LIGHT LEVEL AS A POTENTIAL LIMITING FACTOR IN THE GROWTH OF
ZOSTERA MARINA EELGRASS IN THE PACIFIC NORTHWEST

by

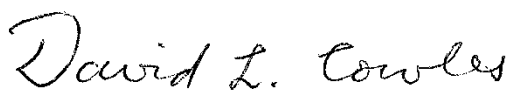MARK JUSTIN HYLARIDES

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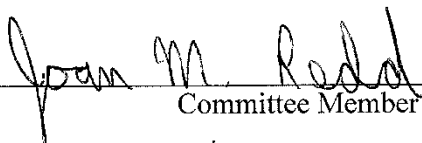the requirements for the
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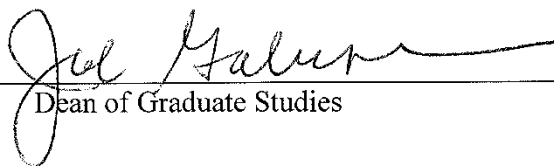
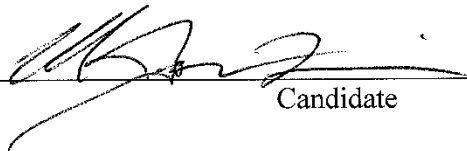Walla Walla University

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ABSTRACT

Eelgrasses serve as important nursery habitats in the Pacific Northwest for diverse marine life and are widely recognized as indicators of coastal ecological health. Seasonal differences in respiration and Photosynthesis-Irradiance (P-I) relationships of a population of marine eelgrass *Zostera marina* in Rosario Bay, WA were investigated in order to characterize its growth potential in different seasons. The local light availability *in situ* for each season was also estimated by combining direct light measurements under different conditions with hourly weather and tide measurements. Measurement of the P-I relationship of blade sections and extrapolation to whole plants demonstrated that sufficient light is present even in the lowest-light season (winter) so that clean eelgrass is able to at least support its metabolism by photosynthesis and experience net growth. However, epiphytic algae living on the blades of *Z. marina* likely reduces its access to light, especially in summer, and could potentially lead to light limiting conditions.

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INTRODUCTION

Background and Life Cycle:

Zostera marina L. is a submerged, marine flowering monocot that belongs to the pondweed family Zosteraceae, and is one of twelve species of eelgrass in the genus *Zostera* ('Zoster' meaning "belt" in Greek) (Mumford 2007, Fonseca & Uhrin 2005). The global distribution of Z. *marina* shows high prevalence in temperate coastal waters of the Atlantic and Pacific coasts and within shallow Mediterranean coastal waters, although it does not commonly occur in the southern hemisphere despite a reported similarity of other seagrass distributions between northern and southern latitudes (Short *et al.* 2007, Fonseca & Uhrin 2005). Studies of Z. *marina* in northern latitudes indicate it inhabits coastal regions from the subtropical to as far as 75 degrees north (Short *et al.* 2007). *Zostera marina* is the only eelgrass species confirmed to be native to the Puget Sound (Fonseca & Uhrin 2005).

Eelgrasses (and seagrasses) such as Z. *marina* are the only submerged marine plants that have an underground root and rhizome system. Typically, the terminal shoot extends laterally from the rhizome, crosses through the substrate, and forms into long, thin blades with rounded tips (Fonseca & Uhrin 2005). For Z. *marina* in the Puget Sound, blade length and width tend to vary with depth such that deeper subtidal populations can reach up to 2m in length and 1-2 cm in width. Conversely, populations in intertidal areas tend to have shorter blades with

1-4 mm width (Mumford 2007). Eelgrasses also contribute to geochemical conditions in the sediment through relatively rapid uptake of nutrients and contaminants into its rhizome (Short *et al.* 2007, Mumford 2007) as well as through provision of oxygen to the sediment via internal lacunae (Mumford 2007, Hemminga & Duarte 2000). In relation to its environment, Z. *marina* thrives in lower intertidal and shallow subtidal areas with muddy to sandy substrate, and is often found in estuaries or lagoons reaching depths of up to 20m in clear waters (Fonseca & Uhrin 2005, Lee *et al.* 2007). Well-established stands of Z. *marina* tend to form extensive canopies or beds of many closely-spaced individuals that play an important role in modifying physical conditions in the water. For instance, eelgrasses can tolerate up to 1.5 ms^{-1} current velocity and serve to shield bottom sediment from erosive forces by dampening wave action (Fonseca & Uhrin 2005). Although eelgrasses do not generally grow along the open ocean coastline likely due to wave action and strong currents, they can grow within the shelter of immobile structures where wave action is less (Lee *et al.* 2007, Fonseca & Uhrin 2005). In the Puget Sound, Z. *marina* beds are prevalent throughout the region in depths ranging from +1.8 to -8.8 m , with an average maximum depth of -3.5 m (relative to Mean Lower Low Water, MLLW) (Mumford 2007).

Zostera marina eelgrass also serves as a habitat, nursery, and feeding grounds for several commercially, recreationally and ecologically important animal species (Fonseca & Uhrin 2005). In the Puget Sound, Z. *marina* hosts several

species of mobile fauna including crab, bivalves, gastropods, and fish including juvenile salmonids as well as several marine-associated birds (Caine 1980, Blackmon *et al.* 2006, Thom *et al.* 1995, Eissinger 2007, Semmens 2008). Other marine organisms in the Puget Sound, including microalgae, macroalgae, copepods, and one known species of isopod, utilize and depend on Z. *marina* for substrate (Mumford 2007, Thom *et al.* 1995).

The life cycle of Z. *marina* is defined by successive annual stages of growth and reproduction, which has been referred to as a "bimodal cycle of clonal growth" by Orth *et al.* 2007. The first year after seed germination is devoted to vegetative growth. In the spring of its second year, this vegetative shoot dies and is replaced by a reproductive, flowering shoot that develops in the same region (Orth *et al.* 2007, Mumford 2007, Fonseca & Uhrin 2005). This shoot flowers and produces seeds during the spring and summer. The seeds are released into the water in mid-summer and rapidly settle in the sediment (Orth *et al.* 2007, Greve *et al.* 2005). The majority of seeds settle near the parent plant and contribute to colony expansion; however, some may be transported in the gut of marine birds such as Black Brant or become attached to gas bubbles released from the plant which can transport the seeds short distances (Mumford 2007, Fishman & Orth 1996, Fonseca & Uhrin 2005). Similarly, the flowering shoot typically dies back after flowering, and may become detached and float away with the seeds to some new site, which is considered the species' most common mechanism of colonization of new areas (Fishman & Orth 1996). Following burial, the seeds

overwinter and germinate the following spring (Mumford 2007). Seedlings develop throughout spring and reach their highest level of vegetative growth during the spring to summer transition (Fonseca & Uhrin 2005). Meanwhile, vegetative growth also spreads horizontally along the substrate by means of rhizomes (underground stems) that branch and produce a tangled mat within the bed (Mumford 2007). During summer months, daughter shoots (or ramets) are continuously created and grow out to the side of the main rhizome every 2-4 weeks, with the apical set of vegetative blades growing toward the surface (Mumford 2007, Fonseca & Uhrin 2005). The transition to autumn is marked by a decline in Z. *marina* vegetative growth, and further declines are observed during the transition to winter. The ramets typically grow into adult vegetative shoots and form their own terminal reproductive shoots the next year (Fonseca & Uhrin 2005), continuing the cycle.

Light intensity and photosynthesis-irradiance (P-I) curves for *Z. marina*

The visible spectrum of light contains photosynthetically-active radiation (PAR) of 350-700 nm wavelengths which is a major factor governing seagrass survival and growth (Lee *et al.* 2007, Hemminga & Duarte 2000). Typically, the blades of plants contain the highest chlorophyll (*a* and *b*) content which is utilized for the transformation of PAR-derived energy into usable carbohydrates via photosynthesis. For a plant cell, cellular respiration and its concomitant

consumption of oxygen occurs in the dark, while in the light the same cell is both respiring (which consumes oxygen) and photosynthesizing (which produces oxygen). Gross photosynthetic rate is the full rate at which photosynthesis takes place and is usually measured by the rate of oxygen production. However, gross photosynthesis cannot be measured directly since cellular respiration always occurs simultaneously and instead it must be calculated: the rate of cellular respiration is typically measured by placing the plant in the dark (conditions under which respiration continues but photosynthesis ceases) and then placing the plant in the light (which allows the measurement of net photosynthesis, or gross photosynthesis minus respiration). Finally, the rate of gross photosynthesis is calculated as the sum of net photosynthesis plus respiration.

The rate of net photosynthesis follows certain predictable patterns of kinetics respective to the levels of PAR available for photosynthesis. This relationship is typically graphed as a 'P-I' (Photosynthesis-Irradiance) curve. A P-I Curve plots net photosynthesis measurements against variable PAR light intensities, and is used to understand how plants photosynthetically respond to light irradiance. Typically, as PAR increases net photosynthetic rate increases up to a limit (called saturation irradiance, I_{sat}). Above this level of PAR, the net photosynthetic rate no longer increases or may even decline, a phenomenon known as photo-inhibition (Hemminga & Duarte 2000). When enough PAR is present to drive a gross photosynthetic rate equal to the rate of cellular respiration, a net photosynthetic rate of zero is attained. The level of PAR needed to achieve zero

net photosynthetic rate is known as the light compensation irradiance, I_c, and is important because this is the lowest level of light at which the plant is able to fix as much carbon as it is using for its cellular respiration. Only at PAR levels greater than the compensation irradiance is there enough light to support growth of the plant. Finally, photosynthetic efficiency (α) is used to describe the slope of the steepest part of the P-I curve with increasing light intensity (Hemminga & Duarte 2000). Photosynthetic efficiency is a measure of how effectively the plant is able to use increased levels of light to support increased levels of photosynthesis.

In addition to characterizing a plant's net photosynthetic response, a P-I curve can be used to investigate photosynthetic response differences among and within plant species. For instance, eelgrasses such as Z. *marina* tend to produce less oxygen as a result of photosynthesis than terrestrial plants do, thus demonstrating comparably lower net photosynthetic rates. This phenomenon has been explained as an adaptation of submersed, aquatic plants to reduced PAR reaching underwater depths (Hemminga & Duarte 2000). In general, the P-I curve for seagrasses is described by an asymptotic function with saturation kinetics, however, some studies with Z. *marina* have reported photo-inhibition at higher light intensities, creating a hyperbolic pattern (Appendix 3, Thom *et al.* 2008). Last, P-I curves of eelgrass such as Z. *marina* provide a baseline from which abiotic and ecological factors that produce changes or shifts in photosynthetic response can be identified and measured.

Factors influencing the amount of daily light irradiance underwater:

a.   Light absorption in water

A submerged, marine existence as a photosynthesizer is dependent upon the availability of light as it penetrates the water column. Since water itself absorbs light, in pure water, light will penetrate three-orders of magnitude less than through air, especially at higher PAR wavelengths. In natural coastal water, solutes and particulates increase absorption at all PAR wavelengths, especially the lower ones, and light penetration diminishes even more. This phenomenon has been especially relevant in cases of water column eutrophication, in which the pronounced attenuation of light caused by the increased solutes and particulates in the water column has been blamed for large-scale deaths of eelgrass populations and even entire eelgrass meadows (Fertig *et al.* 2013). Therefore, water turbidity plays an influential role on Z. *marina* by influencing the spectral properties of light in the water column and its penetration to the bottom where the eelgrass is located.

b.   Daily light period

Growth of Z. *marina* is influenced more strongly by its total length of time exposure to light within a single day (daily light period) than by whether it

actually reached specific levels of light above the compensating intensity during the course of the day (Lee *et al.* 2007). This effect was demonstrated in light manipulation experiments on Z. *marina* beds where changes in the exposure to light compensation and saturation intensities did not appreciably affect *in situ* growth unless they were accompanied by changes in its daily light period (Dennison & Alberte 1985, Lee *et al.* 2007). Daily light period can be measured in *situ* or modeled using compensation and saturation irradiances (and P-I curve kinetics) to predict compensation and saturation daily light periods, or H_{comp} and H_{sat} respectively (Dennison & Alberte 1985, Zimmerman *et al.* 1991). Specifically, H_{comp} has been used to predict the minimum light requirement and/or maximum depth limits for seagrasses, whereas H_{sat} can be used to predict a carbon balance and daily or yearly integrated production of plants (Lee *et al.* 2007). Integrated production of plants combined with the daily light period *in situ* can be used to estimate whether a particular eelgrass bed is receiving enough light to be actively growing or not (Zimmerman *et al.* 1991).

c.   Seasonality

The daily light period changes seasonally for temperate eelgrasses such as Z. *marina*, with the longest periods of illumination occurring during summer months (Hemminga & Duarte 2000). Seasonal studies of eelgrasses find that summer months generally correspond with the highest levels of biomass accumulation of

the year. Prediction of the precise effect is complicated by seasonal changes that may occur in the characteristics of eelgrass P-I curves. For example, maximum photosynthetic rate, respiration, saturation and compensation irradiances (I_k and I_c, respectively) and photosynthetic efficiency (α) may change seasonally (Thom *et al.* 2008, Hemminga & Duarte 2000). The most accurate models must therefore take into account seasonal changes in daily light period and the P-I curve. The mechanisms that lead to biomass changes from these photosynthetic parameters are complex, but seasonal variation in daily light period is thought to appreciably contribute to Z. *marina* annual biomass change.

Zostera marina in the Rosario Strait (the region of interest for this study) differs in seasonal P-I relationships from that of the same species in other regions such as Chesapeake Bay where highly variable temperatures in the water regulate its growth (Mumford 2007, Orth *et al.* 2007). Temperature change in marine waters is a known regulator of Z. *marina* growth responses and has been tightly linked to increased seagrass respiration (Lee *et al.* 2007, Hemminga & Duarte 2000). In particular, Salish Sea waters experience only moderate seasonal shifts in temperature that typically do not exceed a range of change of 8-9°C annually, which is less drastic than those experienced in other temperate marine habitats such as Chesapeake Bay (10-25°C). The Chesapeake region supports a bimodal life cycle of *Z. marina* linked to water temperature where positive growth occurred between 10 and 25°C and leaf senescence and/or inhibited plant growth occurred outside of this range (Orth *et al.* 2007). The narrow range of

temperature changes in Puget Sound (or Salish Sea) waters may mean that eelgrass growth here is regulated more strongly by other factors such as water clarity, dissolved oxygen levels, physical habitat, nutrient inputs and water salinity, although salinity levels also fluctuate very little in general within the Salish Sea based on recent annual data (Mumford 2007, Khangaonkar *et al.* 2012). Also, narrow temperature fluctuation in Salish Sea waters likely contributes to its relatively stable levels of dissolved oxygen (DO) observed. Seasonally-varying levels of dissolved oxygen in nearshore habitats, which lead to periods of hypoxia or anoxia, are also known to severely limit plant growth (Hemminga & Duarte 2000). However, DO levels in the Strait of Juan de Fuca were found to be relatively high at the surface (~7 mg/L) during spring and winter months, whereas in late summer and autumn DO surface levels decreased only slightly to 6-7 mg/L (Khangaonkar *et al.* 2012). Therefore, relatively stable temperatures and dissolved oxygen levels in Rosario Bay and surrounding regions likely minimize the seasonal impacts on Z. *marina* respiration and growth, which is closely linked with instantaneous photosynthetic measurement or P-I curves (Staehr & Borum 2011).

Water turbidity in the Salish Sea fluctuates seasonally and by weather patterns, yet defined patterns can be difficult to characterize due to complicating factors. It is presumed that a sheltered, coastal estuary or lagoon would reach its highest level of clarity during winter months as algal blooms tend to decline, however; ambient daylight also reaches its lowest levels and the shortest daily light periods

occur during the same season. Physical and other abiotic/biotic factors such as storms can also fluctuate substantially (Hemminga & Duarte 2000). Rosario Bay in particular is in a semi-exposed site which is often affected by wave action created to the west in the Strait of Juan de Fuca. Waves pounding on the gravelly beach and abundant kelp deposits quickly stir up particulates in the water and sharply increase turbidity, decreasing light penetration to the Z. *marina* beds. Thus, high variability in light attenuating factors necessitates site-specific and seasonal-specific characterization of the light environment in order to model Z. *marina* photosynthesis and growth.

d.   Other considerations

Large tidal amplitudes occur in Rosario Strait and may strongly influence distribution of Z. *marina* with respect to the depths it occupies. At high tide, increased underwater depth may limit net photosynthesis of Z. *marina* due to diminished light penetration through a deeper water column. In contrast, low tide brings the same population closer to the water surface, which increases light penetration but in hot summer months can lead to increased plant respiration and/or desiccation as well. Thus, both of these tidal extremes tend to limit the subtidal and intertidal depth distributions of Z. *marina* in the Puget Sound, especially during summer months (Mumford 2007). Z. *marina* beds in the Puget Sound experience PAR fluctuations ranging from 0.5 to 30 mol quanta

$m^{-2} d^{-1}$ as a result of large tidal amplitudes, whereas tidal-induced variations in light flux tend to be more limited in habitats where tidal amplitudes are smaller (Thom *et al.* 2008, Zimmerman *et al.* 1991). It has been noted that Z. *marina* living in environments with high tidal amplitude tend to exist at comparably greater depths than populations in environments with low tidal amplitude, although the role of PAR flux on minimum light requirements of eelgrasses remains unclear (Thom *et al.* 2008). Alternatively this phenomenon has been attributed to higher water clarity in certain habitats within the Puget Sound (Mumford 2007). Specific to our study site, strong tidal currents and tidal flushing into Rosario Bay from the more turbid waters east of Deception Pass can also bring higher turbidity conditions into the bay, further restricting light penetration to the eelgrass (Foreman *et al.* 1995, Khangaonkar *et al.* 2012).

e. Whole plant dynamics

In attempting to understand the light requirements of Z. *marina*, whole plant dynamics rather than simple P-I curves of leaves must be considered. In P-I curves as normally done, the net photosynthetic rate of the blade does not account for the respiratory burden of the root and rhizome since the standard technique usually employs a single leaf alone. This is not representative of what is happening in the whole plant. In Z. *marina*, for example, the root and rhizome system is responsible for up to 26% of the total plant respiratory demand

(Hemminga & Duarte 2000). To compensate for this additional respiratory burden, one study added an additional hour of daylight irradiances at saturation levels (H_{sat}) needed to support whole-plant metabolism to the values calculated for leaves alone. The accuracy of this adjusted estimation, however, is uncertain (Zimmerman *et al.* 1991). At any rate, the physiological demands of the rhizome and root system increase the photosynthetic requirement of Z. *marina*, and must be accounted for.

Z. *marina* response to variable irradiance:

a. Minimum irradiance

Previous studies have used 'minimum percent of surface irradiance' required to support growth as a means of describing and comparing Z. *marina* at different temperate latitudes. However, percent surface irradiance is no longer considered a particularly useful indicator of minimum light requirements for Z. *marina* due to the fact that the surface irradiance level itself varies sharply by latitude and other conditions (Lee *et al.* 2007, Hemminga & Duarte 2000). For these reasons, recent work has used daily light compensation period (H_{comp}) to describe the minimum light requirements of Z. *marina*. Although these estimations cannot be uniformly applied, site-specific characterization has increased the confidence of H_{comp} predictive modeling against *in situ* measurement, which helps ascertain

how Z. *marina* survives at or near minimum light requirement thresholds (Lee *et al.* 2007).

The minimum light requirements (H_{comp}) of different Z. *marina* beds vary with the *in situ* availability of light, and generally a positive relationship has been observed between normal irradiance levels experienced and minimum light requirements. For example, Z. *marina* populations existing in intertidal zones, where light availability is greater, tend to have greater minimum light requirements than do populations living at greater depths and/or in more turbid waters that often receive less light (Hemminga & Duarte 2000). Morphological and physiological acclimatizations often accompany low minimum light requirements and permit eelgrasses such as Z. *marina* to survive seasonal changes in light conditions, where light availability may fall below minimum levels for long durations of time. For example, in Pacific Northwest habitats winter-acclimated populations of Z. *marina* had an approximately three-fold greater net primary productivity (NPP) rate under low light than summer-acclimated populations would have had under the same conditions (Thom *et al.* 2008). Such acclimatization is considered integral to the survival of Z. *marina* populations that periodically receive light levels that fall below their minimum light requirements.

b.   Morphological and physiological patterns of Z. *marina* acclimatization to low light levels

The immediate effects of light attenuation in Z. *marina* involve drastic reductions in photosynthetic productivity and carbon reserves, to which Z. *marina* may respond via early changes in its chlorophyll content and then thickness of its leaves (Hemminga & Duarte 2000). Longer-term responses of Z. *marina* to light attenuation include biomass reduction and/or reallocation within the plant (Hemminga & Duarte 2000). For instance, experiments demonstrated that the light compensation point for growth of the leaves is considerably lower than that of the roots and rhizomes, and only at a certain minimum is whole plant carbon balance maintained. Therefore, at low light levels the root and rhizome may decrease in biomass through anaerobic metabolism and/or re-allocation of compounds, despite negligible changes in leaf biomass (Lee *et al.* 2007, Hemminga & Duarte 2000). Similarly, when irradiance falls below a certain minimum to maintain whole plant carbon-balance for Z. *marina*, root and rhizome belowground tissues undergo hypoxia or anoxia because their aerobic metabolism directly depends on oxygen that is produced by and transported from above-ground structures via lacunal airspaces (Hemminga & Duarte 2000). Such longer-term responses of Z. *marina* are additionally subject to environmental variability, which may contribute to plant morphology as well as intra-species differences (Hemminga & Duarte 2000).

Non-structural carbohydrate reserves can be stored and maintained in the rhizomes of Z. *marina* and other seagrasses for use during long periods of underwater light reductions (Hemminga & Duarte 2000). Light attenuation can induce anoxic conditions for the root and rhizome, which shifts the carbon-demand to the non-structural carbohydrates in order to sustain ATP generation through anaerobic respiration (Lee *et al.* 2007). However, it has been shown that use of non-structural carbohydrates are costly to Z. *marina* during periods of low light and anaerobiosis due to the typical "carbon drain" caused by inefficiencies in anaerobic waste product disposal and inhibited translocation of sucrose from above-ground structures when roots become anoxic (Hemminga & Duarte 2000). Therefore, the physiological use of non-structural carbohydrates benefits Z. *marina* plants in low light conditions, but this mechanism proves costly and necessitates periods of replenishment (Hemminga & Duarte 2000).

A recent P-I curve determined for Z. *marina* in the Pacific Northwest and the proposed experiment:

A 5-year study of Z. *marina* in the Pacific Northwest by Thom *et al.* (2008) included development of a P-I curve (Appendix 3) based on replicate Z. *marina* leaf sections exposed to ambient light. The P-I curves were obtained from stalks that had been growing in flowing seawater tanks. Their plants were initially collected from the mouth of Sequim Bay, Strait of Juan de Fuca, and grown in

tanks under ambient temperature and light at 1.5 m depth. For developing the P-I curves, the blades were enclosed in glass jars for short-term incubations and exposed to ambient daylight. Photosynthetic rate was determined by measurement of changes in dissolved oxygen levels in the jars, as measured by an oxygen electrode. Light levels were measured *in situ* in the tanks and were recorded as the average light level the plants were exposed to during each day. Despite many replicates, their resultant curve had wide variability and the r values for each season fell at or below 0.708. Furthermore, the 95% confidence interval was ±21% of the mean net productivity value, which reflected the high variability in their data. Using each day's average *in situ* light intensity is also a potential source of variability because ambient light levels can vary strongly over the course of the day and day length varies by season, making average light values in a day a less useful measure. Measurement error associated with the oxygen probe and shading due to leaf section distributions inside the incubation jars were also considered, although the research team was satisfied with the measurement technique (Thom, personal communication, 2013). Inflation of respiration values could also have been introduced by the effect of tearing the eelgrass plants prior to their placement in experimental respiration jars. Previous respirometry studies of eelgrass blades found that the wound effects of sectioned leaves induce unnaturally high respiration rates which may unintentionally alter results for net photosynthetic rate (Hemminga & Duarte 2000).

In this study different protocols and techniques were employed to generate a more precise P-I curve for *Z. marina* in the Pacific Northwest. For instance, only one population of eelgrass was examined— Z. *marina* in Rosario Bay—and freshly-collected specimens, standardized light levels, and refined techniques were utilized to limit the influence of wound effects in order to produce a more consistent P-I curve. The purpose of this study was to estimate the growth potential of a single population of *Z. marina* eelgrass in Rosario by combining the P-I relationships found for blade samples with hourly local weather data for an entire year. Using models based on physical measurements taken directly in Rosario Bay, this study sought to estimate whether this population was light-limited based on the estimated annual light levels they received at depth.

MATERIALS AND METHODS

Field Work:

a. Selection of research sites and eelgrass sampling methods

Three underwater monitoring and sampling sites were chosen in Rosario Bay, Skagit County, WA among an established population of *Zostera marina* eelgrass (Figure 1). The below-surface depths of the three study sites were as follows below zero tide level in relation to zero tide: the shallowest site, near the inner edge of the eelgrass bed (WGS84 UTM Coordinates, 10U 0524782 5362965), was 3.0m depth, the middle site, near the middle of the eelgrass bed (10U 0524764 5362953), was 4.3m, and the deep site, at the outer edge of the eelgrass bed (10U 0524760 5362933), was 4.6m (Table 1). The three sites were chosen because they represented the shallowest and deepest depth limits as well as a middle region of robust growth for this population of Z. *marina*. The study was conducted during July and August of 2013 and 2014 to represent the "summer" season, in December 27, 2013 to January 3, 2014 for "winter", and on March 20-27, 2014 for "spring".

At each study site, several blade samples from representative plants were harvested for photosynthetic experiments using SCUBA. Also collected was a smaller set of whole, intact plant samples (which included the entire intact plant, including all the blades plus the rhizome and roots) for respiration studies. Blade samples were harvested by snapping off the blade near the plant sheath. Whole
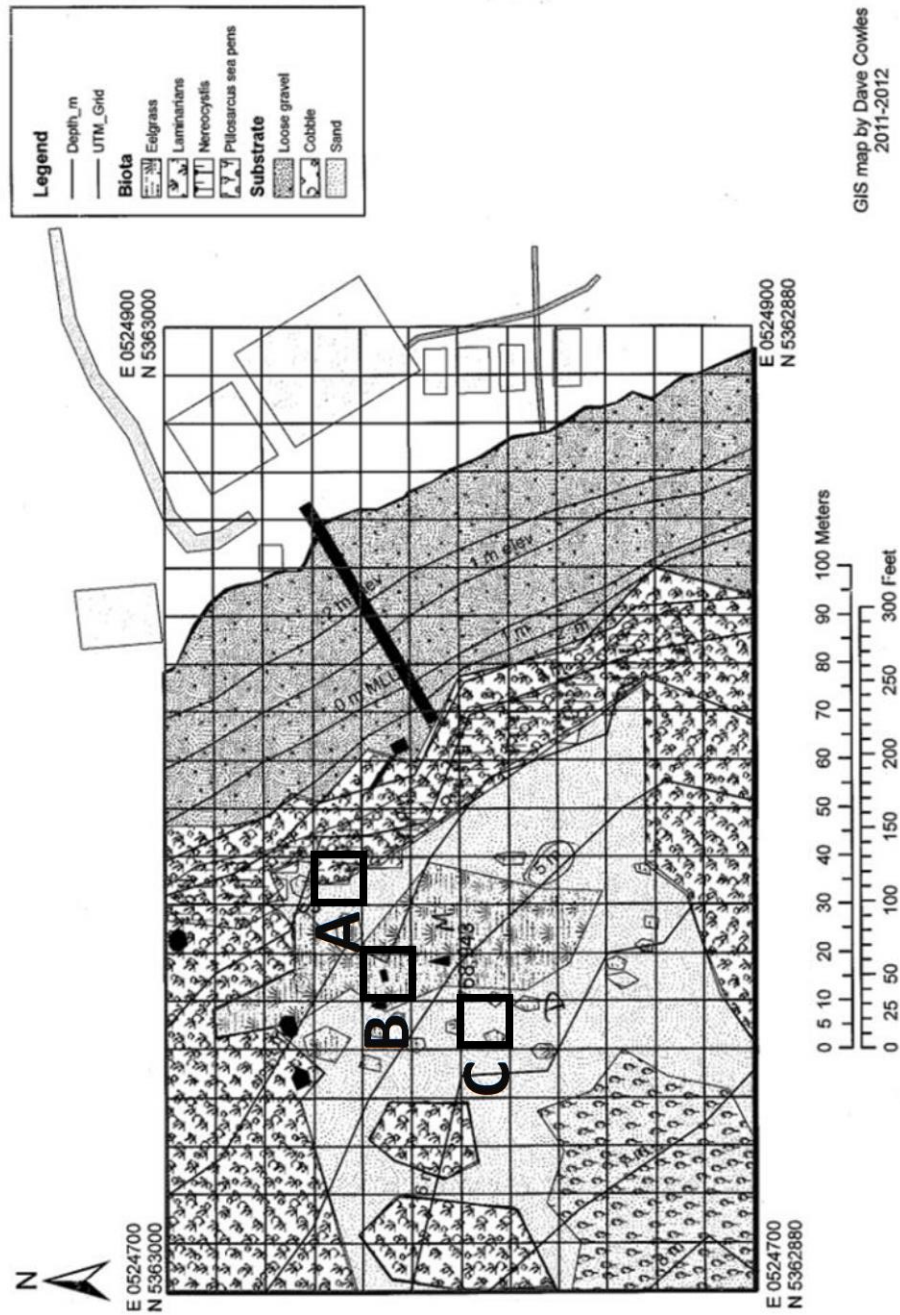
Figure 1. *Geosubstrate Map of Rosario Bay.* The approximate distribution of *Zostera marina* eelgrass in Rosario Bay is shown, and the bolded squares indicate the UTM locations of the three sampling stations (3.0, 4.0 and 4.6m are shown at A, B and C, respectively) where I collected eelgrass for this study.

Table 1. *UTM Coordinates for research sampling sites <u>in situ</u>.* Study site depths are based on zero tide.

Study Site	Depth (m)	UTM Coordinates
Shallow	3.0	10U 0524782 5362965
Medium	4.3	10U 0524764 5362953
Deep	4.6	10U 0524760 5362933

plant samples (rhizome + blades) were obtained by carefully digging up the rhizomes and transporting the whole plant to shore. All blade samples were wiped clean of epiphytes prior to respirometry experiments.

b. Measurements of *In situ* light characteristics at the research site

Seasonal irradiance measurements for both light attenuation in the water column and light transmission through the water's surface were obtained on representative days in order to model the levels of light available to Z. *marina* at its depths in Rosario Bay throughout the year. For light measurements, a 360° light sensor (Li-cor Spherical sensor) connected to a light intensity datalogger (Li-cor LI-1400®) was lowered from a boat stationed at the water surface to measure Photosynthetically-Active Radiation (PAR) at the water surface, immediately below the water surface, and at one-meter depth increments from the surface to the bottom. For light transmission through the surface, PAR was measured directly above and below the surface of the water at different angles of the sun above the horizon. PAR is generally defined as the wavelengths of light within the visible spectrum, roughly 400 to 700 nm. A minimum of four measurements at each height or depth were made including a qualitative assessment of water surface roughness and weather condition at the time of measurement.

Laboratory Work:

a. Respirometry experiments

The respirometry setup is shown in Figure 2. A broad-spectrum lamp (Hubbell LP® with MH-400W lamp) was suspended over the two respirometers used in these experiments. A crank-and-pulley system, which adjusted the lamp height vertically above the respirometers, and gray fiberglass-mesh screens were used to achieve specific light intensities at the surfaces of eelgrass samples suspended in the respirometry chambers. This setup permitted adjustment of lamp height and provided stable illumination. Light intensity intervals within a range of 100 to 700 µmol quanta m^{-2} s^{-1} for photosynthesis experiments were calibrated with the Li-Cor meter with its spherical sensor at the level of the respirometers (the acrylic lid showed a negligible influence on the light path from the lamp). Directly underneath the lamp, two respirometers were placed side-by-side to ensure each respirometer was equally illuminated. Closed, clear acrylic respirometers in water jackets measured photosynthesis and respiration under similar temperatures to that experienced by *Z. marina in situ* (Figure 3). The respirometers were composed of an inner liner composed of a borosilicate petri dish with an acrylic lid and an acrylic outer housing that allowed cooling water to be circulated around them. The lid was sealed to the chamber by a rubber O-ring. Borosilicate dishes were chosen in order to improve the efficiency of the temperature regulation system which involved using a VWR 1160 S® recirculating water bath to cool and circulate water with ethylene-glycol antifreeze
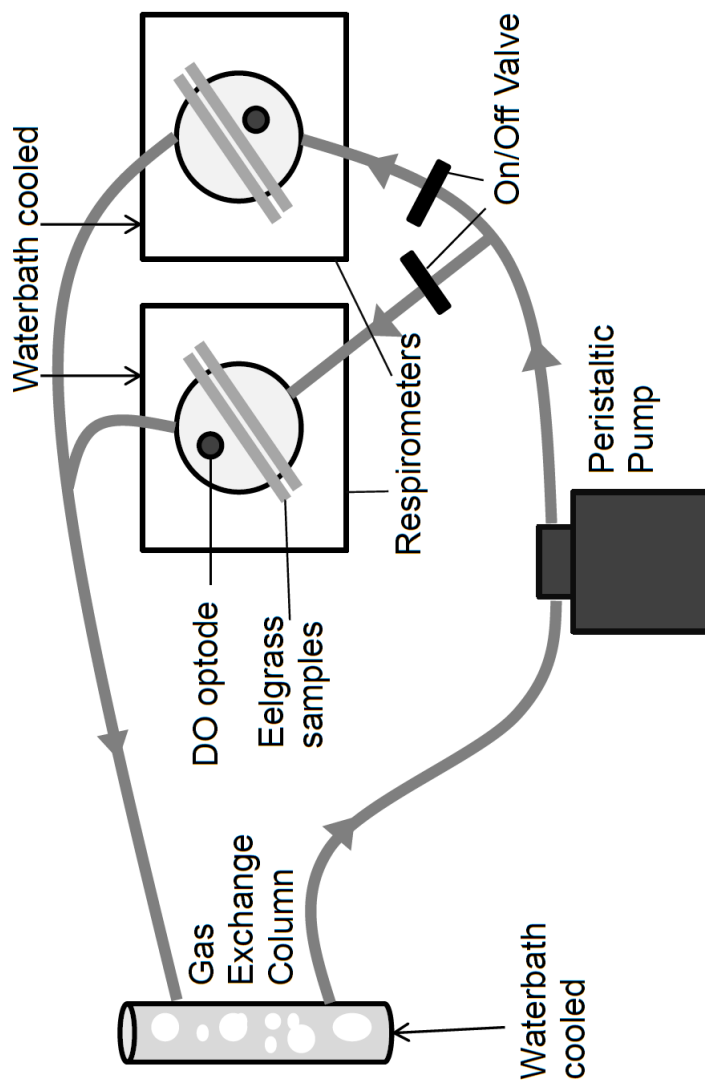
Figure 2. *Schematic diagram of respirometer system setup.* Between experiments or during adjustment of pO_2, the peristaltic pump produced the flow of artificial seawater to the two respirometers, and on/off valves connected to the tubing directed water to fill either respirometer chamber, or both. The water from the respirometer chambers then traveled into the gas exchange column where air or nitrogen gas was bubbled through the water to raise or lower its dissolved oxygen content. The water in both the respirometer chambers and the gas exchange column was maintained at ambient coastal water temperatures using external water baths. During respirometry measurements the water was not pumped through the chambers so that respiration or photosynthesis could be measured as a decrease or increase in oxygen levels in the chambers.
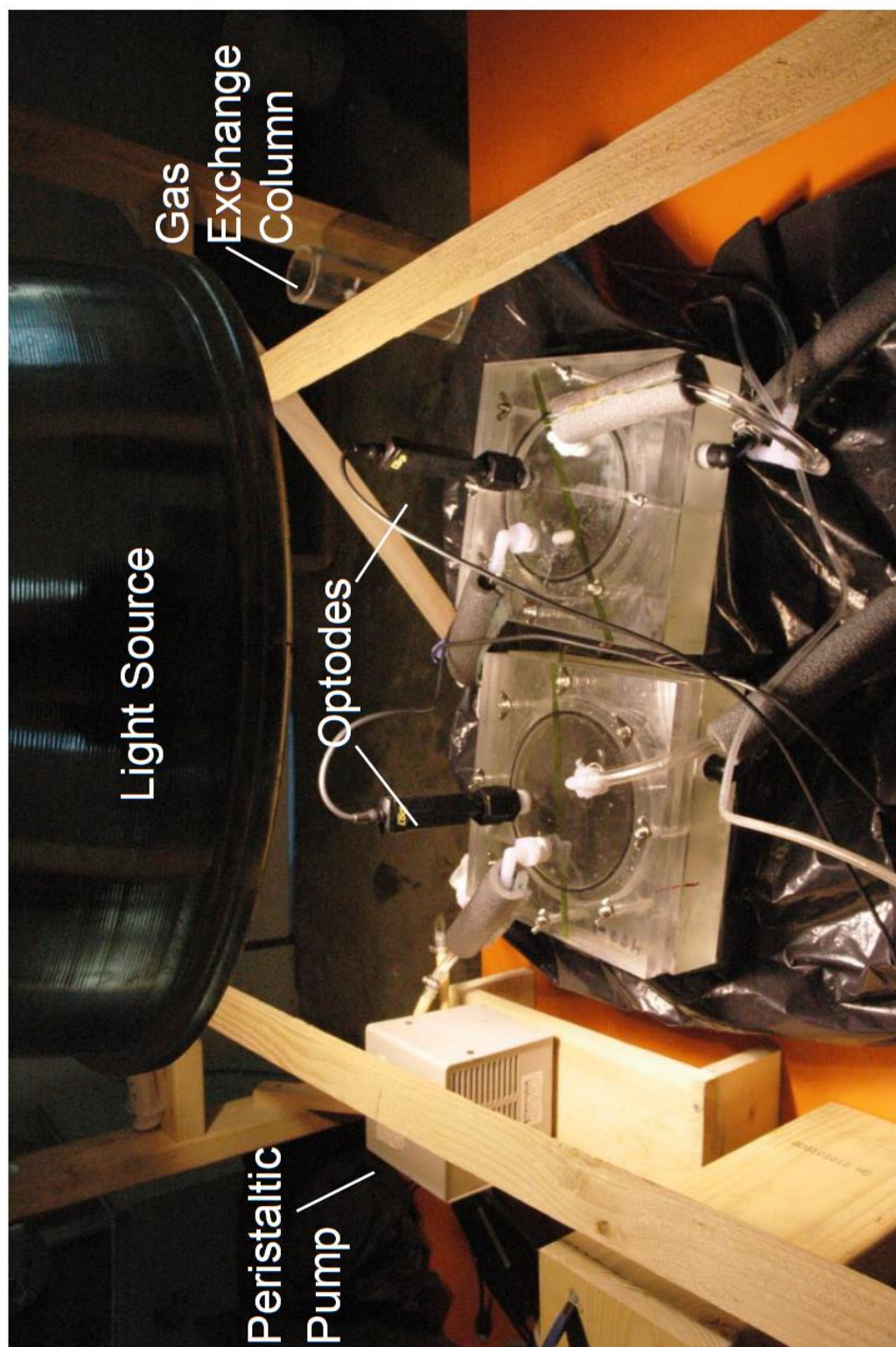
Figure 3. *Photograph of the respirometers with labeled system components.*

through the respirometer jacket around the outside of the borosilicate inner chamber. The volumes of the inner respirometry chambers were 488 mL and 472 mL. Between respirometry "runs" (defined in the following section), the artificial seawater in the respirometry chambers was exchanged by a peristaltic pump. During this exchange process the water could be passed through a countercurrent exchange column through which either air or nitrogen gas was being bubbled so that the pO_2 of the water could be adjusted as needed. Oxygen content of the respirometer water was measured using a Hach HQ34® oxygen optode, which was inserted through a port in the lid of the respirometer. The oxygen optodes measured the levels of dissolved oxygen based on the fluorescence of a special fluor chemical on a membrane exposed to the water. At the beginning of each data collection session and also specifically, the optodes were calibrated for saturating levels of dissolved oxygen in the chamber water by vigorously shaking and measuring the oxygen content of a separate vessel containing similar artificial seawater at seasonally-adjusted temperatures. For all experiments the dissolved pO_2 of the chamber water was kept within 40 to 100% of the air saturation value in order to avoid low and supersaturated oxygen conditions. Water within the chambers was constantly, gently stirred during experiments by a magnetic stir bar on the inner chamber floor.

b. Photosynthesis Experiments using Blades

In each case the experiments on Z. *marina* samples were conducted within 8 hours of their collection from Rosario Bay which until laboratory use were maintained in seawater at ambient coastal water temperatures. To prepare the respirometers for experiments, the respirometry chambers and their tubing were flushed with 5% hydrogen peroxide, and the respirometers were physically cleaned by wiping down the inner surfaces to neutralize potential marine biota and to remove accumulated debris. Then two single eelgrass blades were suspended flat and in parallel next to each other, and sealed inside each respirometer with their cut ends left outside of the chamber to avoid potential wound effects on respiration rate. The blades extended the entire width (14.8 cm) of the respirometer and were sealed in place on both ends by a rubber gasket, which also sealed the chamber. Next, the respirometry chambers were filled with Oceanic® artificial seawater that had been adjusted to 29 ppm, the normal salinity of Rosario Bay, by use of a hydrometer. The chamber water was allowed sufficient time to reach ambient coastal water temperatures matching Rosario Bay for each respective season: 10.5°C in summer, 9°C in spring and 8°C in winter. The dissolved oxygen level in the chamber water was adjusted to 80% of its air saturation value and bubbles were purged from the system. For photosynthesis experiments, paired blades in each respirometer (four blades total) were simultaneously exposed to a series of light intensities in the following order: 0, 100, 150, 300, 200, 400 (excluding winter), 500, and 700 (summer and

winter only) photons m^{-2} s^{-1}. This light exposure sequence constituted a "run" for respirometry experiments using eelgrass blades. Zero-light (dark respirometry) experiments lasted overnight and photosynthesis experiments (using illumination) lasted 1-2 hours at each light intensity (roughly totaling 10.5 hours of light exposure experiments each day) which was conducted during daylight hours to mimic into the normal daily light period for Z. *marina in situ*. Using a series of gradually increasing light intensities was chosen rather than a random light level sequence to assure that the blades, especially in the winter, were not damaged by uncharacteristically high light levels. The oxygen optodes continuously measured dissolved oxygen levels in the respirometers and electronically recorded raw data at one minute intervals. Last, wet mass and linear measurements (length and width) of the eelgrass blades were recorded in order to calculate the surface area at the end of each run.

c. Respirometry of whole plant samples

For experiments with whole plant samples (blade + rhizome), a whole plant sample of Z. *marina* was coiled in each respirometer without any portions outside of it, and the respirometer conditions for temperature and salinity were adjusted similarly to the protocol used for blade experiments. Whole plant samples were used for dark respiration studies only, and respirometry experiments were started at 100% of the air saturation value for dissolved

oxygen due to a substantially greater respiration rate of whole plants. These respirometry experiments were conducted at or shortly after sunset for a few hours. The wet mass of the whole plant and its rhizome were weighed separately and the dimensions of all blades were measured in order to calculate the total leaf surface area of each plant.

d. Dry weight measurements of samples

All blades and whole plant samples were stored at -20°C following each respirometry experiment. At the end of each season, the samples were desiccated at 60°C for 24-36 hours and their dry weights were recorded.

Data Analysis:

a. P-I Curve Analysis

A linear regression was fitted to P-I Curve data in each season to describe the relationship between average net photosynthetic rates and increasing light intensity as observed in laboratory photosynthetic experiments using *Zostera marina* blades, except for data for light levels at which net photosynthetic rates became constant (i.e. light saturating kinetics, or P_{max}). For P_{max}, the net photosynthetic rates observed at each light intensity above saturation were averaged, which was defined by the characteristic 'plateau' in asymptotic

kinetics. Linear regression modeling and P_{max} were used to characterize net P-I

Curves produced for wet and dry *Z. marina* blades which served to predict and

describe the gross photosynthesis of whole plants at variable light levels. Also,

the P-I Curve for gross photosynthesis of Z. *marina* blades (both wet and dry

masses) was determined by adding the mean dark respiration rate to each of the

net photosynthesis measurements. Finally, the measured blade surface areas

and total respiratory rates of whole plants (plus the photosynthetic rates per unit

area of leaf blades) were used to calculate the P-I curve for whole plants in each

season. One-way ANOVA statistical analysis was utilized to determine significant

relationships for chosen data.

b. Modeling the seasonal light environment in Rosario Bay

To model the seasonal light environment in Rosario Bay, a "top-down

approach" was used to predict the amount of light reaching Z. *marina* at its

underwater depths throughout the year-long experimental period. Specifically,

this model traced the path of sunlight from the atmosphere through the water

column and incorporated light-limiting factors along its path to characterize light

availability at eelgrass depths. Year-long data for maximum light levels and cloud

cover in the atmosphere at one hour record intervals were obtained from the

Automated Surface Observing System (ASOS) instrument used by the weather

station KNUW on Naval Air Station Whidbey Island (NASWI) for the study period

of June 2013 through July 2014. Each hour of data was then interpolated into half-hour intervals. This data characterized the local atmosphere near Rosario Bay (NASWI is roughly 8.5 km south) allowing a highly representative model to be created for light levels and total cloud cover.

c. Modeling light limitation in the atmosphere

To estimate the effects of clouds on the light path, the percent cloud cover in three altitude ranges based on the ASOS data were considered (0-5500 ft, 5500-20,000 ft, and 20,000+ ft) where "Total Cloud Cover" described the combined effects of clouds from each altitude range. Due to the likelihood of substantial overlap in cloud coverage and non-equal refraction and absorption of light by clouds among the three altitudes, a hierarchical protocol was developed to preferentially decrease light levels in the atmosphere based on the percent cloud coverage at each altitude range as reported by ASOS (Appendix 1). The lowest and middle-altitude cloud covers were counted first since low clouds such as these tend to be more opaque and strongly block light compared to the thin ice-crystals observed at the highest altitudes (20,000+ ft) which mainly scatter light. More specifically, the lowest and middle cloud cover ranges were compared to determine which range showed the highest level of cloud cover (this range became the starting point). The percent cloud cover of the other lower-altitude range was then multiplied by the proportion of sky not covered by the first cloud

cover range and added to the first cloud cover proportion. For the highest cloud altitude range, the proportion of higher cloud cover was likewise multiplied by the proportion of sky left open by both lower atmosphere cloud cover ranges and added to their combined cloud cover proportion. Total cloud cover in the model ranged from 0 to 1. Therefore, the amount of light reaching the water surface was calculated based on the amount of light measured in situ reaching the bay water surface at the particular sun angle above the horizon for that time of day on a clear day, minus a factor which multiplied the calculated cloud cover (described above) times the amount of reduction in light measured on days with total cloud cover. Also, derived formulas were used to determine the amount of light reaching the water surface for clear days and for cloudy/foggy days with increasing sun angle above the horizon. Fully cloudy days thus had a calculated light equal to full cloud cover, while partly cloudy days had values intermediate between zero and full cloud cover. Foggy days were treated in a similar manner. Sun angle above the horizon and its azimuth position were calculated by the formulas in Blanco-Murel *et al.* (2001). Times of day in which the sun was behind the treeline which is on the eastern and southern horizons from Rosario Bay had the calculated amount of sunlight reaching the water reduced by half.

d. Modeling from surface irradiance to light availability at eelgrass depths

At the water surface, the model fully relied on physical measurements taken periodically in Rosario Bay on representative days and conditions for each season. Percent light transmission through the water surface was plotted against the angle of the sun above the horizon for different weather conditions, and a best-fit linear regression produced the formulas used in the model to estimate the actual amount of light passing through the water surface.

Below the water surface, the light environment was modeled as a function of turbidity and light attenuation in the water column across seasons as well as a dynamic function of depth changes due to tidal fluctuation. First, the effects of season and water surface roughness on light attenuation in the water column were analyzed: a stratification or "layering" effect in the water column for all seasons was confirmed in which light attenuation tended to generally increase with greater depth, and this finding led to an incorporation of seasonally averaged attenuation coefficients segregated by depth range into the model. In addition, a separate tidal model was created and used to estimate depth changes above the Z. *marina* population in Rosario Bay during the study period. This model was based on NOAA tidal data from a nearby standard site in Port Townsend, WA (Station ID: 9444900) in order to accurately quantify the water column height above Z. *marina* in Rosario Bay (Appendix 2).

RESULTS

Photosynthesis-Irradiance (P-I) Curves and Seasonal Morphology of Z. *marina*:

a. The P-I Curve for Z. *marina* blades

As expected, net photosynthesis (P_n) increased rapidly with increasing light levels for *Zostera marina* blades, then leveled off at a maximum rate (Figure 4, Table 2, Appendix 4).   Similar P-I relationships were observed in net productivity, which was based on dry mass of blades (Figure 5, Appendix 5). The formula used to calculate net productivity values (units described as mg C/g dry wt x h) for *Z. marina* blades was based on the P-I relationship determined for wet blades: 1 µmol O_2/ g wet wt x h (X) g wet wt/g dry wt (X) 1 µmol C/1 µmol O_2 (X) 12.011 x 10^{-6} g/ 1 µmol C (X) 1000 mg/1 g = mg C/g dry wt x h (Cowles, personal communication, 2015). Conversion ratios used in this formula were based on average measurements of *Z. marina* blades and whole plant leaves and are shown in Table 3.

b. Seasonal changes in morphology and condition

Table 4 shows morphological measurements of the eelgrass by season. The blades in winter were thin, fragile, and dark green with little epiphyte growth. The more numerous blades in spring were bright green and robust. In the summer the blades were heavily overgrown with epiphytes and were more
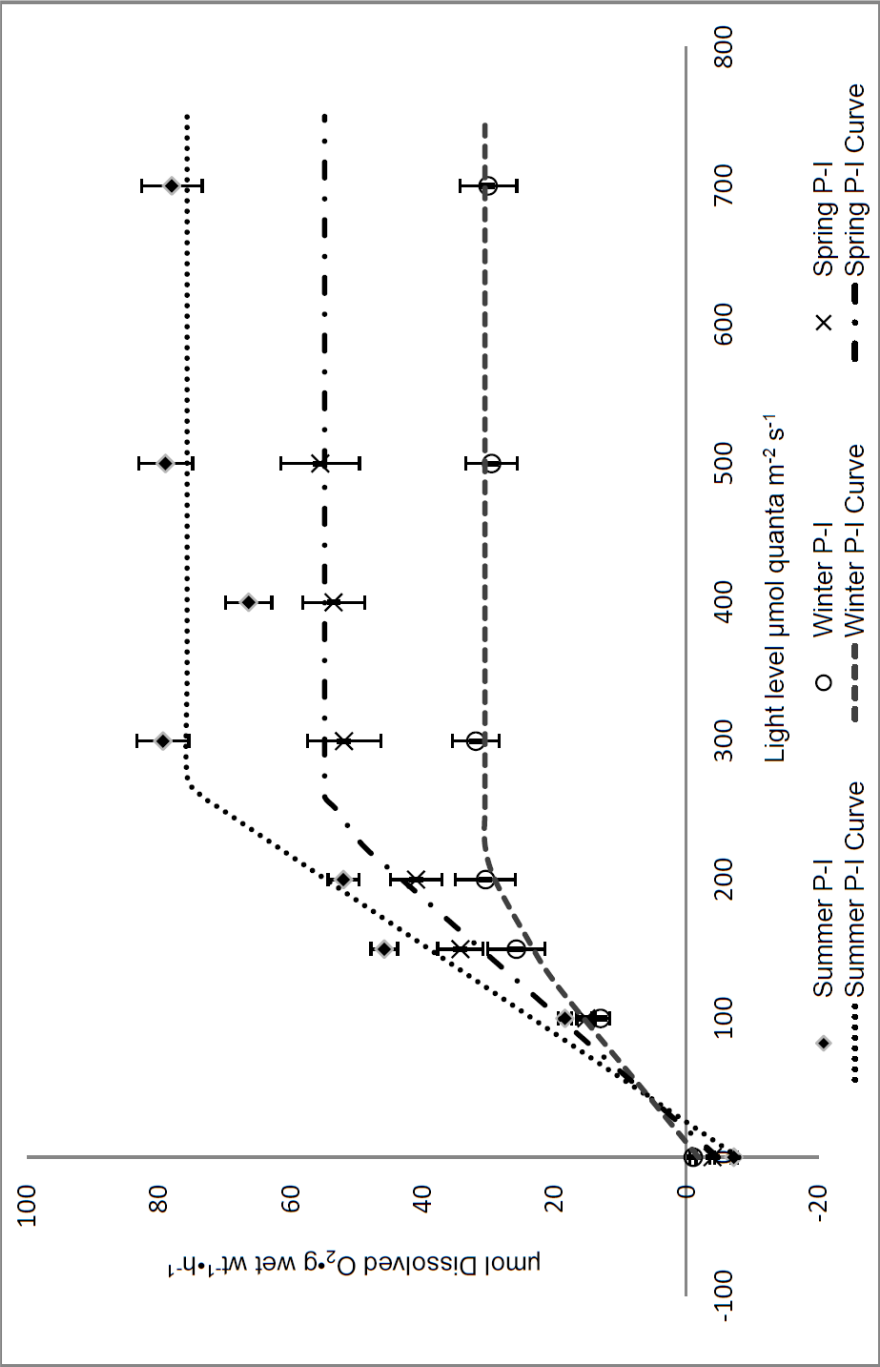
Figure 4. *P-I Curves for Z. marina blades based on wet mass. Zostera marina* blades in Rosario Bay can photosynthesize substantially even at low light levels. Error bars show standard error based on values shown in Table 2.

Table 2. *Linear regression analysis of P-I Curves for gross photosynthesis of Z. marina blades*. Metabolic Rate is based on N= 27 for Summer, N= 14 for Winter, and N= 12 for Spring. SE (Standard Error) for Gross Photosynthetic Rate at Saturation reflected averaged values in the P-I Curve for higher light levels excluded from slope calculation (N= 4, 4 and 3, respectively). Sample count (N) is shown in parentheses and SE = Standard Error.

Season	Summer	SE	Winter	SE	Spring	SE
Metabolic Rate (μmol O_2 g wet wt^{-1} h^{-1})	7.25	0.48	1.06	0.45	4.08	0.37
Gross Photosynthetic Rate at Saturation (μmol O_2 g wet wt^{-1} h^{-1})	82.98	2.86	31.56	0.52	58.97	0.85
Light Level at Compensation (μmol quanta m^{-2} h^{-1})	23.48	-	6.33	-	17.36	-
Light Level at Saturation (μmol quanta m^{-2} h^{-1})	268.77	-	189.05	-	250.95	-
Photosynthetic efficiency (α), N (for slope)	0.309 (4)	-	0.167 (3)	-	0.235 (4)	-
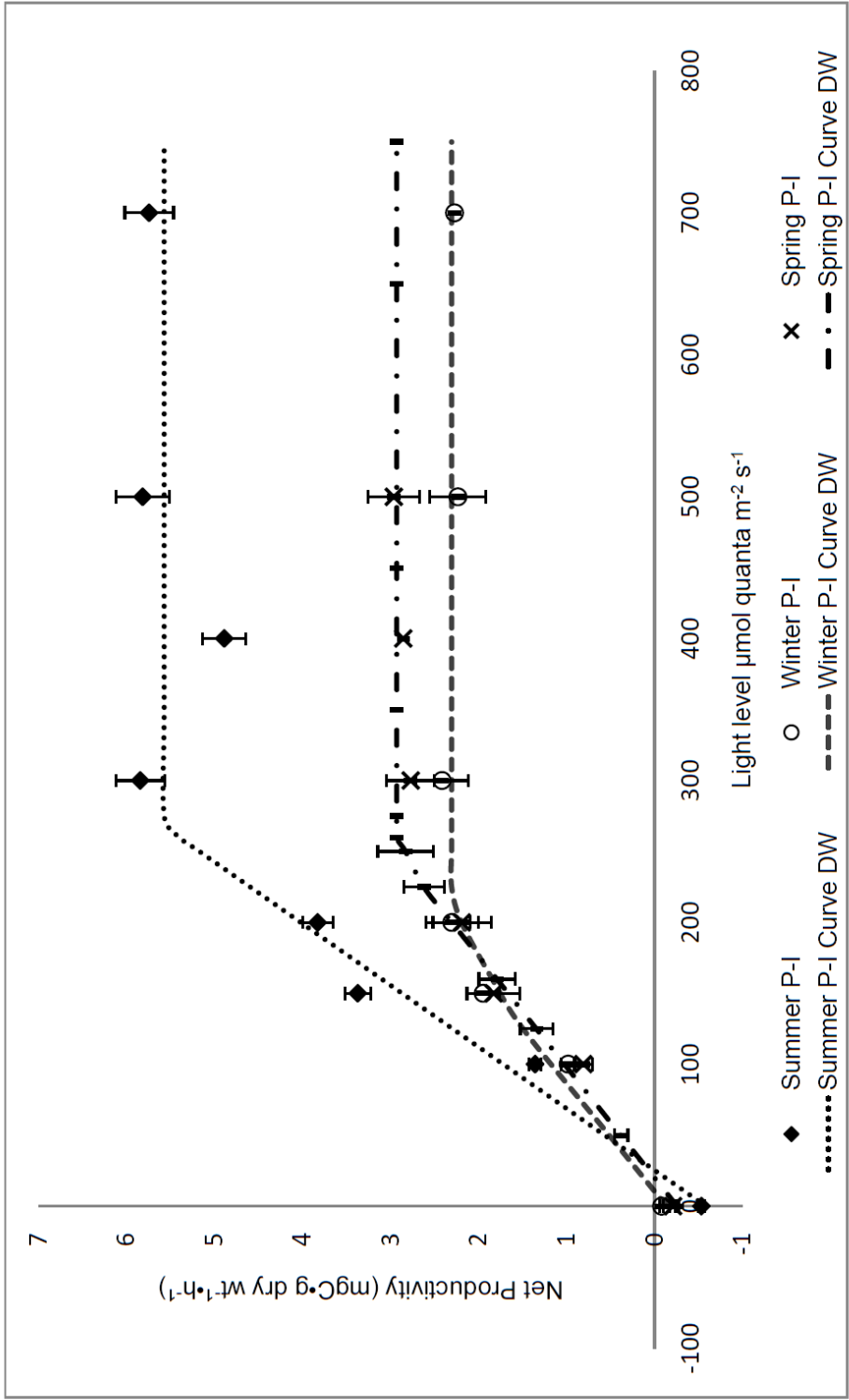
Figure 5. *P-I Curves for Net Productivity of Z. marina blades based on dry mass.* Error bars show standard error based on values in Table 3.

Table 3. *Conversion ratios between wet and dry masses for blades and whole plant leaves*. Values are the ratio by which the average wet mass exceeded the average dry mass for *Z. marina* blades and whole plant leaves in each season.

Conversion ratios (wet-to-dry mass)	Summer	Winter	Spring
Blades	6.13	6.29	4.44
Whole Plant Leaves	9.50	7.81	5.61

Table 4. *Seasonal Comparisons of Z. marina morphology and mass.* Significant P-values are shown in the last column and SE = Standard Error.

Season	Summer	SE	Winter	SE	Spring	SE	P
Averages for Respirometry Experiments							
Surface Area of blades in Respirometer (cm^2)#	23.69	0.372	20.71	0.540	15.81	0.717	<0.01
Total length of blades in respirometer (cm)	28.95	0.025	29.00	<0.01*	29.04	0.064	-
Wet Weight of blades in Respirometer (g)	0.49	0.016	0.44	0.013	0.40	0.020	<0.01
Dry Weight of blades in Respirometer (g)	0.08	0.002	0.07	0.002	0.09	0.006	<0.01
N	28	-	13	-	12	-	
Averages for Whole Plant Samples							
Blade Length (cm)	52.09	2.92	37.87	3.94	25.70	0.95	<0.01
Blade Width (cm)	0.67	0.02	0.58	0.02	0.53	0.01	<0.01
Total Surface Area of Leaves per Whole Plant (cm^2)	383.37	29.02	110.65	14.96	93.50	7.26	<0.01
Leaf Count per Whole Plant	10.50	0.79	4.80	0.33	6.75	0.46	<0.01
Total Wet Weight (g)	22.99	1.59	8.26	0.91	7.66	0.60	<0.01
Wet Weight of Rhizome (g)	6.18	0.55	3.34	0.64	3.45	0.35	<0.01
Wet Weight of Stalk (g)	16.81	1.30	4.92	0.60	4.21	0.31	<0.01
Total Dry Weight (g)	2.51	0.16	1.26	0.18	1.60	0.22	<0.01
Dry Weight of Rhizome (g)	0.74**	0.07	0.64	0.14	0.85	0.15	0.6
Dry Weight of Stalk (g)	1.77	0.11	0.63	0.07	0.75	0.08	<0.01
N	16	-	5	-	12	-	

#Surface area of leaf blades is reported for only one side of the leaf.

*Standard blade length (14.5 cm) assumed for winter respirometry experiments

** Likely a slightly low value for summer due to some burnt rhizome samples sticking to their weighing tin.

fragile. Some blades were a yellowish-green. Further, during the second summer no eelgrass was evident at the shallow site and little at the deepest site. The intermediate depth site (4.0 m), however, had abundant eelgrass. Figures 6 and 7 and Table 5 show the calculated P-I relationship for whole plants.

Modeled Light Availability to Z. *marina* eelgrass at depth:

a. Effects of clouds and fog on light above the water surface

Above the surface of the water, light levels on clear days in Rosario Bay increased logarithmically with increasing angle of the sun above the horizon (Figure 8, upper curve). The light level climbed rapidly even before the sun appeared over the horizon. Overcast and foggy conditions exhibited a decreased overall light level in a pattern similar to one another, so the same curve was used to model both types of conditions (Figure 8, lower curve). At low sun angles, the light level increased approximately linearly, then increased more rapidly at roughly 15 degrees above the horizon before it leveled off. The cloudy/foggy relationship had much more scatter since the extent of cloudiness and fog can vary greatly, but the curve represents average light levels during these conditions. Also, on clear days a positive linear relationship was observed between percent light transmission through the water surface and an increasing angle of the sun above the horizon (Figure 9). For overcast and cloudy days, this
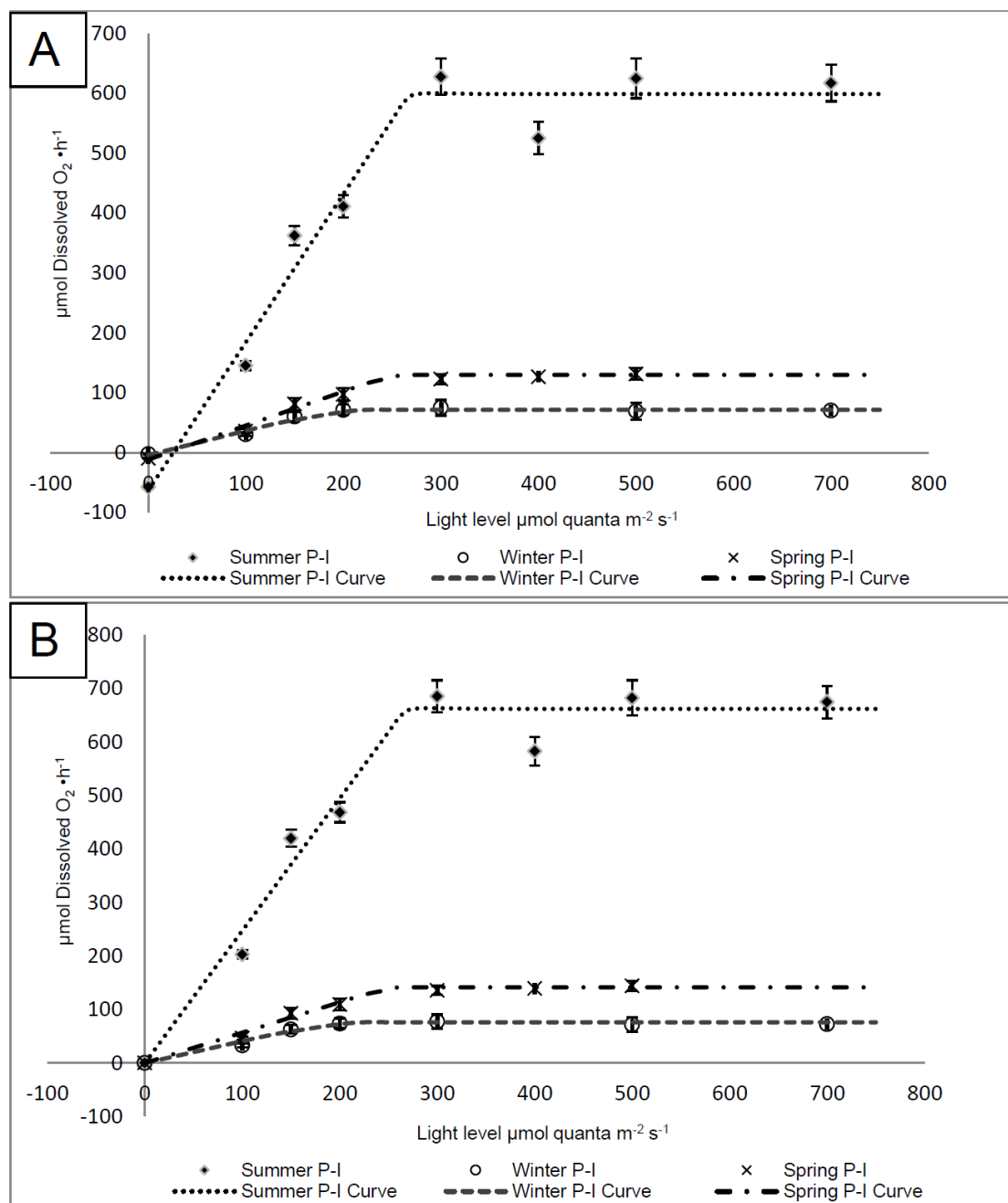
Figure 6. *P-I Curves for Z. marina whole plants based on wet mass.* (A) Net Photosynthesis (B) Gross Photosynthesis.
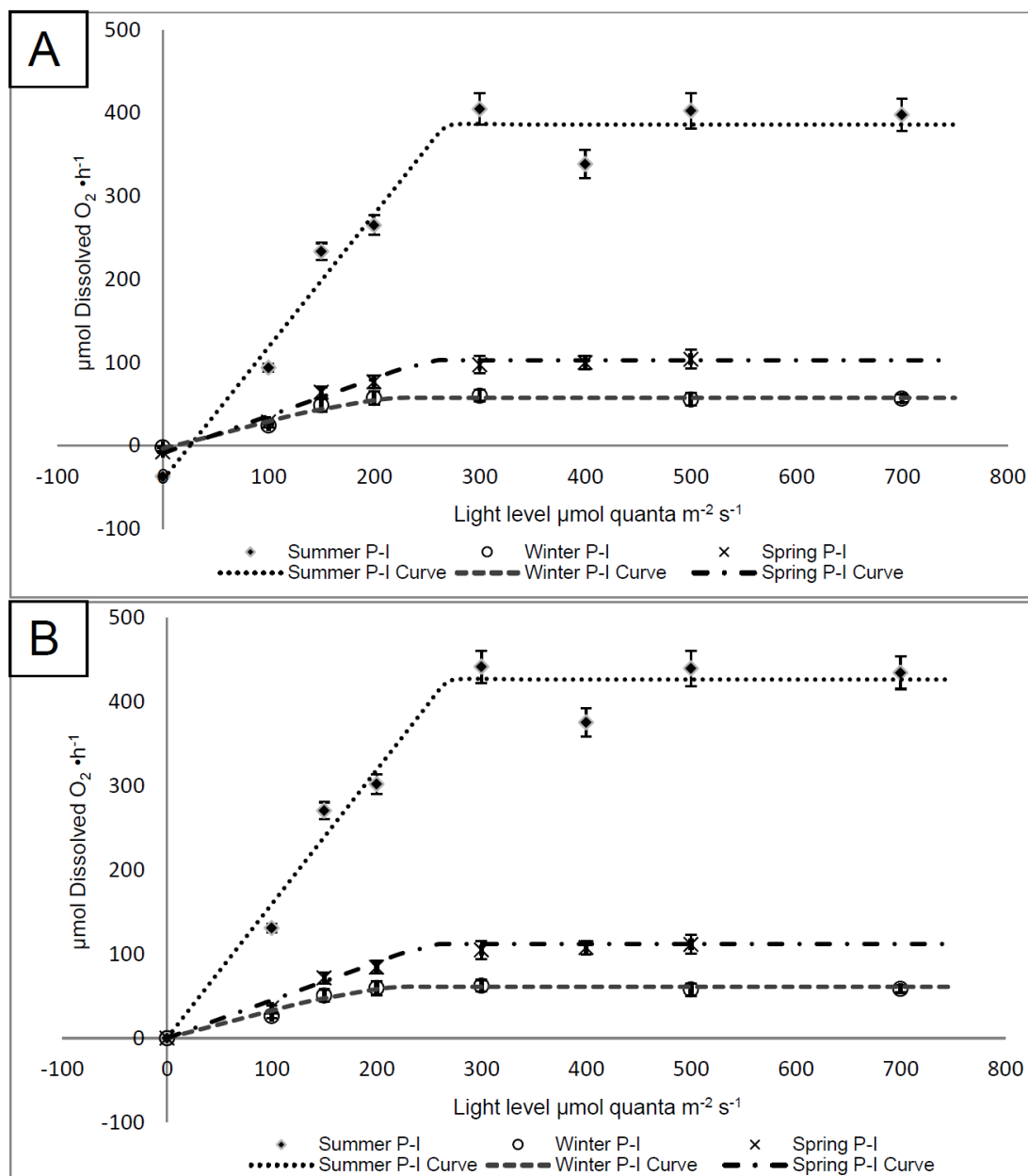
Figure 7. *P-I Curves for <u>Z. marina</u> whole plants based on dry mass*. (A) Net Photosynthesis (B) Gross Photosynthesis.

Table 5. *Whole plant metabolism and gross photosynthesis for Z. marina.*
Sample count (N) is shown in parentheses and SE = Standard Error.

Season	Summer	SE	Winter	SE	Spring	SE
Metabolic Rate (μmol O_2 g wet wt^{-1} h^{-1})	34.64 (16)	1.86	5.90 (5)	0.58	13.14 (12)	0.51
Gross Photosynthetic Rate at Saturation (μmol O_2 g wet wt^{-1} h^{-1})	655.92	-	73.93	-	136.39	-
Light Level at Compensation (μmol quanta m^{-2} h^{-1})	14.19	-	15.08	-	24.65	-
Light Level at Saturation (μmol quanta m^{-2} h^{-1})	268.77	-	189.05	-	250.95	-
Photosynthetic efficiency (α), N (for slope)	2.44 (4)	-	0.39 (3)	-	0.54 (4)	-

Figure 8. *Insolation versus sun angle at Rosario Bay, WA for sunny and cloudy/foggy days.*

Figure 9. *Percent transmission through the water surface versus sun angle above the horizon.*

linear relationship was not observed and instead half of the available surface

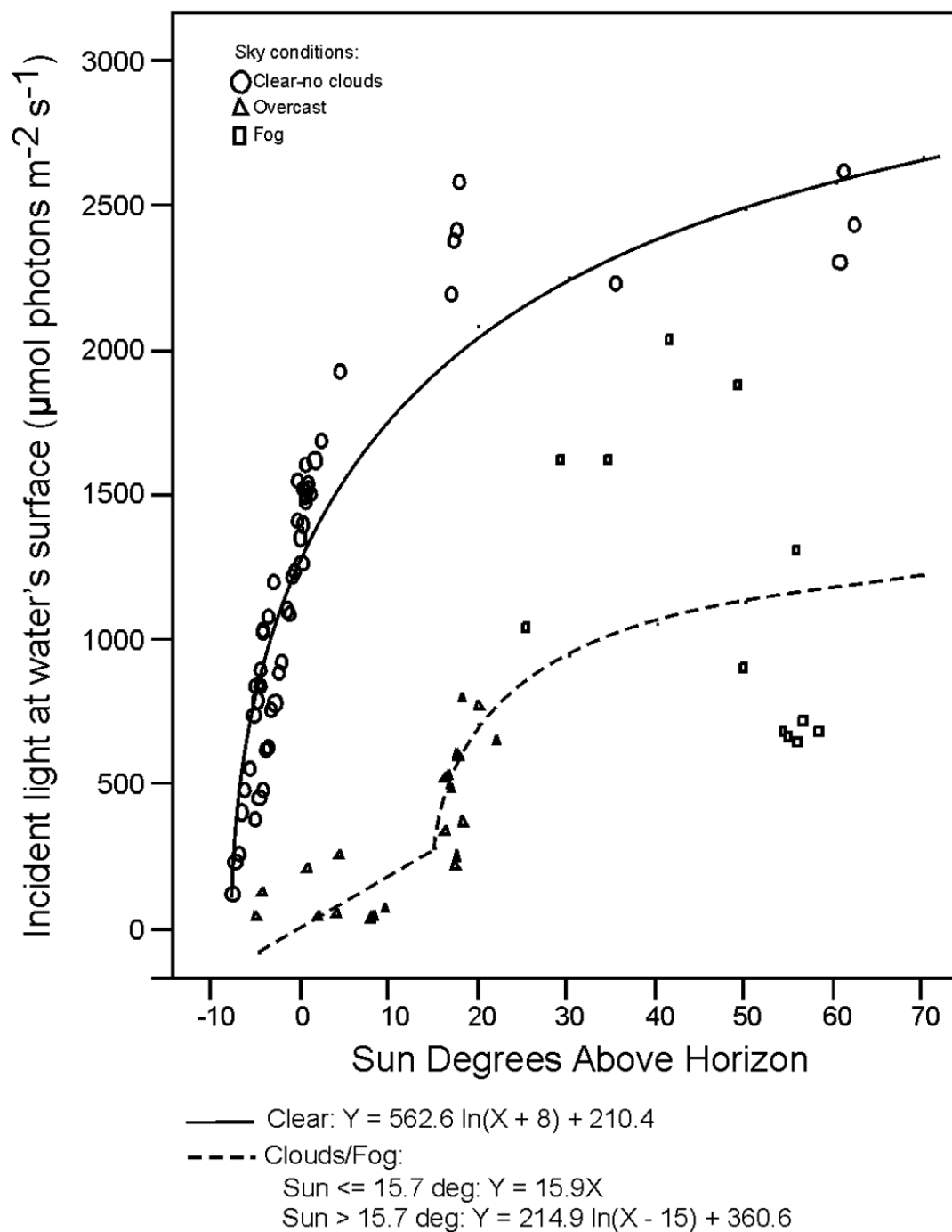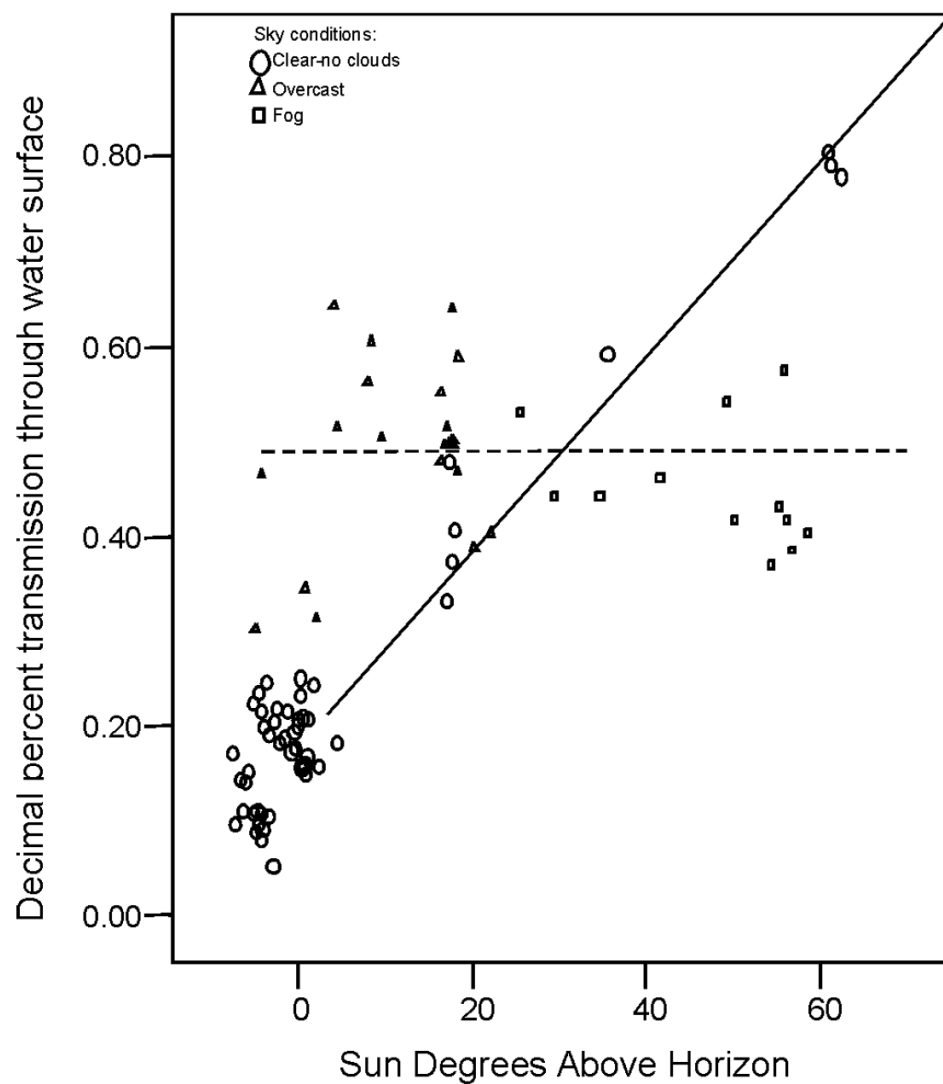irradiance transmitted through the water surface independently of sun angle.

b. The effects of water surface roughness and light attenuation in the water

column

Table 6 shows light attenuation over the three depth ranges for each

season. The surface layer typically had lower light attenuation than the middle or

bottom, while attenuation near the bottom tended to be greatest. Most conditions

of surface roughness had similar levels of light attenuation as well (Table 7).

c. Light levels available traced from the atmosphere to the eelgrass depths

Figure 10a-c shows some representative week-long patterns of insolation

for weeks chosen from each season at three distinct stages of the light path:

calculated light level that would exist on a perfectly clear day, actual surface

irradiance given the true weather and sun angle conditions, and light availability

at the shallowest and deepest depth limits of the *Zostera marina* bed in Rosario

Bay (3.0m and 4.6m below MLLW). The calculated light levels for clear days

were equivalent during the weeks chosen for summer and spring, whereas this

light level was reduced by about 1/5 during the week in winter (Figure 10).

Greater seasonal variability was observed in light levels at the surface of the

Table 6. *The effect of water surface roughness on underwater attenuation coefficients at three depth ranges in Rosario Bay.* Light attenuation coefficients were represented by one meter increments in each depth range. Sample count (N) exceptions: N= 58 for light surf Bottom and N= 8 for medium surf Bottom.

Water surface condition	N	Depth Range					
		Top	SE	Mid	SE	Bottom	SE
Light surf	67	0.049	0.003	0.195	0.006	0.333	0.040
Medium surf	9	0.059	0.008	0.160	0.017	0.323	0.067
Heavy surf	4	0.061	0.007	0.322	0.011	0.434	0.031

Table 7. *Seasonal effects on mean attenuation coefficients at three depth ranges in Rosario Bay*. Light attenuation coefficients were represented by one meter increments in each depth range. Sample count (N) exceptions: N= 39 for Summer Bottom and N= 10 for Winter Bottom.

Season	N	Depth Range					
		Top	SE	Mid	SE	Bottom	SE
Summer	44	0.046	0.003	0.202	0.007	0.396	0.046
Spring	14	0.050	0.009	0.173	0.010	0.176	0.023
Winter	9	0.057	0.004	0.186	0.015	0.229	0.026
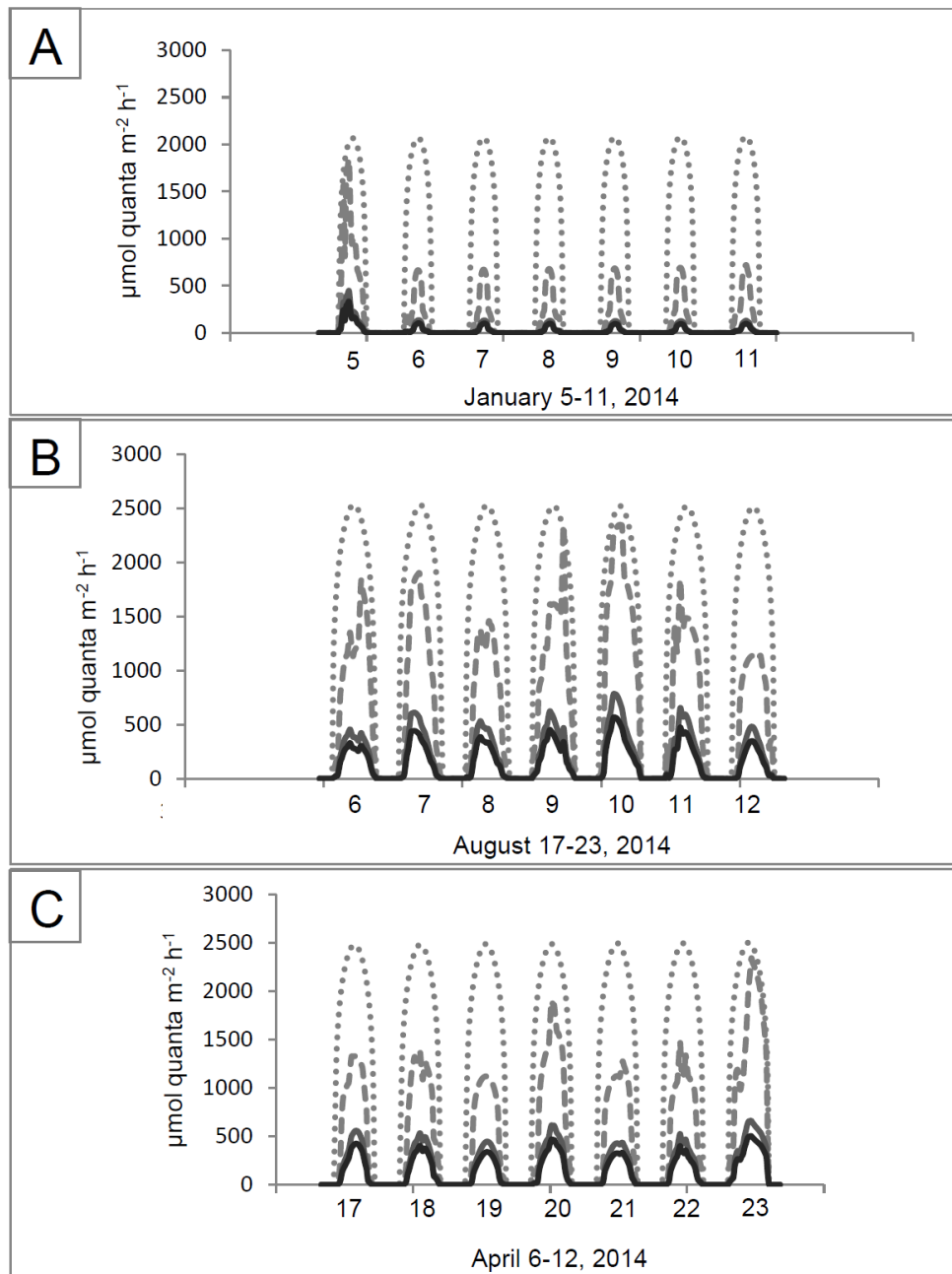
Figure 10. *Representative week-long patterns of insolation for (A) Winter: January 5-11, 2014, (B) Spring: April 6-12, 2014, and (C) Summer: August 17-23, 2014.* Full Sun (dotted line)= the light level that would be present at the water's surface if the day were cloudless and no shadows were present. Surface light (dashed line) = the light level present at the water's surface under the actual conditions of the day. Light at Eelgrass 3.0 and 4.6m (gray and black lines, respectively)= the calculated light level present at 3.0 and 4.6 m depths MLLW, which were the minimum and maximum depths the eelgrass was found at.

water and at eelgrass depths than in the atmosphere, with spring and summer showing much higher light levels than winter at these stages in the light path. In fact, light levels at eelgrass depths approximated 500 µmol quanta m^{-2} h^{-1} for at least part of the day in summer as well as in spring, only less frequently. The outer depth limits (3.0 and 4.6m) showed only moderate differences from one another, with the 4.6 m depth always darker, as expected.

Using annual data from ASOS, the average daily maximum light levels by month estimated the highest level of light available to eelgrass in Rosario Bay for at least part of the day (Figure 11). As expected, a strong decrease in maximum light levels was observed during the winter season, yet the daily average maximum light levels still exceeded photosynthetic compensation requirements of Z. *marina* whole plants in all seasons (see Table 5). The outer depth limits (3.0m and 4.6m) had only modest differences in maximum light level at any season. However, there appeared to be more marked differences between these depth limits in terms of average daily total quanta (Figure 12). The most evident difference in this metric occurred during the summer which showed a gap of roughly 1500 µmol quanta m^{-2} h^{-1} between the two depths, whereas the least difference was found in winter (about 200 µmol quanta m^{-2} h^{-1}).
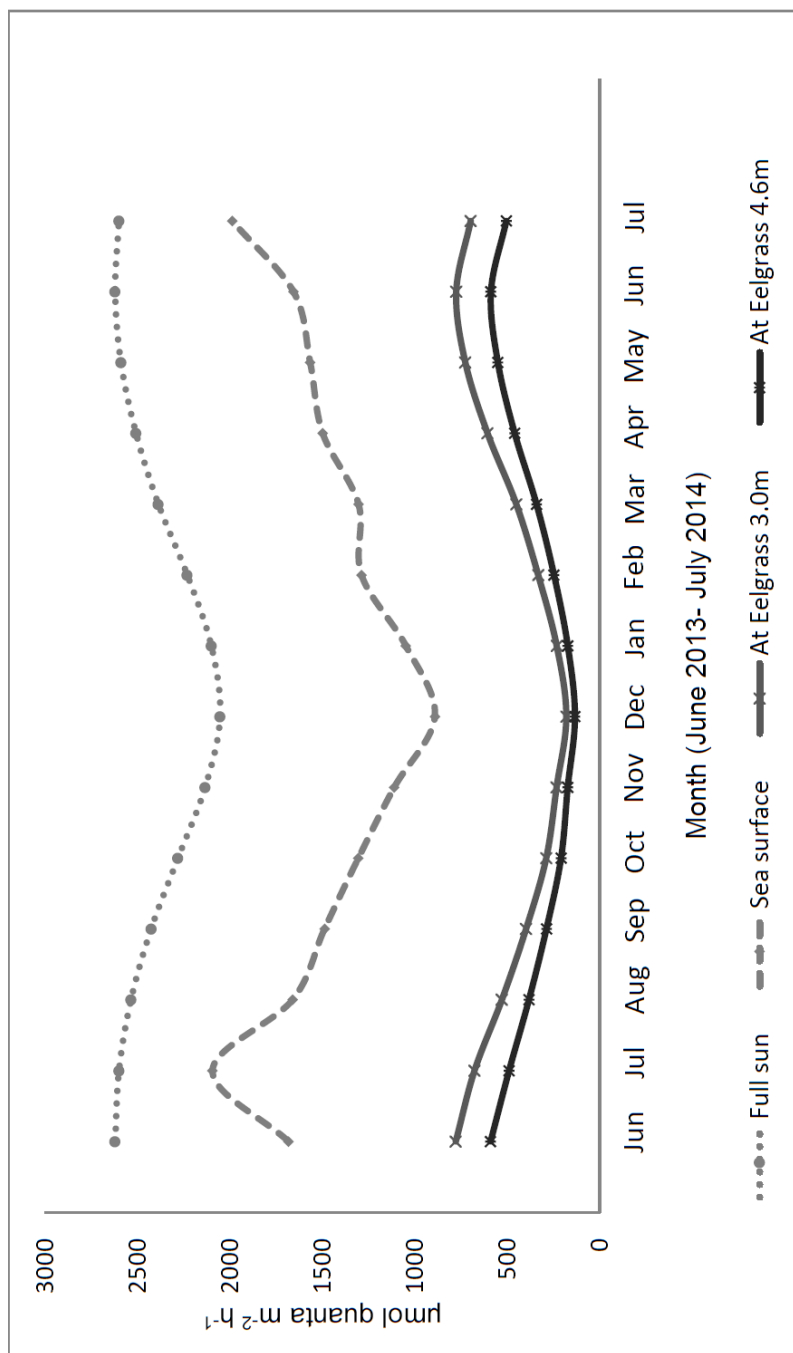
Figure 11. *Modeled daily average maximum light levels, by month.* Full Sun = the light level that would be present at the water's surface if the day were cloudless and no shadows were present. Sea Surface = the light level present at the water's surface under the actual conditions of the day. Light at Eelgrass 3.0 and 4.6m = the calculated light level present at 3.0 and 4.6 m depths MLLW, which were the minimum and maximum depths the eelgrass was found at.
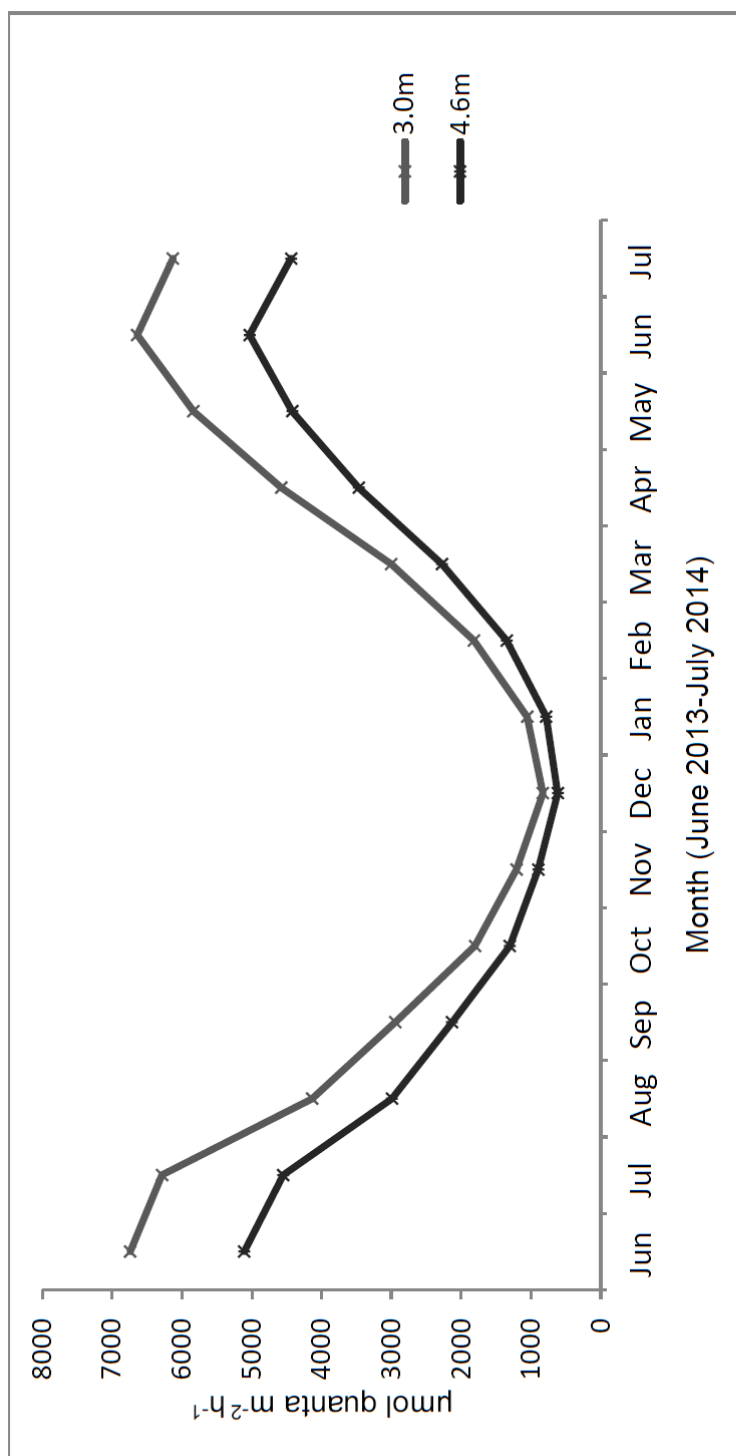
Figure 12. *Average Daily Total Quanta at 3.0m and 4.6m Eelgrass Depths*. The Total Quanta per day are shown at 3.0m and 4.6m depths MLLW, which were the minimum and maximum depths the eelgrass was found at.

Combining *Z. marina* light requirements and modeled light availability at eelgrass depth:

a. Annual estimates for hours of sunlight by month

Figure 13 shows the estimated average daily number of hours that sunlight was present at the eelgrass depths over the study period. The average daily hours of sunlight shown in this figure differed more strongly by season than did the average daily maximum light levels (see Figure 11). The average daily number of hours that light levels met or exceeded both photosynthetic compensation (I_c)and saturation (I_{sat}) requirements of individual *Zostera marina* plants at the two outer depth limits (3.0m and 4.6m) of Z. *marina* in Rosario Bay were plotted: at the compensating light level for whole plants, the two depths showed a tightly-coupled pattern for the daily number of hours that light levels met or exceeded the plant's compensation point, and Z. *marina* received 15-16 hours per day of these light levels in the summer. By contrast, plants were exposed to compensation levels of light for only 6-8 hours per day in the winter. Z. *marina* received 9-11 hours per day of light levels at or above their saturation point in the summer, whereas in the winter they received only 1-2 hours per day of these light levels.
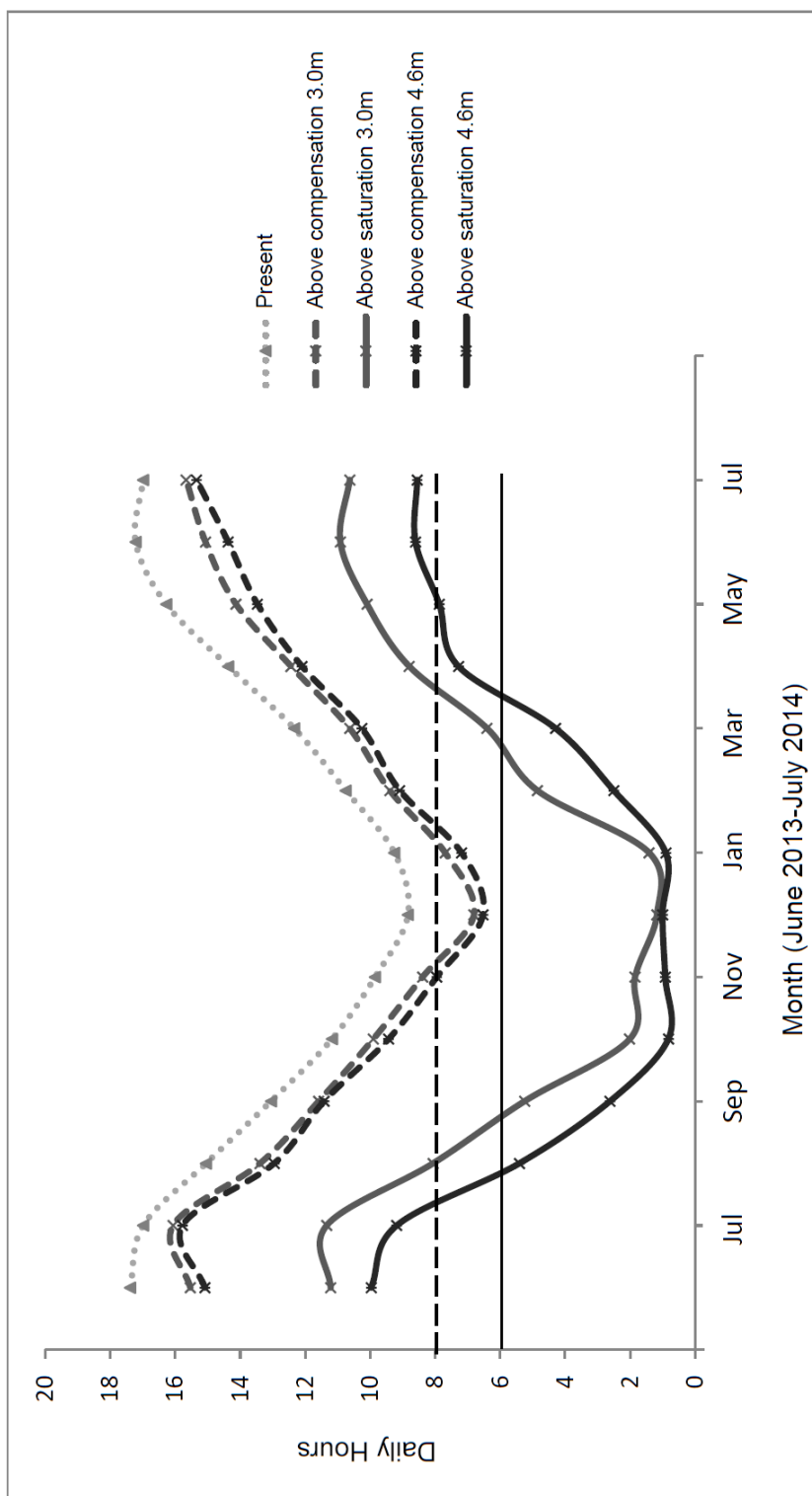
Figure 13. *Average Daily Hours of Sunlight, by Month.* "Present" Daily Hours defines any light level > 0 at the 3.0m depth. The horizontal reference bars at 6 (solid line) and 8 hours (dotted line) show the minimum number of hours of light saturation required for eelgrass long-term survival according to Dennison and Alberte (1985).

b. Whole plant P-R results

The average daily Photosynthesis – Respiration (P-R) calculated for Z. *marina* plants was always positive during the study period throughout the depth range of the eelgrass (Figure 14). Not surprisingly, daily P exceeded daily R by the smallest amount in winter months, while gross photosynthesis in summer months greatly exceeded respiration. Interestingly, the net photosynthesis in spring only moderately exceeded that of winter, although not nearly to the extent characterized by summer. These findings were confirmed utilizing a P/R Ratio (Figure 15) which plotted the ratio between average daily values for gross photosynthesis and respiration of whole plants over the study period. The P/R ratio averaged approximately two in winter and ten in summer.
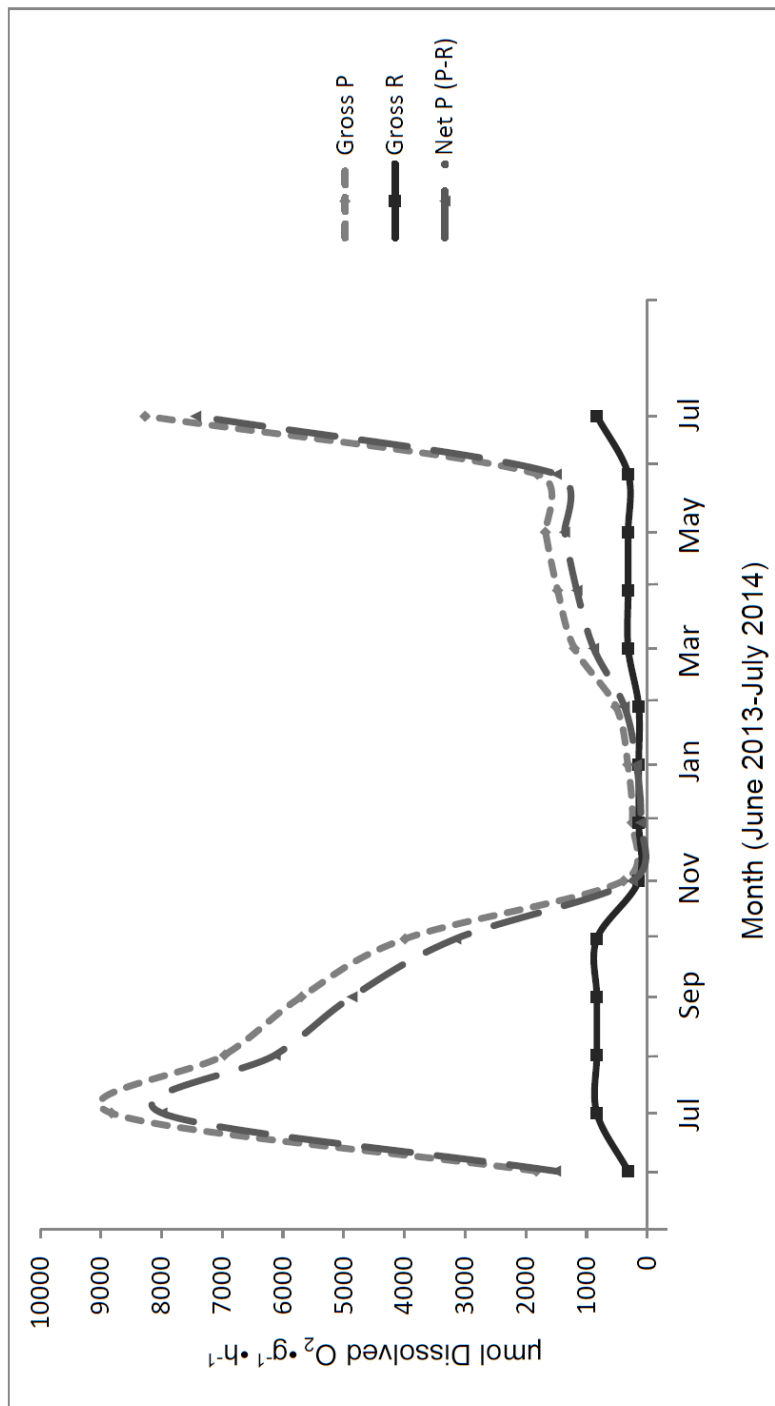
Figure 14. *Average monthly P and R results modeled for Z. marina whole plants at 3.0m depth. P = Total* daily photosynthesis. R = total daily respiration. Total photosynthesis exceeds total respiration at all P – R values >0.

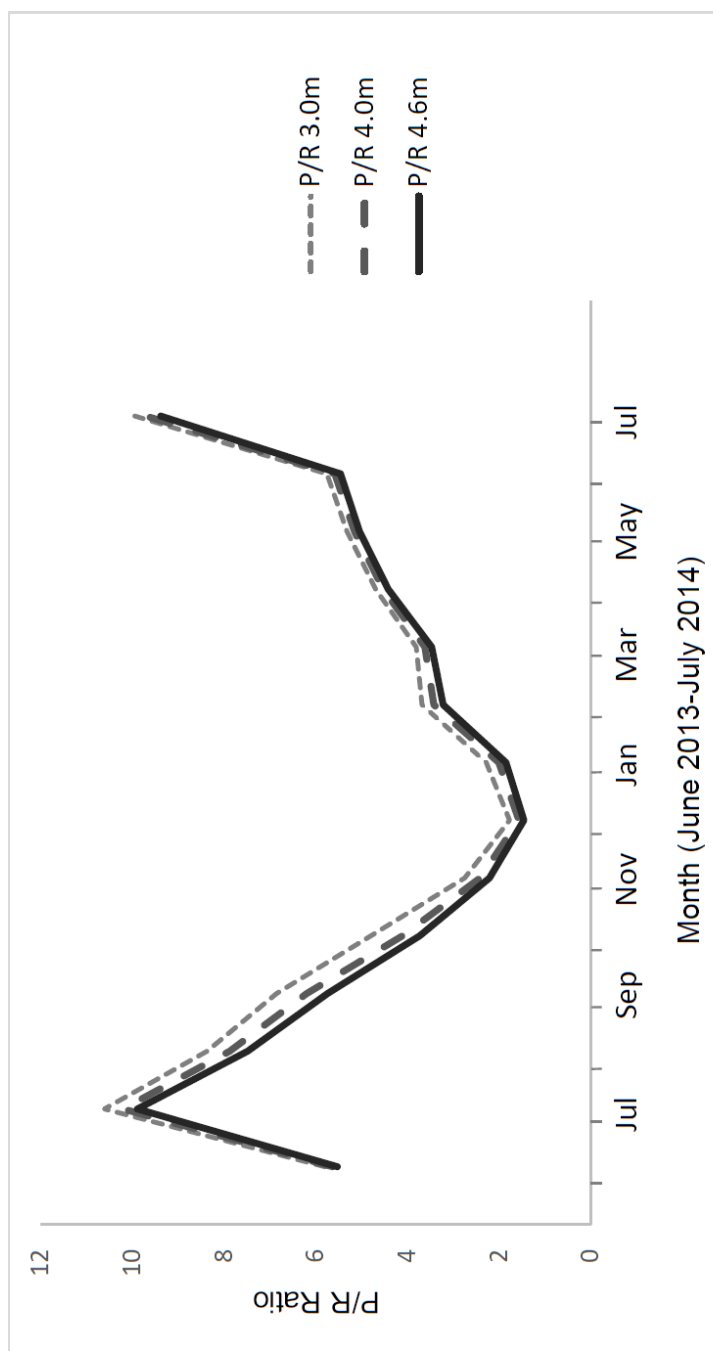Figure 15. *Average monthly P/R ratio for Z. marina whole plants compared at 3.0, 4.0 and 4.6m depths.* P = total daily photosynthesis. R = total daily respiration. Photosynthesis exceeds respiration at all P/R values >1. 3.0, 4.0, and 4.6m represent the rates at the shallowest, middle, and deepest extents of the eelgrass bed below MLLW.

DISCUSSION

Addressing the hypothesis:

In this study, the population of *Zostera marina* eelgrass in Rosario Bay, WA received light levels that exceeded their compensation requirements for at least part of each day and their P-I relationship was always >0, suggesting that they could potentially maintain a positive growth potential throughout the year.

Since Z. *marina* is a long-term established population in Rosario Bay, it was predicted they would be receiving sufficient light to meet their growth requirements throughout the year, however, it was also expected the average light availability would fluctuate and in certain months would fall below the compensation threshold, especially in winter months. Winters in the Pacific Northwest are generally accompanied by shorter day length and a greater frequency of cloud cover than in other seasons, which imposes greater light restrictions on Z. *marina*. It is known that Z. *marina* can change its morphological and physiological characteristics in response to changing environmental light levels (Hemminga & Duarte 2000); however, it was unclear whether the population in Rosario Bay would receive sufficient light levels in the winter months to compensate for their metabolic requirements. The present study demonstrated that Z. *marina* had a potentially positive P/R ratio throughout winter and that this ratio approached a value of two even in December when light levels

were at their lowest during the study period (Figure 15, Figure 12). This finding

was supported by the average daily number of hours that Z. *marina* was exposed

to compensating and saturating light levels: in the winter months they received

more than six hours of compensating light levels with some of that time at

saturating levels (Figure 13). Dennison and Alberte (1985) stated that the

minimum light levels for growth and survival are 6-8 hours of saturation light

levels, at least in the summer. The horizontal bars shown in Figure 13 indicated

that Z. *marina* did not receive at least 6 hours of saturating light levels from

August through April of the study period, even though they reached this threshold

in spring and early summer months. In terms of growth and survival, this study

does not support the claim of Dennison and Alberte since this population has

persisted in Rosario Bay despite receiving less exposure to saturating light

intensities in the late summer and winter months than their model would require

for positive growth. Instead, Z. *marina* in Rosario Bay appears well-adapted to

survive fluctuation in its number of hours at or above light compensation and

saturation at depth. In sum, the initial prediction of this study was supported and

Z. *marina* received sufficient annual light levels in Rosario Bay to at least meet its

compensational requirements based on experimental P-I relationships.

Furthermore, this study also confirmed that a net positive growth potential was

maintained even during the winter months.

Zostera marina plants at the shallow and deep depth limits of the population in

Rosario Bay (3.0 and 4.6m) demonstrated close relationships in terms of P/R

across the study period. Figure 15 showed that the P/R ratio for the shallow sampling site (3.0m) was only slightly higher than that of the deepest site in all months. Also, the daily average maximum light levels in Rosario Bay at the outer depth limits of *Z. marina* apparently differed only to a small degree (Figure 11). Somewhat greater variability in light exposure between these depths was observed for the daily number of hours at saturating light levels, although the daily number of hours at light compensation differed little (Figure 13). This finding suggests that Z. *marina* in Rosario Bay should be able to meet net growth requirements throughout the year within a seasonally changing light environment.

Comparing studies of Z. *marina* in the Pacific Northwest (PNW):

The P-I curves produced by Thom *et al.* (2008) reflected large-scale efforts to characterize Z. *marina* eelgrass in the Pacific Northwest by sampling from several sites along the regional coast and utilizing large numbers of samples to study P-I relationships in the PNW. Surprisingly, their P-I curves did not show strong uniformity, and they also reported a photo-inhibition effect when exposing eelgrass blades collected in the winter to saturating light intensities. These results differed from the findings of another study in Chesapeake Bay (MA, USA) which demonstrated a lack of photo-inhibition by Z. *marina* blades (Zimmerman *et al.* 1991). In the present study, the P-I curves for Z. *marina* blades showed a greater level of uniformity than those produced by Thom *et al.*, and also showed

no photo-inhibition from 500 to 700 μmol quanta m^{-2} h^{-1} in the study population

(Figure 4). The photo-inhibition observed by Thom *et al.* may have reflected

potential tissue damage to the surfaces of eelgrass blades caused by saturating

light intensities used in their experiments that greatly exceeded the levels

typically reaching eelgrass depths during that season (i.e. light intensities

exceeding 700 μmol quanta m^{-2} h^{-1}). This study employed methods that differed

from Thom *et al.* as follows: first, they exposed their eelgrass samples to ambient

sunlight and used the average of several varying periodic light measurements

taken during the experiment to characterize experimental light levels. The halide

lamp used for a light source in this study achieved greater precision in producing

specific, repeatable light intensities, which no doubt resulted in a more uniform P-

I relationship. On the other hand, the spectrum of light emanating from a halide

bulb differs from the solar spectrum. The solar spectrum has comparatively high

levels of spectral irradiance across the visible light spectrum with its highest

irradiance at roughly 500 nm, whereas a halide bulb peaks at 590 nm. The halide

lamp likely does not maximize potential light absorption by chlorophyll *a* and *b*

(which have their highest absorbance in the ranges of 400-500 nm and 625-

700nm, respectively), and therefore it is reasonable to assume that the P-I curve

in this study likely underestimated the photosynthetic potential of Z. *marina* under

natural light at the same irradiance levels chosen for experiments, if only to a

small degree. Second, continuous dissolved oxygen measurements were taken

during the experiments instead of only measuring initial and final dissolved

oxygen levels. Although this difference is expected to be minor, continuous

measurements permitted tracking of real-time changes in oxygen level and monitoring for unforeseen errors that could have strongly altered the data. Last, a spherical sensor was utilized for respirometry calibrations as well as for physical measurements in Rosario Bay which more accurately measured the light field eelgrass are exposed to than the cosine sensor was used by Thom *et al.* In particular, the spherical sensor records light coming from all directions, such as is used by photosynthesizing plants, rather than only downwelling light as measured by the cosine sensor.

Marked differences in seasonal trends were observed in the P-I Curves produced by this study compared to that of Thom *et al.* for *Z. marina* blades. Thom *et al.* (2008) found that the P-I Curve for blades in the winter demonstrated a greater photosynthetic efficiency (α) than that observed in summer and spring for light levels below 500 µmol quanta $m^{-2} h^{-1}$ (Appendix 3). In contrast, in this study the P-I curve for blades in the winter appeared to have lower photosynthetic efficiency than in the other seasons (Figures 4 and 5). The differences between these studies may be attributed to different experimental methods used (described in the following section) as well as population-specific characteristics of *Z. marina* in Rosario Bay: for this study population, the blades of winter plants tended to have a greater average surface area than those in the spring despite a lower leaf count (Table 4). The greater surface area can be explained in the *Z. marina* life cycle in which leaves in the winter tend to be carried over from the preceding summer and are mature, whereas new leaves begin to appear in

spring. It was not reported in Thom *et al.* (2008) whether the blade samples

collected in winter also had a greater average surface area than blades collected

in other seasons. Also, it is known that the respiratory rate of Z. *marina* plants

decreases in the winter (Hemminga & Duarte 2000), which was also seen in this

study (Tables 2 and 5). This response may be due at least in part to losses or

decay in leaf tissue in the winter such that the percentage of rhizome mass on

eelgrass plants increases. Previous work showed that rhizome tissue

demonstrates lower respiration rates per gram than leaf tissue (Hemminga &

Duarte 2000). Although a reduced respiration rate in the winter would be

expected for samples used by Thom *et al.*, this response was not reported and

respiration rates could not be compared between these two studies. In addition,

cold winter waters tend to lower the respiration rate of eelgrass which could

create conditions where the light compensation threshold of plants would be

more easily reached. However, these cold water temperatures may reduce their

photosynthetic potential at the same time, and at this time the effect of micro-

variations in water temperature on the respiration of eelgrass samples collected

in different habitats cannot be determined, which may have had a small influence

on P-I Curve produced by Thom *et al.* Last, it was qualitatively noted during

sample collection dives that leaf abundance in this study population appeared

dramatically lowered in the winter. It was considered that Z. *marina* plants in

Rosario Bay perhaps compete less with their neighbors for light in the winter

which may have contributed to a lower photosynthetic efficiency of blades

observed in this study.

In comparing the net productivity P-I curve (Figure 5) with that of Thom *et al.*

(2008), the P-I curves in winter and summer showed dramatic differences

between these two studies with respect to other seasons. For Thom *et al.*, the

winter P-I curve had the greatest photosynthetic efficiency with comparable

kinetics to that of spring until approximately 150 µmol quanta m^{-2} h^{-1}. At 500 µmol

quanta m^{-2} h^{-1} and above, winter showed photo-inhibition and its net productivity

fell steeply. Finally, their summer P-I curve showed the smallest rise in net

productivity with increasing light levels (Appendix 3). The net productivity P-I

curve in this study, however, showed that winter blades had the lowest

photosynthetic efficiency whereas summer blades had the greatest, and no

photo-inhibition was observed in any season at saturating light levels (Figure 5).

Also, the light intensities plotted along the X-axis of the P-I Curve for net

productivity by Thom *et al.* greatly exceeded both the light intensities utilized for

P-I Curves in this study as well as the maximum light intensities that eelgrass in

Rosario Bay experience at depth (Figure 11). Specifically, the average daily

maximum light intensities experienced by Z. *marina* at the outer depth limits in

Rosario Bay did not exceed 1000 µmol quanta m^{-2} h^{-1} at any time, and almost

always fell in the range used for light experiments. In the experimental design of

this study, protocols were established to avoid the negative impacts of potentially

damaging light intensities above the levels typically experienced by eelgrass *in*

situ, and light intensity exposures were gradually increased during the

experiments rather than exposing the blade samples to random light level

exposures. Last, the influence of a potential increased respiration rate at sites of

wounded eelgrass tissue was minimized by sealing the clipped ends of their blades outside of the respirometry chambers, which may be a novel approach to respirometry studies. Even with modifications in the design and protocols of the present study, a general agreement was observed with that of Thom *et al.* in the numerical range of net productivity values for P-I Curves from both studies, and this fact could potentially validate both approaches toward understanding Z. *marina* photosynthesis in the Pacific Northwest despite the differences described previously.

Extrapolating blade experiments to whole plants:

Hemminga and Duarte (2000) cautioned against efforts to extrapolate laboratory-measured leaf photosynthetic measurements to whole plants in the field since a laboratory-derived P and R may differ from those in the field among other uncertainties. In this study, whole plant respiration was directly measured, and little difference was expected to be found in the field. However, the laboratory-derived P (gross photosynthesis) in this study involved extrapolation of photosynthetic measurements for blade sections to the combined average surface area of leaves on a whole plant, which involved inherent limitations of studying complex systems in a laboratory setting. For light experiments using blade samples, the maximum amount of surface area was illuminated on one side of the blades by orienting the blade surface perpendicular to the light path.

By extrapolating to whole plants, the P-I Curves for whole plants (Figures 6 and 7) also reflected this idealized light exposure and assumed an equal, fully maximized illumination of all of its leaves on one side, which likely does not reflect how eelgrass receives natural light *in situ*. Eelgrass leaves often rest in the vertical position such that they are oriented parallel to the light path, and they move in a dynamic manner with the water currents. Furthermore, Z. *marina* experiences an increase in light blockage due to self-shading with increasing leaf abundance in the population, especially during late spring and summer. It should also be noted that the P-I Curve for whole plants does not take into account other environmental factors that could reduce the photosynthetic potential of eelgrass, and the light requirements to maintain compensational growth of whole plants would likely be slightly greater than estimated. Interestingly, the light compensation point in the summer was lower for whole plants than it was for blades (Tables 2 and 5). Hemminga and Duarte (2000) claimed that the light compensation point for whole plants should be greater than that of individual blades, and the findings for light compensation in winter and spring months supported this view. It is expected the rhizome respiration would increase the metabolic demand above that of the respiration rate for the combined leaves of a whole plant, and thus the light compensation point for whole plants in summer should exceed that of blades. In this study, however, whole plants in the summer demonstrated a lower respiration rate than would be predicted based on a hypothetical extrapolation (analogous to the laboratory-derived P) of the respiration rate of blade sections to whole plants. Yet the assumption that blade

sections used in photosynthesis experiments were representative of all leaf tissues may not be entirely accurate. While cleaning leaves of epiphytes for respiration studies, it was observed that Z. *marina* leaves often varied in age and condition, even originating from the same plant. Unfortunately, this study was not equipped to address variation in age and/or health of blade samples nor could it reproduce in the laboratory the environmental pressures they experienced at depth *in situ*. The age of eelgrass plants would likely influence the thickness and quality of leaf tissues, and light compensation requirements for thicker leaves likely exceed the threshold for blades reported in the study. It has been demonstrated that thicker leaves contain lower levels of chlorophyll *a* per gram of tissue which serves to reduce their photosynthetic potential (Hemminga & Duarte 2000). In regards to the decreased respiration rate of whole plants in summer, it is possible that some of the leaves that were counted on whole plants had substantially lower respiration rates than would be predicted from photosynthetic blade measurements due to leaf tissue aging and/or declining health. Alternatively, the instantaneous photosynthetic efficiency (α) of whole plants could be described as being unexpectedly high in the summer compared to that of blades, although it is more likely that this phenomena reflects a decreased respiration rate.

Other unexpected differences were found between the P-I Curves for blades and whole plants in this study. Figures 6 and 7 showed net and gross whole plant P-I Curves for Z. *marina* based on wet and dry masses of their leaves, respectively.

For both wet and dry leaves, whole plant P-I curves in the summer were more elevated with respect to other seasons than that observed in the P-I Curve for blades. For instance, the summer P-I curve for whole plants had a six-fold greater P_{max} than the winter curve (Figure 6), whereas for blades only a 2.5-fold increase in the summer P_{max} was found compared to winter (Figure 4). This finding is likely due to the greatly increased abundance of leaves in the summer, suggesting that an increased number of leaves has an important and possibly synergistic role in overall photosynthetic potential, especially at saturating light intensities. Interestingly, Hemminga and Duarte (2008) stated that for eelgrass blades the P_{max} can exceed respiration by 20-fold (it is usually about 5-fold) and that whole plants should have a lower ratio. However, this finding was based on comparisons of instantaneous P_{max} ratios between blades and whole plants, and in this study, the daily P_{max} /R ratio for whole plants showed a 20-fold greater P_{max} in the summer than the respiration rate (Figure 15). In contrast, the instantaneous P_{max} for blades exceeded blade respiration by 10-fold in summer and spring months, whereas this ratio differed by 20-fold for blades in winter months (Table 2). These results suggest that summer plays a dominant role in supporting Z. *marina* growth and that during the summer Z. *marina* experiences its highest growth potential in Rosario Bay.

Unexpected differences arose between the physical measurements of *Z. marina* blades and whole plants. For instance, two-fold differences were observed in the conversion ratios from wet-to-dry mass between blades and whole plant leaves

(Table 3). These ratios were expected to closely resemble one another by season, although one might have expected a small difference to occur due to the inclusion of plant stalks for whole plants whose combined mass with the leaves could have adjusted this ratio. However, the differences between these ratios were much higher than anticipated, and led to considerations of how water mass may have contributed to this unexpected finding. In the respiration studies of whole plants, the leaves were weighed directly after experiments by coiling them into a small bundle and blotting the exterior of this bundle dry with a paper towel to remove excess water. Yet, it is likely that these coiled leaves retained much higher quantities of water than individual blades could have post-blotting, which could have inflated the mass values obtained. The potential implications of using an inflated mass for the leaves of whole plants were considered in the analysis portion of this study: the g/cm^2 ratio was multiplied, based on averages from blade section measurements, by the average total surface area calculated for individual whole plant leaves (respective to each season) to estimate a theoretical wet mass of whole plant leaves. This value was used as a multiplication factor to extrapolate P-I relationship found for blades to whole plants. Alternatively, if direct measurements of leaf mass were instead used as the multiplication factor, the P/R ratio of whole plants would approximately double each month in all seasons. It was concluded that the multiplication factor developed and the method utilized to extrapolate photosynthetic blade measurements to whole plants produced a conservative estimate of gross photosynthesis for whole plants. In addition, this approach allowed exclusion of

discolored and decaying tissues from the calculation as they did not likely contribute to photosynthesis substantially, which would not have been possible using direct measurements of whole plant leaf mass.

Also, maintaining low water temperatures in the inner respirometry chambers matching that of coastal waters in Rosario Bay was difficult due to restricted ventilation in the laboratory space. As a result, the eelgrass samples at times experienced small variations in temperature which in most cases rose above the set point by 1-3 degrees. It has been shown that eelgrass plants increase their respiration rate in response to temperatures raised higher than they typically experience at depth (Hemminga & Duarte 2000). The blades used in light experiments were likely influenced by minor variations in temperature above that of coastal waters, and it is likely that the actual respiration rate is slightly lower than the respiration rate reported here. Temperature fluctuations such as these would be the norm in shallow bay environments where eelgrass is typically found, but would be less common in a deeper, more exposed bay such as Rosario Bay.

Limitations of this study:

The models used in this study ignored the influence of wind on water turbidity because its effects are difficult to predict. Sustained wind from the west or southwest can lead to larger waves and turbidity from stirred-up sediments in the

bay. However, the effects of surface waves on water turbidity are not instantaneous and sediment stirred up in the water column may take days to settle down. Therefore, actual turbidity in the water cannot be fully determined without continuous measurements. Lacking the capability of taking continuous measurements, the average seasonal values for turbidity where chosen to represent annual water conditions, which is likely approximates seasonal averages in marine water turbidity. These measurements were taken on a periodic basis where light and medium wave action and its effects on turbidity were inherently a part of the model and presumed to be captured in the average values obtained. During the summer of 2014, sample collections were restricted to the 4.0m depth station because the Z. *marina* population in Rosario Bay had an unanticipated die-off in early July. This phenomenon likely reflected its biannual life cycle where adult eelgrass plants tend to decompose and give way to newly seeded plants on the seabed floor late in the summer. Seed deposition is usually within 1 m of the shoot, but floating flowering shoots can disperse widely (Thom *et al.* 2012). Also, the development of belowground structure is known to be important for eelgrass resilience since the rhizome tends to be the primary storage location of carbohydrates contributing to their long-term survival (Thom *et al.* 2012). Unfortunately, trending losses in eelgrass abundance also have been observed in the Puget Sound during 2005-2007 (Dowty *et al.* 2007), and continued monitoring of this population in Rosario Bay is necessary.

Also, it should be noted in Table 7 that the deepest layer of the water column attenuated light to a greater extent in the summer than in the other seasons. This discrepancy was likely due to shading effects on the light sensor bulb caused by the increased density of Z. *marina* plants typical in summer months. Ultimately, only half of the last meter which defined the deepest layer of the water column was calculated into the model based on the expectation that the average eelgrass blade was about ½ m from the bottom.

Another important consideration of this study involves epiphytic and red algae growth on the aboveground structures of eelgrass plants which likely blocks substantial quantities of available light from reaching the eelgrass tissues. These organisms can reside in thick layers on the surface of eelgrass blades, and while collecting samples it was observed that these layers were very prominent in the spring and especially the summer. Those seasons corresponded with increased water temperature compared with winter. Furthermore, epiphytic and red algae layers on the eelgrass surfaces likely do not benefit the eelgrass in any direct fashion and perhaps serve only to block light from reaching the eelgrass (Dr. Cowles, personal communication, 2015). Concerns of rising water temperatures in this region may upregulate epiphytic growth and potentially decrease the amount of light Z. *marina* plants can acquire underwater. Future studies may focus on understanding the variables driving Z. *marina* distribution in Rosario Bay, which would help in monitoring this eelgrass population at their subtidal and depth limits by characterizing the relative importance of factors known to promote

eelgrass presence in the Pacific Northwest, including sediment type and nutrient content and pH (Thom *et al.* 2012).

Conclusion:

In summary, this study supported the hypothesis that Z. *marina* in Rosario Bay received more than sufficient light levels at underwater depths to maintain its monthly and annual metabolic requirements. The P-I Curves developed for Z. *marina* whole plants provided a theoretical and idealized framework to understand the photosynthetic potential of Z. *marina* plants, Combined with estimations of the actual light levels in Rosario Bay at underwater depths, this study reported a positive monthly and annual growth potential for the study population of Z. *marina* throughout the year, even in the winter. However, certain factors blocking light such as epiphytic growth were not measured in this study, and potentially represent an increasing threat to Z. *marina* with warming ocean waters in the Pacific Northwest. In the future, a better understanding of known variables including sediment type, nutrient content, and pH that promote eelgrass distribution in Rosario Bay would also assist in monitoring this population.

ACKNOWLEDGMENTS

LITERATURE CITED

Blackmon, D., Wyllie-Eschevarria, T. and Shafer, D.J. 2006. The role of seagrasses and kelp in marine fish support. Published by Wetlands Regulatory Assistance Program, Army Engineering and Research Development Center, US Army Corp of Engineers, MS.

Caine, E.A. 1980. Ecology of two littoral species of Caprellid amphipods (Crustacea) from Washington, USA. *Marine Biology* 56:327-335

Dennison, W.C. and Alberte, R.S. 1985. Role of daily light period in the depth distribution of *Zostera marina* (eelgrass). *Marine Ecology- Progress Series* 25: 51-61

Dowty, P., Schanz, A., and Berry, H (eds.). 2007. Eelgrass Stressor-Response Project: 2005-2007 Report. Nearshore Habitat Program, Washington Department of Natural Resources, Olympia, Washington. 228 pp. http://www.dnr.wa.gov/Publications/aqr_nrsh_05_07_biennial_report.pdf

Eissinger, A.M. 2007. Great Blue Heron in Puget Sound. Puget Sound Nearshore Partnership, Report No. 2007-06. Published by Seattle District, US Army Corp of Engineers, Seattle, WA

Fertig, B., Kennish, M.J. and Sackowicz, G.P. 2013. Changing eelgrass (*Zostera marina* L.) characteristics in a highly eutrophic coastal lagoon. *Aquatic Botany* 104: 70-79

Fishman, J.R. & Orth, R.J. Effects of predation on Z. *marina* L. seed abundance. *Journal of Experimental Marine Biology and Ecology* 198: 11-26

Fonseca, M. S. and Uhrin, A.V. 2005. The status of eelgrass, *Zostera marina*, as Bay Scallop Habitat: Consequences for the Fishery in the Western Atlantic. *Marine Fisheries Review* 71 (3): 20-33

Foreman, M. G. G. *et al*. 1995. A tidal model for the eastern Juan de Fuca Strait and the southern Strait of Georgia. *Journal of Geophysical Research* 100 (No. C1): 721-740

Greve, T.M., Krause-Jensen, D., Rasmussen, M.B. and Christensen, P.B. 2005. Means of rapid eelgrass (*Zostera marina* L) recolonization in former dieback areas. *Aquatic Botany* 82: 143-156

Hemminga, M.A. and Duarte, C.M. 2000. Seagrass Ecology. Cambridge University Press, p. 99-145

Khangaonkar, T. *et al*. 2012. Puget Sound dissolved oxygen modeling study: development of an intermediate scale water quality model. Publication No. 12-03-049. Pacific Northwest National Laboratory PNNL-20384 Rev 1. Washington State Department of Ecology, WA

Lee, K., Park, S.R. and Kim, Y.K. 2007. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *Journal of Experimental Marine Biology & Ecology* 350: 144-175

Li, W., Kim, S.H., Kim, J.W., Kim, J. and Lee, K. 2013. An examination of photoacclimatory responses of *Zostera marina* transplants along a depth gradient for transplant-site selection in a disturbed estuary. *Estuarine, Coastal and Shelf Science* 118: 72-79

Li, W., Park, J., Park. S.R., Zhang, X. and Lee, K. 2010. Chlorophyll a fluorescence as an indicator of establishment of *Zostera marina* transplants on the southern coast of Korea. *Algae* 25 (2): 89-97

Mumford, T.F. 2007. Kelp and eelgrass in Puget Sound. Technical Report in support of the Puget Sound Nearshore Partnership. Washington Department of Natural Resources, Olympia, WA

Orth, R. J., Marion, S.R. and Moore, K.A. 2007. A summary of eelgrass (*Zostera marina*) reproductive biology with an emphasis on seed biology and ecology from the Chesapeake Bay region. (No. ERDC/TN-SAV-07-1). Engineer research and development center Vicksburg, MS coastal and hydraulics lab.

Orth, R.J. *et al*. 2006. A global crisis for seagrass ecosystems. *Bioscience* 56 (12): 987-996

Short, F., Carruthers, T., Dennison, W. and Waycott, M. 2007. Global seagrass distribution and diversity: A bioregional model. *Journal of Experimental Marine Biology & Ecology* 350:3-20

Staehr, P.A. and Borum, J. 2011. Seasonal acclimation in metabolism reduces light requirements of eelgrass (*Zostera marina*). *Journal of Experimental Marine Biology and Ecology* 407: 139-146

Thom, R., Miller, B. and Kennedy, M. 1995. Temporal patterns of grazers and vegetation in a temperate seagrass system. *Aquatic Botany* 50 (2): 201-205

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

Thom, Ronald M., Heida L., Diefenderfer, J. V., and Borde, A.B. 2012. Restoring resiliency: case studies from Pacific Northwest estuarine eelgrass (*Zostera marina* L.) ecosystems. *Estuaries and coasts* 35: pp 78-91

Zimmerman, R.C., Reguzzoni, J.L., Wyllie-Echeverria, S.W., Josselyn, M. and Alberte, R.S. 1991. Assessment of environmental suitability for growth of *Zostera marina* L. (eelgrass) in San Francisco Bay. *Aquatic Botany* 39: 353-366

APPENDIX 1

Calculating Total Cloud Cover

Based on advice from Roger Trombetta, the meterorologist at the Naval Air Station Whidbey Island (NASWI), the following values of cloud cover were used for each of the specified codes reported by the NASWI ASOS system:

ASOS Code	Meaning	Cloud Cover Value
CLR	Clear	0%
FEW	Few	12.5%
SCT	Scattered	37.5%
BKN	Broken	75%
OVC	Overcast	100%
RA	Rain	100%
TS	Thunderstorm	100%
DZ	Drizzle	100%
FOG	Fog	100%
VV	Low visibility (fog)	100%
HZ	Haze	100%
SN	Snow	100%
GS	Dispersed snow grains	50%
PL	Ice pellets	100%
GR	Hail	100%

APPENDIX 2

Calculating Tidal Height at Rosario Bay

A tidal program was developed and utilized to calculate high and low tide times and heights at Bowman Bay (next to Rosario Bay and the nearest tidal station) with those predicted for Port Townsend, WA (t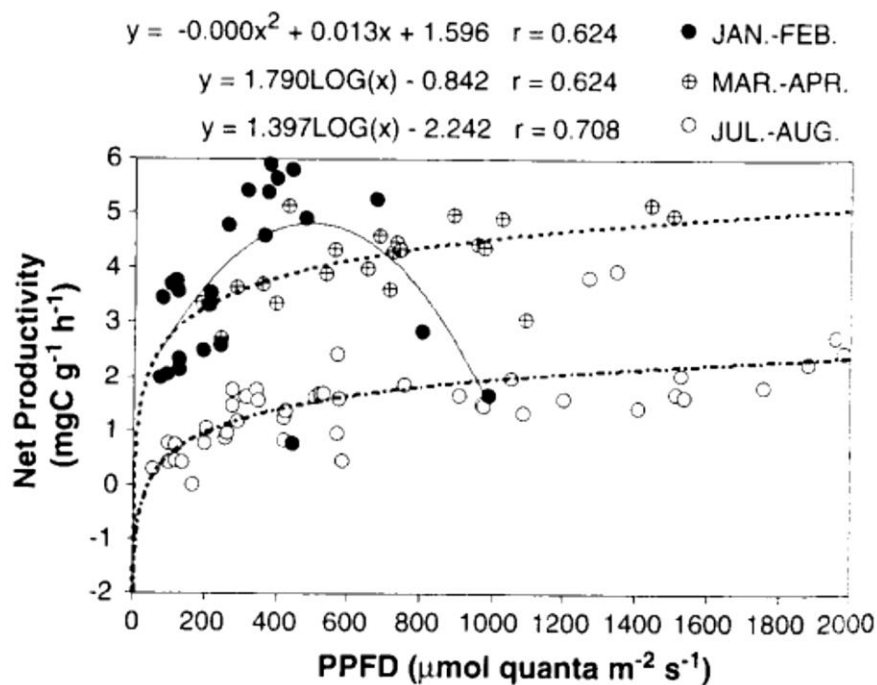he nearest calibrated tidal station) from the NOAA site (http://tidesandcurrents.noaa.gov/NOAATidesFacade.jsp?Stationid=9444900). Specifically, each incoming or outgoing tide was approximated by a sine wave with the NOAA-predicted times and heights for the bounding high/low tide as nodes. A cross-check of the model using NOAA-predicted tides for Bowman Bay confirmed a high degree of confidence in our tidal model since it predicted tide heights within 5 cm of the NOAA-predicted tides for this site (predicted tides for Bowman Bay were available for current periods, unlike Rosario Bay). Tidal fluctuations in Rosario Bay predicted by this model dynamically adjusted the overhead height of the water column above Z. *marina* eelgrass at depth, which principally modified the depth range of the middle layer in the water column and thus the amount of light attenuated in this layer.

APPENDIX 3

P-I curve for *Z. marina* in the Pacific Northwest for three seasons

by Thom *et al.* (2008).

$$y = -0.000x^2 + 0.013x + 1.596 \quad r = 0.624 \qquad \bullet \text{ JAN.-FEB.}$$
$$y = 1.790LOG(x) - 0.842 \quad r = 0.624 \qquad \oplus \text{ MAR.-APR.}$$
$$y = 1.397LOG(x) - 2.242 \quad r = 0.708 \qquad \circ \text{ JUL.-AUG.}$$

Note that the winter curve is hyperbolic indicating photo-inhibition in net productivity at 500 PPFD (photosynthetic photon flux density), whereas summer and spring showed asymptotic curves.

APPENDIX 4

Data for P-I Curves using Z. marina blades based on wet weight. Sample
count (N) is shown in parentheses and SE = Standard Error. Negative
values at 0 light level represent respiration rate. The units for light level
are µmol quanta m^{-2} s^{-1}, and Net P is represented by µmol dissolved O_2 g
wet wt^{-1} h^{-1}.

Light Level	Summer Net P	SE	Winter Net P	SE	Spring Net P	SE
0	-7.248 (27)	0.479	-1.0568 (14)	0.450	-3.988 (12)	0.368
100	18.422 (27)	0.960	12.984 (14)	1.308	15.177 (12)	1.416
150	45.838 (27)	2.014	25.756 (10)	4.323	34.267 (12)	3.458
200	52.022 (27)	2.364	30.462 (11)	4.585	40.998 (12)	3.890
300	79.420 (25)	3.951	31.949 (12)	3.602	51.929 (12)	5.558
400	66.431 (25)	3.524	-	-	53.491 (10)	4.685
500	79.025 (28)	4.106	29.552 (12)	3.904	55.533 (12)	5.948
700	78.053 (19)	4.574	30.060 (4)	4.314	-	-

APPENDIX 5

Data for Net Productivity of <u>Z. marina</u> blades based on dry weight. Sample count (N) is shown in parentheses and SE = Standard Error.

Light Level	Summer Net Prod.	SE	Winter Net Prod.	SE	Spring Net Prod.	SE
0	-0.533 (27)	0.035	-0.080 (14)	0.037	-0.213 (12)	0.020
100	1.355 (27)	0.069	0.981 (14)	0.107	0.809 (12)	0.076
150	3.372 (27)	0.146	1.946 (10)	0.298	1.827 (12)	0.184
200	3.827 (27)	0.171	2.301 (11)	0.332	2.186 (12)	0.207
300	5.843 (25)	0.275	2.414 (12)	0.272	2.769 (12)	0.296
400	4.887 (25)	0.245	-	-	2.853 (10)	0.228
500	5.814 (28)	0.302	2.233 (12)	0.295	2.962 (12)	0.317
700	5.742 (19)	0.277	2.271 (4)	0.188	-	-