the areas cleared of the debris, *Z. marina* individuals began to recolonize naturally. During each of the three years following the initial debris removal activities, the areal extent of *Z. marina* increased (Table IV.2 and Figure IV.8).

One hundred *Z. marina* individuals removed from a proposed project site were transplanted in a 2.5 m² area. Ninety (36 m²) individual *Z. marina* ramets were counted on 3 September 1997 (10 weeks after transplanting). On 7 September 1998 (62 weeks from transplanting), 92 (36.8 m²) individuals were present. On 23 September 1999 (116 weeks from transplanting), 102 (40.8 m²) individuals were present. On 23 August 2000 (164 weeks from transplanting), 110 (44 m²) individuals were present (Table IV.3).

The 36 m² portion of dock that was removed to allow unobstructed sunlight to reach the submarine environment promoted the natural recolonization of *Z. marina* into the affected area. During the first year following the dock removal, rhizomal recolonization of *Z. marina* occurred along the perimeter of the affected area. During the subsequent years, seed-germinated *Z. marina* individuals naturally established patches within the affected area and continued to increase in distribution (Table IV.4 and Figure IV.8). After the first year, the newly established *Z. marina* individuals

covered 6% of the 36 m² area where the dock had been removed. After the second year, the *Z. marina* population had grown to cover 17% of the area.

Beneath the southern edge of Gravel dock, reflective panels were installed to increase the amount of submarine light in the subtidal environment. In the area affected by the panels, approximately 9-11% of full sunlight was reflected into the subtidal waters. In the area affected by dock shading, approximately 1-3% of full sunlight was recorded in the subtidal waters. The results of this increase of submarine PAR led to the natural recolonization of 18 m² of *Z. marina* at an average density of 28 shoots m⁻² (Table IV.5). (See *Measurements of Physical Parameters* section for seasonal PAR data)

Vegetation Characteristics

In 1997, prior to the initiation of any restoration treatments, the mean *Z. marina* shoot count for the existing population was 70 shoots m⁻² (S.E. = 9.36). In 1998, the first year following initial treatments, the mean shoot count was 98 shoots m⁻² (S.E. = 4.04). In 1999 and 2000, the mean *Z. marina* shoot count was 101 and 97 shoots m⁻² (S.E. = 6.25 and 10.08 respectively). Overall, the highest density measured was 146 shoots m⁻² (S.D. = 43.96); transect A (1997) and the lowest was 38.4 shoots m⁻² (S.D. = 6.68); transect E (1997).

In 1998, for transects located south of Gravel dock, the average blade width for the *Z. marina* individuals within this area was 1.96 mm (S.D. = 0.15). Generally, blade

widths increased with depth. The smallest widths were found in transects A and B (Table IV.6). Blade height followed a similar trend to blade width. In 1998, for transects located south of Gravel dock, the average blade height for the *Z. marina* individuals is 0.71 m (S.D. = 0.30). Generally, blade heights increased with depth. The smallest values were found in transects A and B (Table IV.7). These results correspond with those for blade widths (increased depth, increased blade width and height). An indirect comparison of biomass, calculated by combining the density count of *Z. marina* and the mean measured blade widths and heights per quadrat of the transects located south of Gravel dock, indicated no significant difference ($\alpha = 0.11$) between transects by depth.

Since the initial mitigation efforts, the overall *Z. marina* areal coverage has increased by approximately 59 m² at a mean density of 73 shoots m⁻² (Table IV.8). This increase in area includes the transplant site (2.5 m²), the area beneath the reflective panels (18 m²), the area from which debris was removed (32.5 m²), and the area from which the dock was removed (6 m²).

The area affected by the reflective panels is being populated rhizomatically (clonal) by *Z. marina* individuals found south of the panels in full sunlight. The area from which debris was removed is being populated by *Z. marina* using two reproduction strategies; rhizomes and seeds. Clonal expansion originated from the lower intertidal

population found adjacent to the debris removal area, and has progressively invaded from its perimeter. Seedling establishment has initiated within the debris/dock removal area. The newly established seedling populations were not found to be rhizomally connected to the previously existing population or to adjacent seedling populations. The seedling populations hence have rhizomatically expanded since their initial establishment. At the current rate of expansion, they will reach adjacent populations within a few years.

Measurement of Physical Parameters

Seasonal measurement of the submarine light conditions of three locations were conducted during each season for one year. Sensor #2, located beneath the reflective panels, indicated that submarine PAR was approximately 9-11% of full sunlight (measured by sensor #1). Sensor #3, located beneath the dock where reflective panels were not installed, indicated that submarine PAR was approximately 1-3% of full sunlight (Figures III.9-13). These percentage results were found to be similar for each season while overall PAR was highest during the summer and autumn months. The greatest difference in PAR affected by the reflective panels versus having no panels was during the winter months.

Sediment textures, in the area south of Gravel dock, varied between sand and loamy sand. Transects A, B and C are a homogeneous subset (consisting of >95% sand), while transects D and E have sediment with more fines (consisting of 70-80% sand,

17-29% silt, 1-3% clay; loamy sand). All measured sediments contained between 1-3% organic matter, with the higher organic matter percentages found in the loamy sands. Percentage of sand was found to decrease with depth while percentage of silt increased.

DISCUSSION

This case study utilized a blend of methods for the purpose of creating new *Z. marina* habitat. Due to the limited available area in which to attempt the methods, some of the treatment areas were combined. This combining of methods inherently makes it difficult to clearly define the individual effects of each. In addition, because this site was the only one available for this research, replication was not possible. Repeating these methods at other sites would help to substantiate these results.

Overall, an areal increase of *Z. marina* of 59 m² has resulted from using a combination of restoration methods. The most significant results from each treatment immediately followed their installation, with continued *Z. marina* expansion during subsequent years.

As indicated by Table 6, the areal increase of *Z. marina* recolonization was highest the year immediately following the debris removal activities. The greatest amount of newly created habitat was exposed to adjacent *Z. marina* populations immediately

following this treatment; however, the *Z. marina* population continued to expand in subsequent years. Transplantation of 100 *Z. marina* individuals was accomplished in an area that had been cleared of debris. Largely because of the transplant, density values for the overall site in 1998 are higher than those in 1999.

The high overall density values recorded in 2000 were largely due to the recolonized *Z. marina* individuals within the dock removal area. With the section of dock removed following the removal of debris from the area, two limiting factors (low-light and restricted sediment surface) were thus removed allowing for recolonization to begin. Because this area is in the intertidal area of the site, plants are smaller but more densely spaced. Following their initial seedling establishment in 1999, the population has been rapidly growing in area rhizomatously.

The high transplant success of the 100 *Z. marina* individuals was most likely due to the similarity in site characteristics and proximity to the donor site. The most obvious deterrent to establishment was a piece of rubber hose lodged at one end into the sediment which previously scoured the area as currents moved it across the sediment surface. With its removal, this area was primed for transplant success.

Low levels of PAR are known to be limiting to *Z. marina* growth (Alcoverro *et al.* 1999, Zimmerman *et al.* 1991). Development of overwater structures, such as

commercial docks, have systematically reduced the areal extent of *Z. marina* in coastal marine environments globally (Burdick and Short 1999, Shafer 1999). The installation of the reflective panels beneath Gravel dock, Anacortes, Washington, succeeded in increasing the ambient submarine light environment from 1-3% to 9-11% of full sunlight. This increase in available submarine PAR, while still significantly lower than that of full sunlight, appears to be sufficient to promote rhizomatous recolonization of *Z. marina* in the affected area (Duarte 1991, Fitzpatrick and Kirkman 1995, Olesen and Sand-Jensen 1993). The stem density of the *Z. marina* individuals is low, yet the individual biomass appears to be more than adjacent individuals found in full sunlight conditions and at the same depth. This indicates that recolonized *Z. marina* individuals in the affected area have responded to the low level of PAR similarly to those individuals found at deeper depths in full sunlight conditions (Fonseca *et al.* 1990). At deeper depths, there is less available PAR, and in response, *Z. marina* individuals exist at lower stem densities yet greater individual above-ground biomass (Bulthuis and Woelkerling 1983, Vermaat 1996).

In addition, the difference in submarine PAR increases with reflective panels versus areas having no panels was greatest during the winter months. This may be accounted for by the lower sun angle during the winter, allowing a greater percentage of full sunlight to reach further beneath the dock. It must be remembered, however, that even though a greater percentage of full sunlight is being reflected into the submarine

environment during the winter months, the overall amount of full sunlight is much less than the amount available during the summer months.

The area affected by the reflective panels is measured as approximately 60 m². It is not possible to precisely delineate the area where light conditions are improved by the panels. In addition, between the reflective panels and the southern edge of Gravel dock is dock decking which is not covered by paneling. If this area were also covered, the amount of reflected PAR might increase from current levels, increasing the density of the newly recolonized *Z. marina* individuals.

In reviewing the treatment results, the removal of submarine debris that limited the area in which *Z. marina* could exist allowed for the quickest repopulation of individuals; 46% of the affected was recolonized within three years. Removing the section of dock thus eliminated the next most limiting environmental condition, light, and allowed for recolonization to begin in that area; 17% of the affected area was recolonized within two years, with the expectation that 100% will be recolonized within 3 additional years. Lastly, enhancing the submarine light environment below the existing dock created an environment that was conducive to natural recolonization in an area devoid of *Z. marina*; 30% of the affected area was recolonized within two years, however at a lower density than those individuals at similar depths in full sunlight.

At this site, the most effective treatment that has resulted in long-term benefit to *Z. marina* distribution was the removal of submarine debris. In areas where *Z. marina* had once existed, as is the case with the treatment areas of this site, a combination of treatments that first identified the most limiting factors (e.g., submarine debris and light) then attempted to reduce or eliminate them made an important contribution to the increase in the existing *Z. marina* distribution.

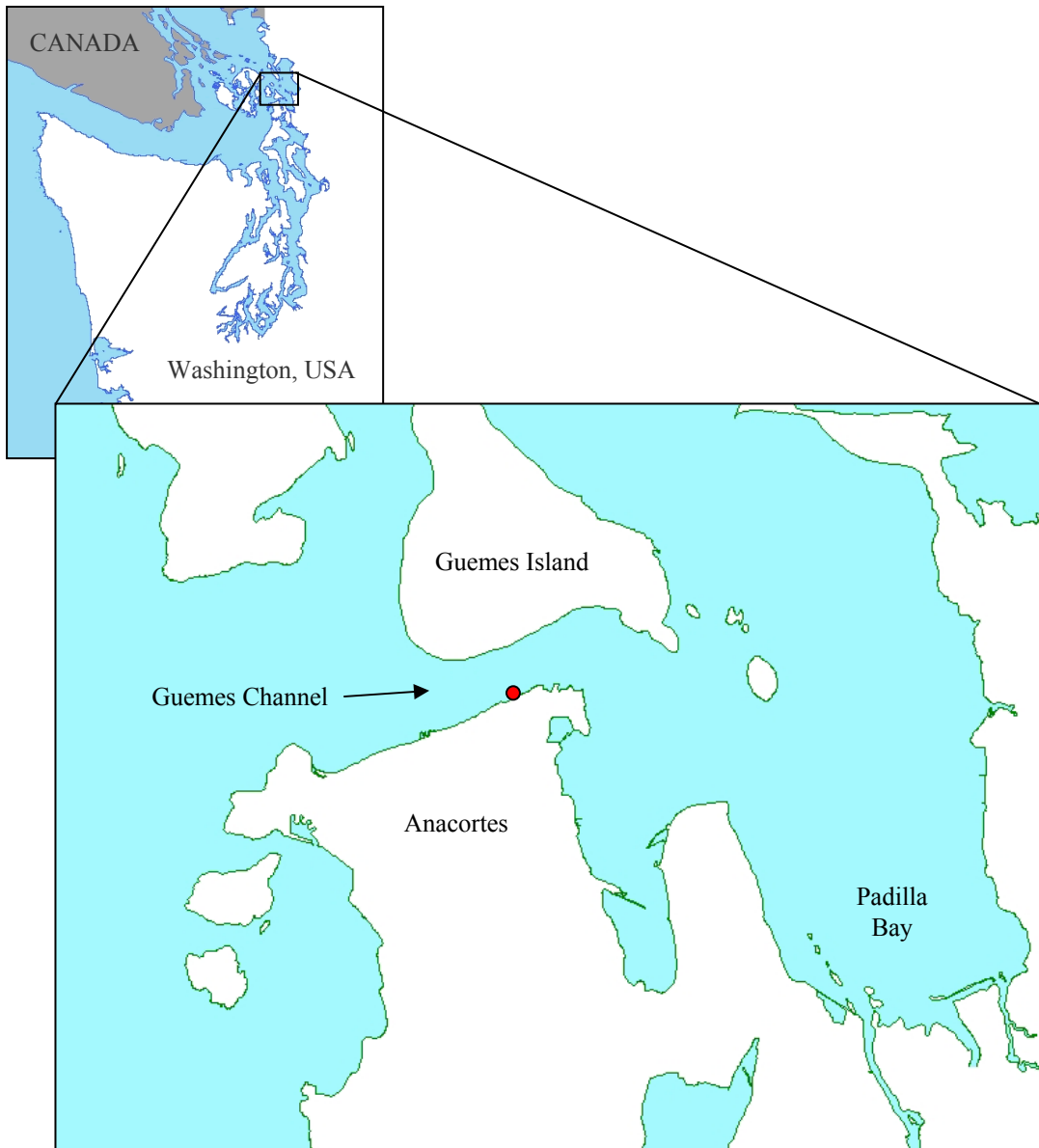


Figure IV.1. Site Map.

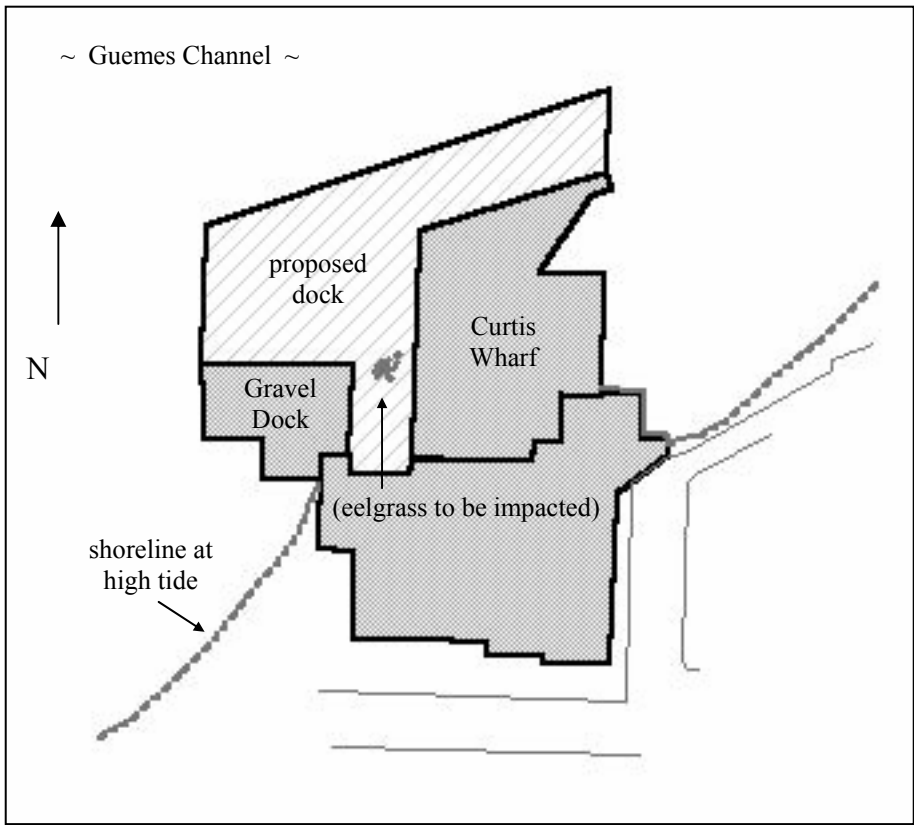


Figure IV.2. Site Configuration.

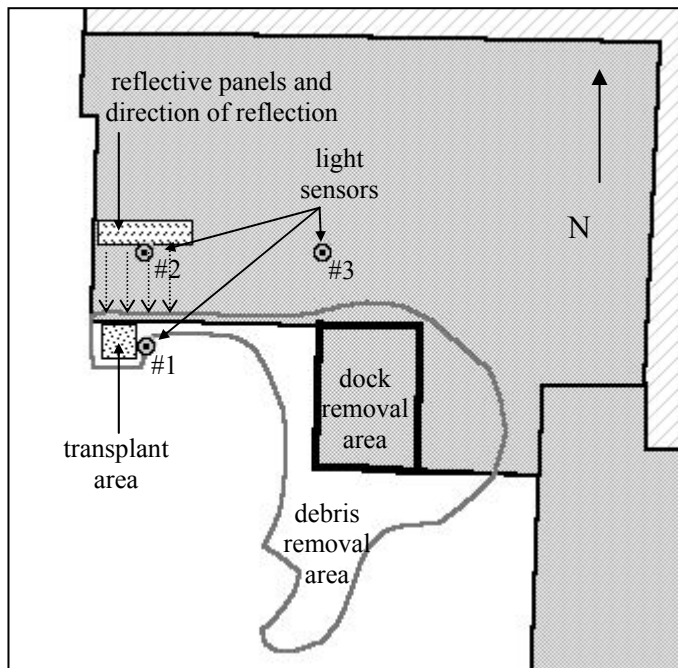


Figure IV.3. Treatments Location Map.



Figure IV.4. Reflective Panels (photo).

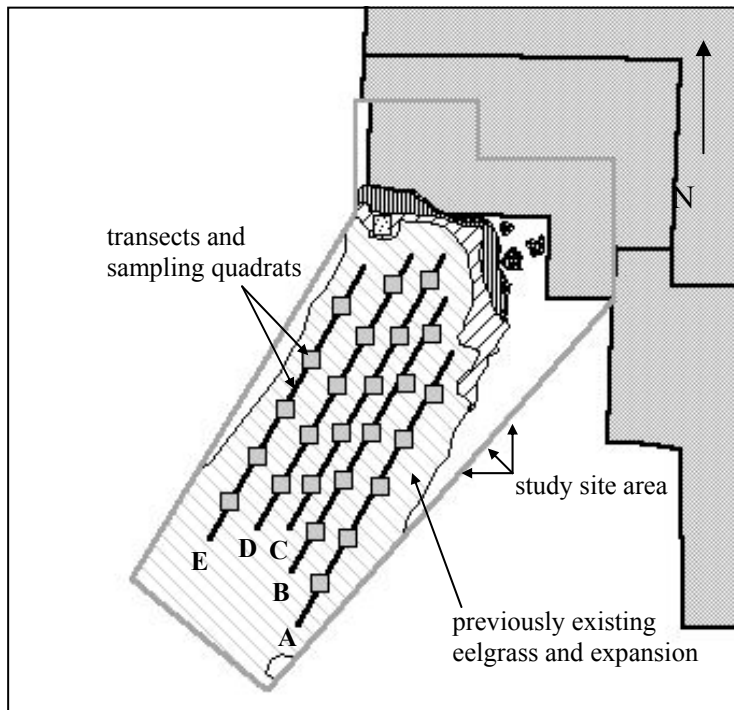


Figure IV.5. Measurement Transect Locations.



Figure IV.6. Removed Rubble.



Figure IV.7. Track-hoe removing rubble during low tide.

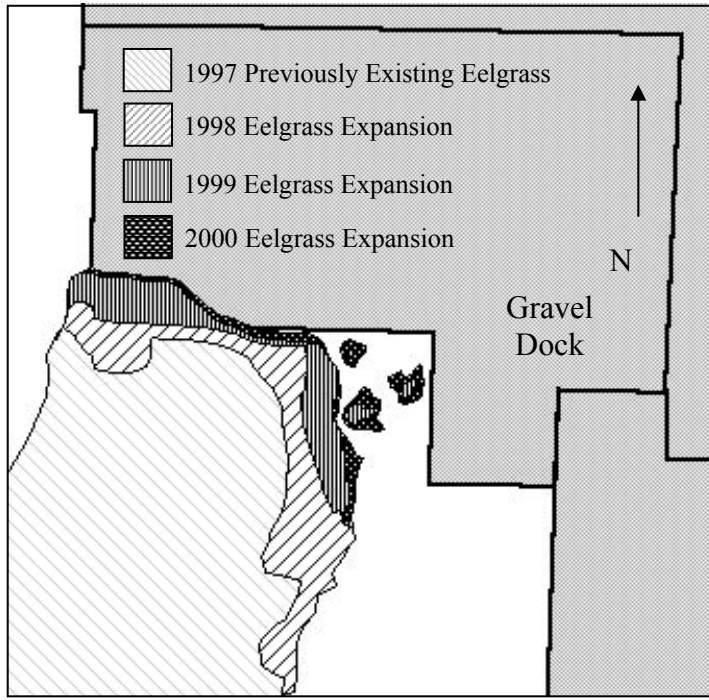


Figure IV.8. Areal Increase of *Z. marina*.

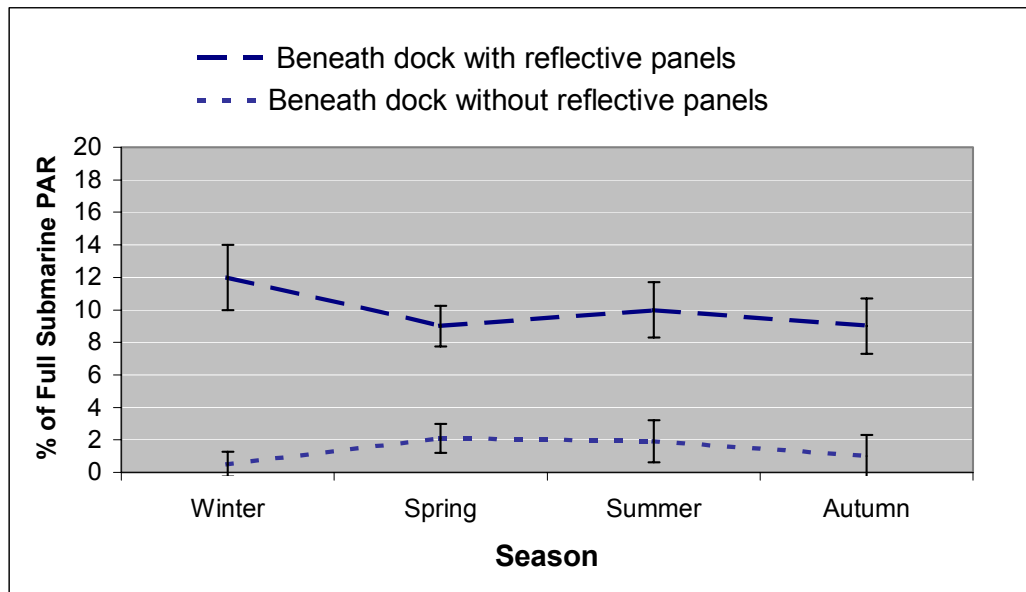


Figure IV.9. Seasonal Submarine PAR Comparison.

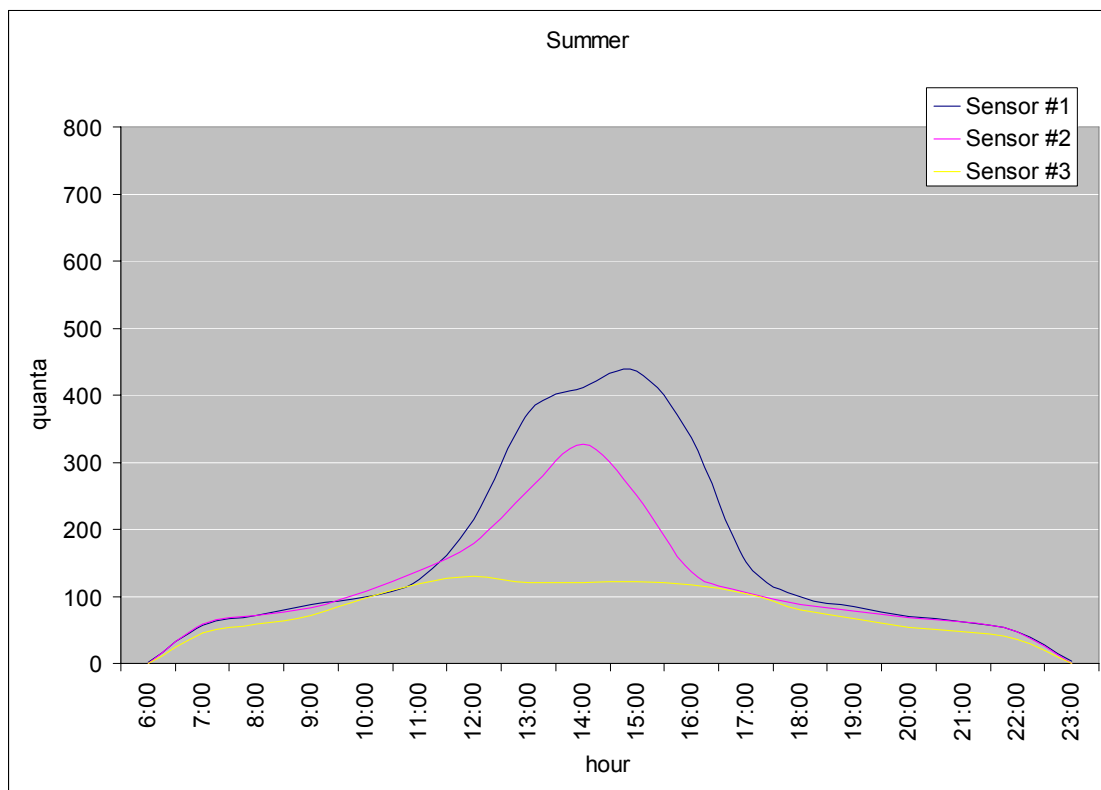


Figure IV.10. Mean Daily PAR Measurements (Summer).

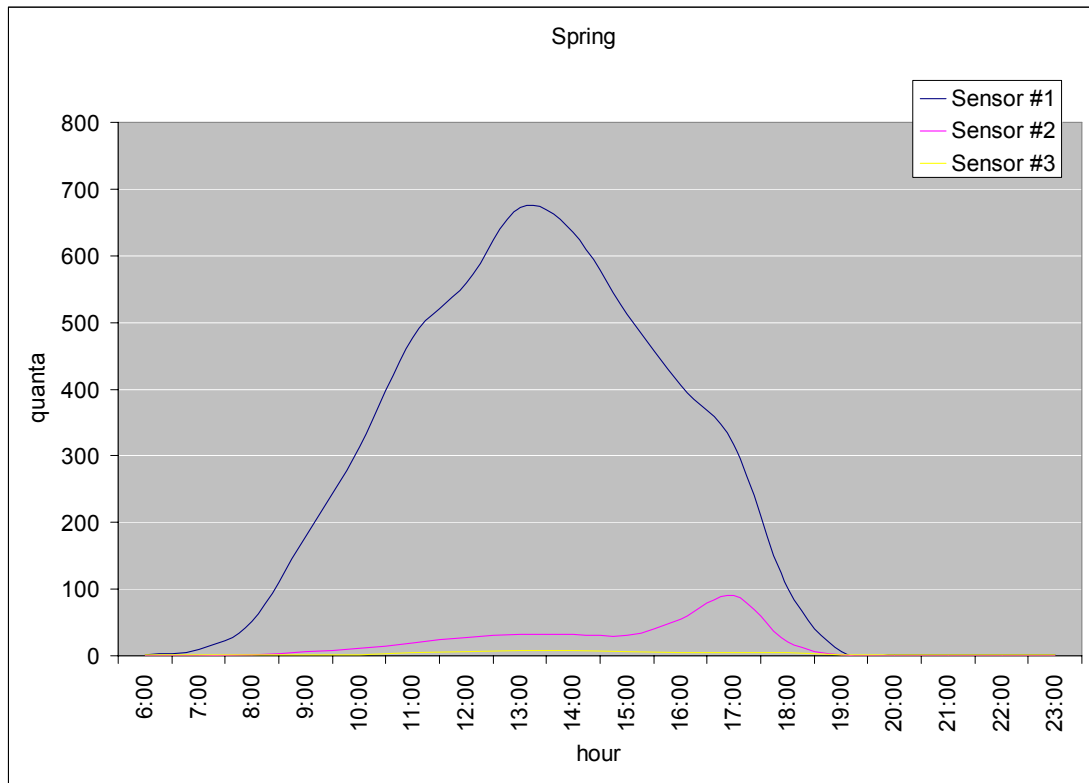


Figure IV.11. Mean Daily PAR Measurements (Spring).

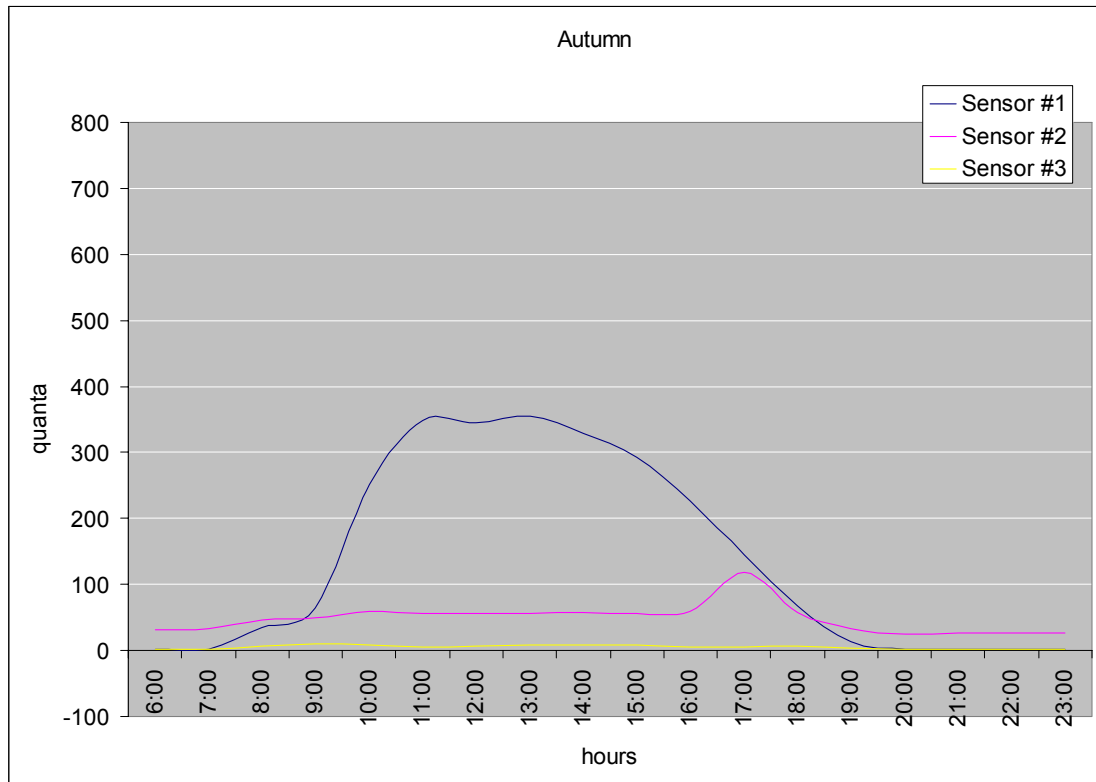


Figure IV.12. Mean Daily PAR Measurements (Autumn).

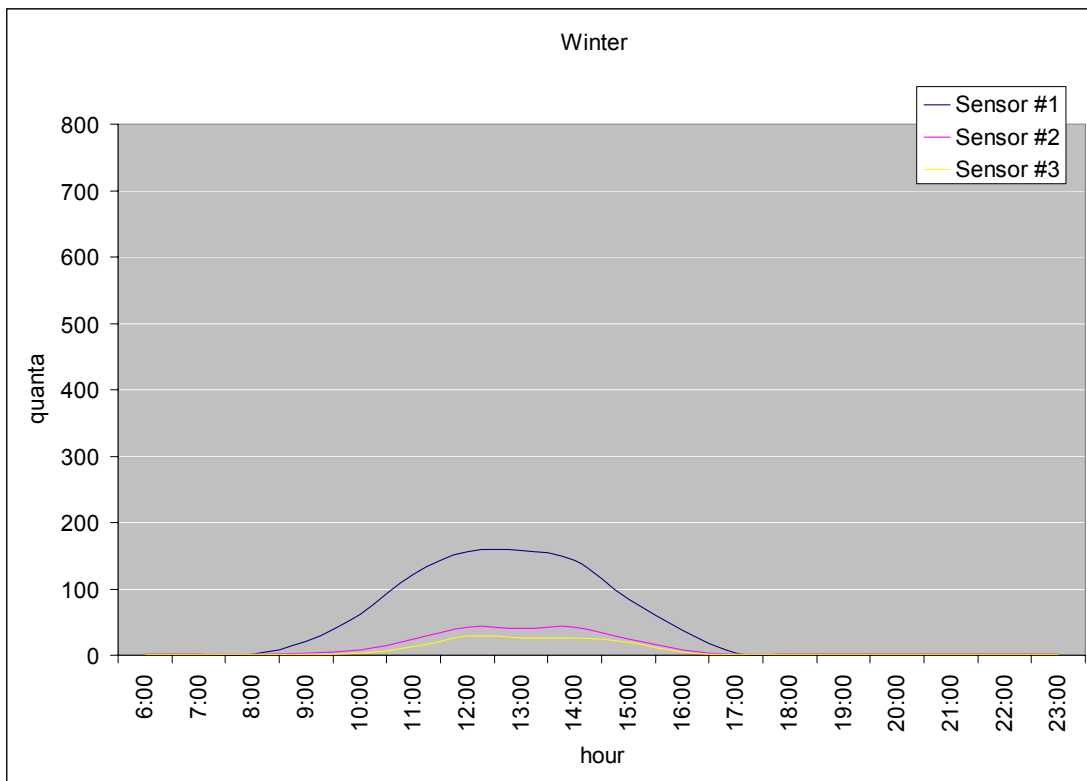


Figure IV.13. Mean Daily PAR Measurements (Winter).

Table IV.1. Transect Depths.

Transect	Depth (m; MLLW)
A	- 0.1
B	- 0.4
C	- 0.8
D	- 1.0
E	- 1.3

Table IV.2. Areal Increase of *Z. marina* in Debris Removal Treatment.

Year Measured	Increase in Area (m ²)	Mean Density (shoots m ⁻²)
1998	32.5	92
1999	Overlapped with Dock Removal and Reflective Panel Treatments	
2000		
	Affected Area (m ²)	Percent Repopulated
<i>TOTAL</i>	70	46%

Table IV.3. Stem count and density of transplants.

Year	# of <i>Z. marina</i> shoots	Density (shoots m ⁻²)
1997	90	36
1998	92	37
1999	102	41
2000	110	44
	Affected Area (m ²)	Percent Repopulated
<i>TOTAL</i>	2.5	100%

Table IV.4. Areal Increase of *Z. marina* in Dock Removal Treatment.

Year Measured	Increase in Area (m ²)	Mean Density (shoots m ⁻²)
1998	Not Yet Removed	
1999	2	27
2000	4	145
<hr/>		
	Affected Area (m ²)	Percent Repopulated
<i>TOTAL</i>	36	17%

Table IV.5. Areal Increase of *Z. marina* in Reflective Panel Treatment.

Year Measured	Increase in Area (m ²)	Mean Density (shoots m ⁻²)
1998	Not Yet Installed	
1999	15	27
2000	3	34
<hr/>		
	Affected Area (m ²)	Percent Repopulated
<i>TOTAL</i>	approx. 60	30%

Table IV.6. Blade Width (mm) Data by Transect (1998).

Transect	Count	Mean	Standard Deviation	Standard Error
A	5	1.80	0.00	n/a
B	5	1.87	0.08	n/a
C	5	2.00	0.44	n/a
D	5	2.11	0.19	n/a
E	5	2.00	0.00	n/a
Total	25	1.96	n/a	0.03

Table IV.7. Blade Height (m) Data by Transect (1998).

Transect	Count	Mean	Standard Deviation	Standard Error
A	5	0.09	0.00	n/a
B	5	0.14	0.05	n/a
C	5	0.79	0.14	n/a
D	5	0.93	0.19	n/a
E	5	1.10	0.24	n/a
Total	25	1.96	n/a	0.30

Table IV.8. Overall Population Increase and Density of *Z. marina*.

Year Measured	Increase in Area (m ²)	Mean Density (shoots m ⁻²)
1998	35	85
1999	17	27
2000	7	123
<i>TOTAL TO DATE</i>	<i>59</i>	<i>73</i>

CHAPTER V: DISSERTATION SUMMARY

"The acid test of our understanding is not whether we can take ecosystems to bits on pieces of paper, however scientifically, but whether we can put them together in practice and make them work"

A.D. Bradshaw

The previous chapters of this dissertation have been aimed at an overall goal for ecological restoration of a seagrass species; *Zostera marina*. What does it mean to attempt ecological restoration? In answering this question, one must first feel confident that the terms are well understood. This alone can be a challenge. The term 'ecology', the totality or pattern of relations between living organisms and their environment, can be nebulous to most. 'Restoration' can be defined in a variety of scopes, however most definitions revolve around the idea of repairing some ecological system following disturbance. These terms by far are not limited to what is written here, but broadly capture their idea.

As an ecological scientist charged with attempting practical, functional, and cost effective restoration methods, I often ask others attempting similar practices the following question: So what's the big deal? By this, I mean to inquire about what it is they are attempting with their activities and for what reason. Most are prepared to explain in length their methods and expected results, but often are lacking the reason for their attempts. Here are my reasons for the attempts described in previous chapters.

Seagrass communities, as W. M. Kemp (1984) stated, are conspicuous components of many shallow estuarine and coastal environments. They are positioned between the terrestrial environment on which we live and the vast oceans to which we are drawn. Seagrass communities are one of few coastal marine gateways through which so many of us pass and to which many of us travel. Understanding them, however, is yet a new science in comparison to many other disciplines. What we do know about this community is yet substantial for our limited understanding.

The physical structure of seagrass communities can significantly dampen the hydraulic capacity of waves and tides, reducing erosional processes and stabilizing depositional and transportation environments (Short *et al.* 1992). Biochemically, seagrasses alter marine sediments in ways that enhance microbial activity and contribute to nutrient cycling (Pedersen *et al.* 1999). This in turn stimulates the lower orders of the food web. Populations of fish and invertebrates tend to utilize seagrass communities, which then become sought grounds for bird populations feeding upon those within the seagrass community (Kemp 1984). All components contribute to raising the diversity of the ecosystem. With diversity comes ecological stability.

Historically, coastal marine science has been driven by oceanographic concerns.

Since the birth of restoration ecology as a science, terrestrially oriented as it may have been, it has required the combination of knowledge from many other established

sciences in order to capture a thread of truth. Through restoration ecology, an avenue for investigating the ecosystem functions of seagrass communities has become available. For the past few decades, knowledge about seagrass communities has grown substantially. The previous chapters have been an attempt to add to this body of knowledge. In the world of science, we strive to do this as a profession; learn, share, unify. Please, take from this body of knowledge.

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APPENDIX A (FROM CHAPTER III)

Step-By-Step Guide to Seagrass Planting Method Involving O₂ Tablets

This method of utilizing tablets which effervesce O₂ is aimed at raising the oxidation/reduction (redox) potential of sediments for the purpose of creating a more benign rooting environment than that which naturally occurs. The seagrass species *Zostera marina* (eelgrass) responded positively to being transplanted into a growth medium whose redox potential had been artificially raised (Chapter III).

Below is a step-by-step guide to utilizing this method for the transplantation of *Z. marina*. Assumed in this guide is the predetermination of an appropriate donor site (Fonseca *et al.* 1998, Shreffler and Thom 1993), number of *Z. marina* individuals that will be harvested from the donor site, the area into which the plants will be transplanted, genetic concerns between donor and transplant sites (Alberte *et al.* 1994, Waycott 1998, Williams and Davis 1996), appropriate time-of-year for seagrass transplantation, and the suitability of the transplant site in terms of appropriate environmental conditions (e.g., depth, sediment grain size, fetch, current, wave action, available submarine light).

Required Items:

- Two O₂-effervescing tablets ('OTABS') per planting unit; tablet diameter = 31.75 mm, height = 19.05 mm. Available from Pemble-Halverson, Inc. (\$.32 per tablet, purchased in bulk), W12291 820th Avenue, River Falls, WI 540223, USA. Tele:715.425.5807
- Three donor adult *Z. marina* individuals per planting unit.
- Ice chest(s) to transport harvested *Z. marina* individuals.
- S.C.U.B.A. diver(s) or mask/snorkel for intertidal or higher subtidal sites.

Steps:

1. **Donor Harvesting** In harvesting the donor *Z. marina* individuals, care must be taken to ensure roots are intact (Davis and Short 1997). Plants can be rinsed of all native sediment and transported as bare root individuals. Donor plants should be placed in an ice chest half filled with seawater, and immediately transported to the site of transplantation.
2. **Excavation of Planting Hole** While using S.C.U.B.A. or mask/snorkel, excavate a hole in the marine sediments by hand to a depth no greater than 15 cm and a width of 16 cm (comparable to a 6-inch diameter seagrass core; Fonseca *et al.* 1998)
3. **Installation of PU** Open two oxygen tablets and place their exposed surface face-up, side-by-side in the middle-bottom of the excavated hole. Fill the hole with sediment to such a depth that the tablets are covered by a minimum of 1-2 cm. Next, place the rhizomes of three *Z. marina* individuals horizontally oriented to the sediment surface. All three rhizomes should cross in the middle of the excavation pit, directly above the two oxygen tablets. Following the placement of the rhizomes into the hole, fill in the remainder of the hole with

sediment. Ultimately, the depth at which the *Z. marina* rhizomes are planted should be equal to their original depth at the donor site.

4. **Post-Installation Activity** Additional methods may be employed to help secure the newly planted individuals and eliminate the resuspension of backfilled sediments due currents or other disturbances. These methods may include the use of bamboo skewers (Davis and Short 1997), metal staples (Fonseca *et al* 1998), or wooden tongue depressors (Merkel 1988) as anchors. In addition, a biofiltration material may be attached or weighted to the sediment surface above the backfilled hole to reduce sediment resuspension. Rock fragments placed on the backfilled sediments or biofiltration material may help to accomplish this.

In addition to utilizing the O₂-effervescing tablets for the purpose of raising *in situ* redox potentials, oxygen should be considered in the maintenance of seagrass individuals grown in a greenhouse or outside flow-through marine tank, especially when live individuals are transplanted into the system. Because environments with higher redox potentials have been shown to encourage more healthy growth rates and root condition, replanting of individuals in controlled marine facilities may benefit by the bubbling of oxygen into the water column or by any other aeration method which stimulates the infusion of oxygen into the depths of the water and sediments.

APPENDIX B (FROM CHAPTER IV)

In 1997, mean *Z. marina* shoot count was 70 shoots m⁻² (S.E. = 9.36). As depth increased, mean shoot count decreased from 145.6 to 38.4 shoots m⁻² (Table IV.9). Tukey's Honestly Significant Difference (Tukey's HSD) statistic indicated that shoot densities along shallow transects (A and B) of the existing *Z. marina* population south of the Gravel dock are significantly different ($\alpha = 0.05$) in shoot count than the other transects (C, D, and E) which do not have significantly different shoot densities from each other. The Area beneath the dock was not analyzed statistically for differences between transects due to the few numbers of *Z. marina* individuals who fell within sampling quadrats. Consequently, all *Z. marina* plants were recorded and mapped for this area and whose results are included in the previous section.

In 1998, the mean *Z. marina* shoot count was 98 shoots m⁻² (S.E. = 4.04). Shoot count did not decrease directly with depth, but rather was highest in transect B and lowest in the middle transect C (Table IV.10). Tukey's HSD statistic indicated that transect B is significantly different ($\alpha = 0.05$) in shoot count from transect C, and that all other transects are not significantly different from each other. Between 1997 and 1998, the mean shoot count increased significantly from 70 to 98 shoots m⁻² ($\alpha = 0.002$).

In 1999, the mean *Z. marina* shoot count was 100.8 shoots m⁻² (S.E. = 6.24). Shoot count did not decrease directly with depth, but rather was lowest in the middle transect C, similar to the previous year (Table IV.11). Tukey's HSD statistic indicated that transect B is significantly different ($\alpha = 0.05$) in shoot count from transect C, and that all other transects are not significantly different from each other. Overall shoot densities were not significantly different between 1998 and 1999.

In 2000, the mean *Z. marina* shoot count was 96.8 shoots m⁻² (S.E. = 10.1). Similar to the previous year, shoot count was highest in transect B, however proceeded to decrease with depth to transect E (Table IV.12). Tukey's HSD statistic indicated that there is no significant differences ($\alpha = 0.05$) in shoot count between transects. Overall shoot densities were not significantly different between 1999 and 2000.

In 1998, the average blade width for the *Z. marina* individuals within this area is 1.96 mm (S.D. = 0.15). Generally, blade widths increased with depth. The smallest widths were found in transects A and B and were not significantly different from each other, but Tukey's HSD statistic indicated that they were both significantly smaller ($\alpha = 0.05$) than transects C-E, which were statistically similar to each other (Table IV.13).

Blade height followed a similar trend to blade width. In 1998, for transects located south of the Gravel Dock, the average blade height for the *Z. marina* individuals is

0.71 m (S.D. = 0.3). Generally, blade heights increased with depth. The smallest values were found in transects 1A and 1B and were not significantly different from each other, but Tukey's HSD statistic indicated that they were both significantly smaller ($\alpha = 0.05$) than transects 1C-1E, which were statistically similar to each other (Table IV.14). These results spatially mirror those of the blade widths. An indirect comparison of biomass, calculated by combining the density count of *Z. marina* and the mean measured blade widths and heights per quadrat of the transects located south of the Gravel Dock, indicated no significant difference ($\alpha = 0.11$) between transects by depth; there was no identified gradient in indirect biomass with depth.

Table IV.9. Shoot Count Data (m² quadrats) by Transect (1997).

Transect	Count	Mean	Standard Deviation	Standard Error
1A	5	145.60	43.96	19.66
1B	5	84.80	15.08	6.74
1C	5	40.00	8.00	3.58
1D	5	41.60	6.08	2.72
1E	5	38.40	6.68	2.99
Total	25	70.00	46.80	9.36

Table IV.10. Shoot Count Data (m² quadrats) by Transect (1998).

Transect	Count	Mean	Standard Deviation	Standard Error
1A	5	103.20	9.96	4.45
1B	5	116.00	14.68	6.57
1C	5	80.80	18.20	8.14
1D	5	88.80	20.28	9.07
1E	5	101.60	21.84	9.77
Total	25	98.00	20.20	4.04

Table IV.11. Shoot Count Data (m² quadrats) by Transect (1999).

Transect	Count	Mean	Standard Deviation	Standard Error
1A	5	115.20	12.16	5.44
1B	5	117.60	13.24	5.92
1C	5	79.60	19.80	8.85
1D	5	84.40	19.48	8.71
1E	5	107.60	23.92	10.70
Total	25	100.80	31.20	6.24

Table IV.12. Shoot Count Data (m² quadrats) by Transect (2000).

Transect	Count	Mean	Standard Deviation	Standard Error
1A	5	107.60	14.24	6.37
1B	5	117.20	15.24	6.82
1C	5	88.00	24.12	10.79
1D	5	86.00	23.08	10.32
1E	5	84.40	21.60	9.66
Total	25	96.80	50.40	10.08

Table IV.13. Blade Width (mm) Data by Transect (1998).

Transect	Count	Mean	Standard Deviation	Standard Error
1A	5	1.80	0.00	0.00
1B	5	1.87	0.08	0.04
1C	5	2.00	0.44	0.02
1D	5	2.11	0.19	0.08
1E	5	2.00	0.00	0.00
Total	25	1.96	0.15	0.03

Table IV.14. Blade Height (m) Data by Transect (1998).

Transect	Count	Mean	Standard Deviation	Standard Error
1A	5	0.09	0	0.00
1B	5	0.14	0.05	0.02
1C	5	0.79	0.14	0.06
1D	5	0.93	0.19	0.08
1E	5	1.1	0.24	0.11
Total	25	1.96	1.50	0.3

VITA

PERRY FLEMING GAYALDO

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- Education:** **University of Washington, Seattle, Washington**
Ph.D., 2002. GPA: 3.7; Ecosystem Sciences
Field of Research: Seagrass Restoration
- University of Washington, Seattle, Washington**
M.S., 1996. GPA: 3.7; Restoration Ecology
Field of Research: Native Shrub-Steppe Restoration
- University of California at Berkeley, Berkeley, California**
B.S., 1991. GPA: 3.0
Major: Soil Science / Conservation and Resource Management
- Experience:** **Affiliate Associate Professor**
University of Washington; Seattle, Washington
Landscape Architecture Department (2002 - present)
- Research Associate**
University of Washington; San Juan, Washington
Friday Harbor Marine Laboratories (2002 - present)
- Coastal Marine Science Fellow**
National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NOAA/NMFS);
Western Regional Center, Seattle, Washington (2001 - present)
- Coordinate national and regional coastal marine restoration projects including, seagrass meadows, oyster cultures, estuarine habitat, in-stream fish habitat, mangrove forests, coral reefs, kelp forests.
- Marine Ecosystem Science/Research and Ecological Restoration**
University of Washington; San Juan, Whatcom, Island, and Skagit Counties, Washington (1997 - present)
- Developed seagrass restoration techniques.
 - Developed mangrove restoration designs and restoration techniques.
 - Evaluated marine sediment chemistry, including reduction/oxidation (REDOX) potential.
 - Collected and monitored marine plant growth and biomass data in research plots.
 - Designed seagrass sampling techniques.
 - Utilized Geographical Information Systems (GIS) for data organization, analysis, and interpretation.
 - Conducted laboratory controlled growth chamber and greenhouse experiments to study sediment chemistry and particle size characteristics on the success of eelgrass restoration and establishment.
 - Examined data collected at numerous eelgrass locations in the northern Puget Sound to determine relationships between eelgrass existence and environmental conditions/characteristics.
 - Statistically and spatially analyzed vegetative and environmental data with detrended correspondence analysis (DCA; ordination), canonical correspondence analysis (CCA; ordination), and two-way indicator species analysis (TWINSPAN; classification).
- University Instructor**
University of Washington; Seattle, Washington (1998 - 2002)
- Ecosystem Design, Team-Taught - Department of Landscape Architecture & College of Forest Resources,***
- An interdisciplinary field ecology course based at the University of Washington's Cedar Rock Biological Preserve; Shaw Island (San Juan County), Washington.
 - Course material includes ecological evaluation, scientific interpretation, and human intervention through design. The course focuses on site geological history, ecosystems and environmental gradients, hydrology and soils, plant physiology, data analysis, site evaluation, conservation/preservation techniques, sustainability, and restoration techniques.

- Multi-dimensional analytical approaches are taught for restoration and design purposes.
- Ecosystem Evaluation, Friday Harbor Marine Laboratory**
- An interdisciplinary field ecology course based at the University of Washington's Friday Harbor Marine Laboratory Preserve; San Juan Island (San Juan County), Washington.
 - Course material includes three elements: 1) evaluation of preserve property including plant identification, hydrologic mapping, soil analysis; 2) ecosystem science laboratory including mathematical problems sets, evaluation of global preserves, GIS, and remote sensing; 3) independent research.

Board Member & Restoration Ecologist for International NGO

Mangrove Action Project (Organization of Earth Island Institute); San Francisco, California (2001 - Present)

- Active board member/steering committee
- Lead volunteers in foreign mangrove restoration efforts working with foreign NGO's and local fisherfolk; Thailand, Malaysia, Costa Rica.

Restoration Ecologist/Scientist (Coastal Marine Ecosystems)

Port of Anacortes; Anacortes, Washington (1996 - 2001)

- Designed and implemented successful eelgrass restoration projects on Guemes Channel & Fidalgo Bay.
- Designed and implemented transplant/restoration of eelgrass from a construction/donor site to a restoration/project site.
- Implemented eelgrass sampling strategies.
- Designed light enhancement techniques for under-dock environments.
- Collected and analyzed sediment for particle size and redox characteristics.
- Performed statistical analysis of vegetation and sediment data.

University Guest Lecturer (department and course number, years of lectures)

University of Washington, Seattle:

- **Wetland Ecology** (Environ. Horticulture & Urban Forestry 475; 1996, 1997, 1998, 1999, 2000, 2001)
- **Restoration Ecology** (Environ. Horticulture & Urban Forestry 473; 1996, 1997, 1998, 1999, 2000, 2001, 2002)
- **Statistical Analysis of Environmental Data** (Environ. Horticulture & Urban Forestry 502; 1995, 1996, 1997, 1998, 1999, 2000, 2001)
- **Restoration Ecology Network** (Environ. Horticulture & Urban Forestry 462; 2000, 2001)
- **Intro to Urban Ecology** (Urban Horticulture & Forestry 202; 1997, 1998, 1999)
- **Ecology of Urban Environments** (Urban Horticulture & Forestry 201; 1997, 1998, 1999, 2000)
- **Landscape Architecture & Cultural Design** (Landscape Architecture 403; 2000)
- **Environmental Ethics and Identity** (Community and Environ. Planning 461; 2001)
- **Public Presentation** (Urban Horticulture & Forestry 561; 1997, 1998, 1999, 2000, 2001)

Washington State University, Port Hadlock:

- **Watershed Stewardship - Water Watcher Training** (2000, 2001)

Graduate Research Assistant

University of Washington; Seattle, Washington (1996 - 2001)

College of Forest Resources, Ecosystem Science Division

- Managed and led restoration activities on the Union Bay Natural Area; activities include ecosystem restoration, plant propagation, site evaluations, invasive plant control and removal, volunteer coordination, research/experiment monitoring and evaluation, student and class coordination, and public lectures.
- Supervised the restoration ecology laboratory (Merrill Hall).
- Provided scientific assistance to Professor Kern Ewing.

Natural Resource Manager – Union Bay Natural Area

University of Washington; Seattle, Washington (1996 – Present)

- Designed, organized and led restoration projects for the Union Bay Natural Area (a.k.a. U.W.'s Montlake Landfill); 66 acres.
- Assisted U.W. faculty & staff with academic/research projects.
- Coordinated volunteers.

Mangrove Restoration/Ecologist (Thailand)

Mangrove Action Project (MAP); Southern Thailand (1997)

- Volunteered to aid *Yadfon* (a Thai non-governmental organization) with restoration efforts in southern Thailand.
- Worked with local fishermen to develop transplantation techniques/methods and to conduct watershed analyses to determine feasibility of restoration.

Graduate Teaching Assistant

University of Washington; Seattle, Washington (1996 - 1999)

College of Forest Resources, Ecosystem Science Division

- Academically supported Professor Kem Ewing in teaching three courses:
 1. Wetland Ecology (EHUF 475)
 2. Restoration Ecology (EHUF 473)
 3. Statistical Analysis of Environmental Data (EHUF 502)
- Developed and delivered lectures on coastal restoration (seagrass and mangrove restoration) and statistical analyses.
- Provided assistance to students.

Shrub-Steppe Ecosystem Research/Science and Ecological Restoration

University of Washington, ENSR Consulting & Engineering, and U.S. Army; Yakima Firing Range, Yakima, Washington (1994 - 1996)

- Developed native shrub-steppe restoration & management plan for Yakima Firing Range/U.S. Army (approx. 300,000 acres).
- Evaluated soil characteristics as it applied to shrub-steppe restoration.
- Collected and monitored aridland plant growth and biomass data in research plots
- Designed shrub-steppe sampling techniques.
- Statistically and spatially analyzed vegetative and environmental data with detrended correspondence analysis (DCA; ordination), canonical correspondence analysis (CCA; ordination), and two-way indicator species analysis (TWINSPAN; classification).

Environmental Project Manager

ENSR Consulting & Engineering; Redmond, Washington (1991-1995)

- Designed and managed large- and small-scale wetland and aridland restoration projects
- Authored Environmental Impact Statements (EIS) and SEPA Checklists for clients
- Applied for and acquired environmental permits subject to clients' needs
- Performed as client contact and representative, including public meetings and legal counsel
- Conducted field sampling, including wetland evaluations and delineations, soil coring and sampling, SCUBA sedimentation data collection, and quantification of plant biomass and extent

Gray Literature:

Gayaldo, Perry, Sandy Wyllie-Echeverria, and Kem Ewing. 2001. Transplantation and alteration of submarine environment for restoration of *Zostera marina* (eelgrass); a case study at Curtis Wharf (Port of Anacortes), Washington. Proceedings: Puget Sound Research Conference. February 12-14, 2001. Bellevue, WA.

Expected Publications:
(to be submitted in 2002)

Gayaldo, Perry. Ecological analysis of *Zostera marina* in Puget Sound, Washington; A latitudinal study of selected vegetation and sediment characteristics.

Gayaldo, Perry, and Kem Ewing. Reduction/oxidation (REDOX) manipulation for the purpose of eelgrass (*Zostera marina*) restoration.

Gayaldo, Perry, Sandy Wyllie-Echeverria, and Kem Ewing. Transplantation and alteration of submarine environment for restoration of *Zostera marina* (eelgrass); a case study at Curtis Wharf (Port of Anacortes), Washington.

Gayaldo, Perry, and Roxanne Hamilton. Ecosystem Design; A multidisciplinary approach to ecosystem evaluation and design considerations for habitat preservation at the University of Washington's Cedar Rock Biological Preserve, San Juan Archipelago, WA.

Gayaldo, Perry and Roxanne Hamilton. Teaching Ecosystem Evaluation and Design Considerations for Coastal Marine, Prairie, Forested, and Wetland Communities; A unique approach to ecosystem preservation management.

Skills & Training:	<ul style="list-style-type: none"> • Ecosystem/Ecological Restoration (including soil science, plant physiology, hydrology, horticultural practices, and watershed analysis) • Statistical and spatial analyses of environmental data; relevant programs include SAS, SPSS, PC-Ord, Canoco, DCA, CCA, TWINSpan • SCUBA Instructor (PADI # 84871) • Geographic Information Systems (GIS); ArcView Certified • Global Positioning Systems (GPS) for data collection and site mapping • Management of large-scale restoration projects • Windows (IBM-PC) and Apple (Macintosh) operating systems • Soil sampling and analysis • Wetland restoration, delineation, and design • Forested watershed analysis • Aerial photo-interpretation and stereoscope use • Bilingual (Spanish) 						
Honors & Activities:	Coordinator, Graduate Student Association; College of Forest Resources, University of Washington (1996-1998) Graduate and Professional Student Senator; University of Washington (1995)						
Special Interests:	Singer/Songwriter, Acoustic guitar, Surfing, sailing, scuba diving, cross-country skiing, mountain biking, hiking, snow & water skiing						
Personal References:	<table border="0" style="width: 100%;"> <tr> <td style="vertical-align: top; width: 50%;"> <p>Dr. Kern Ewing (Ph.D. Committee Chairperson)</p> <p>Associate Professor Ecosystem Sciences Division University of Washington Box 354115 Seattle, WA 98195-4115 206.543.4426 206.685.2692 fax kern@u.washington.edu</p> </td> <td style="vertical-align: top; width: 50%;"> <p>Dr. John Skalski (Ph.D. Committee)</p> <p>Professor, Aquatic and Fishery Sciences University of Washington Box 358218 Seattle, WA 98101-2509 206.616.4851 206.616.7452 fax jrs@u.washington.edu</p> </td> </tr> <tr> <td style="vertical-align: top;"> <p>Dr. Sandy Wyllie-Echeverria (Ph.D. Committee)</p> <p>Research Analyst School of Marine Affairs University of Washington Box 355685 Seattle, WA 98107-6715 206.468.4619 206.543.1417 fax zmseed@u.washington.edu</p> </td> <td style="vertical-align: top;"> <p>Jennifer Steger (professional collaborator)</p> <p>Coastal Marine Biologist National Oceanic and Atmospheric Administration 7100 Sand Point Way NE Seattle, WA 98115 206.526.6604 206.526.6667 jennifer.steger@noaa.gov</p> </td> </tr> <tr> <td style="vertical-align: top;"> <p>Roxanne Hamilton (course collaborator)</p> <p>Lecturer Landscape Architecture University of Washington Box 355734 Seattle, WA 98195 206.543.2848 206.685.4486 fax rhamilto@u.washington.edu</p> </td> <td></td> </tr> </table>	<p>Dr. Kern Ewing (Ph.D. Committee Chairperson)</p> <p>Associate Professor Ecosystem Sciences Division University of Washington Box 354115 Seattle, WA 98195-4115 206.543.4426 206.685.2692 fax kern@u.washington.edu</p>	<p>Dr. John Skalski (Ph.D. Committee)</p> <p>Professor, Aquatic and Fishery Sciences University of Washington Box 358218 Seattle, WA 98101-2509 206.616.4851 206.616.7452 fax jrs@u.washington.edu</p>	<p>Dr. Sandy Wyllie-Echeverria (Ph.D. Committee)</p> <p>Research Analyst School of Marine Affairs University of Washington Box 355685 Seattle, WA 98107-6715 206.468.4619 206.543.1417 fax zmseed@u.washington.edu</p>	<p>Jennifer Steger (professional collaborator)</p> <p>Coastal Marine Biologist National Oceanic and Atmospheric Administration 7100 Sand Point Way NE Seattle, WA 98115 206.526.6604 206.526.6667 jennifer.steger@noaa.gov</p>	<p>Roxanne Hamilton (course collaborator)</p> <p>Lecturer Landscape Architecture University of Washington Box 355734 Seattle, WA 98195 206.543.2848 206.685.4486 fax rhamilto@u.washington.edu</p>	
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