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Recommended Citation

Miller, Cale A.; Love, Brooke; and Yang, Sylvia, "Moderate Increase in TCO2 Enhances Photosynthesis of Seagrass Zostera japonica, but Not Zostera marina: Implications for Acidification Mitigation" (2017). *Environmental Sciences Faculty and Staff Publications*. 47. [https://cedar.wwu.edu/esci_facpubs/47](https://cedar.wwu.edu/esci_facpubs/47)
Moderate Increase in TCO$_2$ Enhances Photosynthesis of Seagrass Zostera japonica, but Not Zostera marina: Implications for Acidification Mitigation

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Photosynthesis and respiration are vital biological processes that shape the diurnal variability of carbonate chemistry in nearshore waters, presumably ameliorating (daytime) or exacerbating (nighttime) short-term acidification events, which are expected to increase in severity with ocean acidification (OA). Biogenic habitats such as seagrass beds have the capacity to reduce CO$_2$ concentration and potentially provide refugia from OA. Further, some seagrasses have been shown to increase their photosynthetic rate in response to enriched total CO$_2$ (TCO$_2$). Therefore, the ability of seagrass to mitigate OA may increase as concentrations of TCO$_2$ increase. In this study, we exposed native Zostera marina and non-native Zostera japonica seagrasses from Padilla Bay, WA (USA) to various levels of irradiance and TCO$_2$. Our results indicate that the average maximum net photosynthetic rate ($P_{\text{max}}$) for $Z$. japonica as a function of irradiance and TCO$_2$ was 3x greater than $Z$. marina when standardized to chlorophyll ($360 \pm 33$ \(\mu\)mol TCO$_2$ mg chl$^{-1}$ h$^{-1}$ and $113 \pm 10$ \(\mu\)mol TCO$_2$ mg chl$^{-1}$ h$^{-1}$, respectively). Additionally, $Z$. japonica increased its $P_{\text{max}}$ \sim 50\% when TCO$_2$ increased from \sim 1,770 to 2,051 \(\mu\)mol TCO$_2$ kg$^{-1}$. In contrast, $Z$. marina did not display an increase in $P_{\text{max}}$ with higher TCO$_2$, possibly due to the variance of photosynthetic rates at saturating irradiance within TCO$_2$ treatments (coefficient of variation: 30–60\%) relative to the range of TCO$_2$ tested. Our results suggest that $Z$. japonica can affect the OA mitigation potential of seagrass beds, and its contribution may increase relative to $Z$. marina as oceanic TCO$_2$ rises. Further, we extended our empirical results to incorporate various biomass to water volume ratios in order to conceptualize how these additional attributes affect changes in carbonate chemistry. Estimates show that the change in TCO$_2$ via photosynthetic carbon uptake as modeled in this study can produce positive diurnal changes in pH and aragonite saturation state that are on the same order of magnitude as those estimated for whole seagrass systems. Based on our results, we predict that seagrasses $Z$. marina and $Z$. japonica both have the potential to produce short-term changes in carbonate chemistry, thus offsetting anthropogenic acidification when irradiance is saturating.

Keywords: Zostera marina, Zostera japonica, seagrass, ocean acidification, photosynthetic potential, mitigation, TCO$_2$
INTRODUCTION

The uptake of CO₂ from anthropogenic fossil fuel emissions by the global oceans is shifting the acid-base balance of the carbonate system in a process known as ocean acidification (OA). Increasing CO₂ concentration outpaces the natural buffering capacity of seawater and increases the total CO₂ (TCO₂), which is the sum of all forms of carbonic acid and its conjugate bases (Doney et al., 2009; Hönisch et al., 2012). The dissolution of anthropogenic CO₂ in nearshore waters interacts with a host of other processes that drive the dynamics of nearshore carbonate chemistry, such as biological metabolism, riverine discharge and associated organic matter composition, tidal pumping, upwelling, nutrient input, and eutrophication (Feely et al., 2008, 2010; Cai, 2011; Duarte et al., 2013; Waldbusser and Salisbury, 2014; Wallace et al., 2014). The synergy of these factors induces high variability to the carbonate system, and results in periodic and episodic decreases in pH and aragonite saturation state (ΩAR) that are more extreme than the ~0.4 pH and ~1.5 ΩAR decreases predicted for global average ocean waters by the year 2100 (Cai et al., 2013; Duarte et al., 2013; Waldbusser and Salisbury, 2014). Among these drivers, photosynthesis and respiration are the dominant processes controlling coastal ocean carbonate chemistry (Gattuso et al., 1998; Sunda and Cai, 2012; Waldbusser and Salisbury, 2014). The diurnal variability of biological photosynthesis and respiration can, therefore, either dampen or amplify the magnitude of extreme carbonate chemistry events, which may occur from episodic influxes of fresh water or upwelling. Gaining a better understanding of how these biological signals modify and potentially ameliorate acidification is imperative, particularly when the effects of acidification can impact the economic and social stability of coastal human communities that are dependent on ocean resources (Ekstrom et al., 2015).

Many marine calcifiers and a variety of other marine species will be negatively affected by OA as decreases in pH, calcium carbonate saturation state and the substrate-to-inhibitor ratio—[HCO₃⁻]/[H⁺]—have been shown to inhibit calcification, growth, and acid-base regulation (Pörtner, 2008; Kroeker et al., 2013; Thomsen et al., 2015; Waldbusser et al., 2015; Fassbender et al., 2016). Conversely, some autotrophic organisms may directly benefit from the increase in seawater CO₂ associated with acidification, which can stimulate photosynthesis and increase growth (reviewed in Kroeker et al., 2010, 2013; Koch et al., 2013). For example, photosynthetic rates of many seagrass species have been shown to increase with TCO₂ (Beer and Koch, 1996; Thom, 1996; Zimmerman et al., 1997; Invers et al., 2001; Cox et al., 2016; Ow et al., 2016). In addition, some studies have concluded that the carbon uptake by seagrass beds may mitigate acidification on specific spatial and temporal scales when irradiance is high (Manzello et al., 2012; Unsworth et al., 2012; Hendriks et al., 2014).

OA mitigation by seagrass arises from the removal of CO₂ from seawater through photosynthesis, which raises pH and reduces TCO₂, thus minimizing acidification on short timescales (Marbá et al., 2006; Unsworth et al., 2012). This direct action of OA mitigation by seagrass is, however, reversed during times of dark respiration, which transiently increases TCO₂.

For most seagrass systems, periods of high photosynthetic activity are robust enough to reduce TCO₂ in spite of the enhanced remineralization of allochthonous organic matter, which accumulates within seagrass beds and fuels the production of TCO₂ via respiration (Koch et al., 2006; Duarte et al., 2013; Hendriks et al., 2014; Baumann et al., 2015). Since most seagrass systems tend to be net autotrophic, or at least seasonally net autotrophic, they can sequester copious amounts of carbon on seasonal timescales that correspond to changes in above-ground biomass, while carbon can be sequestered on longer timescales by below-ground retention in the sediment (Duarte et al., 2005, 2010; Chung et al., 2011; McLeod et al., 2011; Fourquarean et al., 2012, 2016; Unsworth et al., 2012; Marbá et al., 2015; Poppe, 2016). Estimated values of organic carbon retained in seagrass sediments to a depth of 1 m range from 9.1 to 625 mg ha⁻¹, with actual measurements extending to 829 mg ha⁻¹ (Fourquarean et al., 2012). Despite the importance of carbon storage, it is the instantaneous photosynthetic carbon uptake that drives OA mitigation potential on short timescales.

Presumably, extreme acidification events in nearshore coastal waters will increase in frequency, duration, and magnitude as a result of anthropogenic OA (Harris et al., 2013; Hauri et al., 2013). For early life stage calcifiers undergoing rapid growth and development, sensitivity to OA is heightened and driven by the duration and intensity of exposure (Kurihara, 2008; Talmage and Gobler, 2009; Hettinger et al., 2012; Waldbusser et al., 2015). Therefore, an increase in acidification severity will likely result in chemical conditions that periodically surpass physiological thresholds of resident organisms already living near their tolerance limits (Grantham et al., 2004; Waldbusser and Salisbury, 2014). Instantaneous carbon uptake rates by seagrasses, however, may be able to expand periods of favorable carbonate chemistry for these sensitive species, or dampen the episodic extremes of acidification on hourly timescales, thus lessening the exposure to extreme acidification.

In the U.S. Pacific Northwest (PNW), two Zostera seagrass species are among the seagrasses commonly found in soft-sediment habitats in the Salish Sea: native Zostera marina L. and non-native Zostera japonica Ascher & Graebner (Harrison and Bigley, 1982). The native seagrass Z. marina has specifically been identified by Washington State as a biological means to ameliorate acidification (Washington State Blue Ribbon Panel on Ocean Acidification Ocean Acidification., 2012). In addition, both Z. marina and Z. japonica have strong habitat-associations with organisms vulnerable to OA, such as bivalves (Ferraro and Cole, 2012; Mach et al., 2014; Dunmbauld and McCoy, 2015). The non-native Z. japonica has colonized previously unvegetated mudflats and is found in the mid to upper intertidal zone, whereas Z. marina has a distribution extending from the lower intertidal to shallow subtidal region; species overlap between Z. marina and Z. japonica can occur in the lower intertidal zone on flat shorelines (Harrison, 1982; Thom, 1990; Kaldy, 2006; Ruesink et al., 2010). The increasing presence and distribution of Z. japonica in the PNW warrants an inclusion of this non-native species when examining the potential of seagrass in the Salish Sea to mitigate OA.
Research has shown that seagrass carbon uptake rates are species-specific and vary in response to altered carbonate chemistry (Campbell and Fourqurean, 2013; Koch et al., 2013). Thus far, Z. marina has been the focus of many studies examining its photosynthetic response to increases in TCO₂ (Beer and Koch, 1996; Thom, 1996; Zimmerman et al., 1997; Koch et al., 2013); however, this is not the case for Z. japonica. There have not been any published studies to our knowledge examining Z. japonica response to CO₂. Only a handful of studies have examined Z. japonica photosynthesis and physiology for populations established in the PNW (see Shafer et al., 2011; Shafer and Kaldy, 2014; Kaldy et al., 2015). Shafer and Kaldy (2014) found that Z. japonica local to the central Oregon coast has a substantially greater photosynthetic rate than Z. marina under the same chemical conditions. While this study provides insight into the relative photosynthetic differences between the two species, a comprehensive understanding of variable TCO₂ and irradiance response is needed in order to determine how this difference drives OA mitigation potential. In addition, it is important to examine if the local adaptations that exist throughout a species’ distribution (Backman, 1991 and references therein; Shafer et al., 2011) result in photosynthetic differences that are dissimilar between central Oregon and Salish Sea populations.

In this study, we conducted a series of laboratory experiments to better understand how Z. marina and Z. japonica may alter carbonate chemistry under conditions of elevated TCO₂ and variation in light intensity, as would occur over a diurnal period. We aimed to determine how the photosynthetic carbon uptake of Z. marina and Z. japonica shift the carbonate system on short timescales potentially counteracting acidification. Specifically, we conducted one experiment per species to (1) examine the differences in species’ photosynthetic rates (i.e., carbon uptake rates), (2) quantify the response of photosynthetic rates to differing levels of TCO₂, and (3) use those results to estimate how the photosynthetic carbon uptake could induce hourly changes on the carbonate system over a diurnal cycle at various biomass to water volume ratios.

**MATERIALS AND METHODS**

**Sample Site and Collection**

Padilla Bay, Washington, is a tidally dominated estuary in the Salish Sea, and is a part of the National Estuarine Research Reserve System (48°31′14.1″N, 122°35′24.4″W). The Z. marina and Z. japonica meadows in Padilla Bay constitute a submerged and emergent total area of ~4,000 ha, where Z. marina accounts for ~3,000 of the total area (Bulthuis, 2013). The higher intertidal region is dominated by Z. japonica, which is morphologically different than Z. marina and has a leaf surface area and mass that is ~5x less than that of Z. marina. Z. japonica constitutes ~22% of the seagrass biomass in Padilla Bay, and the spread of the non-native species has increased the total areal extent of seagrass acreage. In the intertidal where Z. japonica overlaps with Z. marina, a 250 m transect was marked for shoot collection, which occurred every 25 m (48°29′36.6″N, 122°29′8.5″W). Two weeks prior to shoot collection, two HOBO Pendant Temperature/Light 64k data loggers were attached to the transect marking poles ~1 m above the sediment (above the height of the canopy) to capture in situ irradiance at the sample site which was used to generate a realistic but idealized light field to drive model visualizations. HOBO data (in units of lux) were converted to daylight photon flux density (PFD) as described by Thimijan and Heins (1983). The measurements from the two sensors were averaged and smoothed, and this curve was scaled to the maximum value recorded by land-based PAR measurements at the Padilla Bay farm station. It is important to note that the HOBO light sensors only measure in the planar flux and not the spherical or scalar flux, which is a more comprehensive irradiance flux; therefore, the sensor data are used to define the shape of the curve, while the scaling should bring the overall range of irradiance values within realistic bounds.

Approximately 200 shoots of each species were collected from Padilla Bay along the entire 250 m transect. Healthy-looking adult Z. marina shoots with intact rhizomes were collected by hand during low tide (0.8 m MLLW) on August 16, 2015 from Padilla Bay, placed in a cooler, and transported to Shannan Point Marine Center in Anacortes, Washington, within 1 h of collection. Ten days later on August 26, 2015, adult Z. japonica shoots with intact rhizomes were collected in the same manner at low tide (~0.0 MLLW) for a second experiment. One hundred of the most healthy-looking shoots (i.e., shoots without visible damage and well-preserved rhizomes) were then haphazardly selected, rinsed with seawater, and dispersed among four separate 40-L acrylic flow through tanks under low PFD (~50 µmol photons m⁻² s⁻¹) on a 12:12 L:D cycle for ~48 h before experimentation.

**Experimental Design**

We used a 5 × 5 factorial design that targeted 25 treatment CO₂ and light combinations: estimated PFD levels 0, 40, 200, 500, and 750 µmol photons m⁻² s⁻¹, and pCO₂ of 140, 250, 400, 650, and 900 µatm, where the 400 pCO₂ treatment is the approximate ambient condition for the open ocean. Treatment pCO₂ values were selected because they correspond to an observed range of diurnal in situ variability of the carbonate system in Padilla Bay (Love et al., 2016). Even though pCO₂ was the variable used to prepare treatments by gas equilibration, TCO₂ is a more appropriate metric for our experiment given that CO₂ and HCO₃⁻ are presumably both utilized for photosynthesis; therefore, we identify treatment levels by initial TCO₂ rather than pCO₂ henceforth. In order to achieve the full factorial design, we used closed experimental incubation vials housing leaf segments rather than whole shoots in large open aquaria. Each treatment combination had quadruplicate replication and duplicate blanks for a total of 100 leaf-segment and 50 blank vials. Blanks had the same PFD exposure and initial TCO₂ but lacked leaf segments, and were used to account for any changes in seawater chemistry induced by microbial activity.

During each experiment, 150 incubation vials (20 ml borosilicate scintillation vials with a polyethylene cone-shaped liner) were placed in five clear acrylic water bath trays (56.5 × 7.62 × 3.81 cm) fitted with flow-through seawater for temperature control. Each water bath tray was mounted to a single 1.5 cm clear acrylic sheet, and placed directly above an individual bulb in a light fixture housing five T5 high output
54W 6,500 K Spectralux bulbs. Incubation vials were nearly fully submerged (water line stopped at cap) when placed in water bath trays. Vinyl mesh wraps were constructed and fitted to incubation vials to attenuate light. Mesh covers either had one, three, or six layers, which provided a PFD range from ~40 to 500 µmol photons m\(^{-2}\) s\(^{-1}\).

### Treatment Water and Leaf Segment Preparation

To obtain a spectrum of TCO\(_2\) concentrations, seawater pumped from Guemes Channel was collected, 0.2 µm filtered, sterilized via autoclave, and distributed into four 20 L polycarbonate carboys. Four mixtures of pure CO\(_2\) (research grade-5) and compressed ambient air stripped of CO\(_2\) using a regenerative molecular sieve adsorber (Twin Tower Engineering, CAS2-11) were generated using four sets of Sierra SmartTrak mass flow controllers. Treatment water was bubbled for at least 72 h with gas mixtures that were first passed through humidifiers (one liter Nalgene bottles filled halfway with tap water). Carboys were held in an incubator, which maintained water temperature at approximately 12.5°C. Further details are described in the equilibration system portions of Love et al. (2017). A fifth CO\(_2\) treatment was produced by mixing, equilibrated, identical volumes of the two lowest CO\(_2\) mixtures.

Preparation of seagrass tissue used for incubations began approximately 20 h before each experiment. This began by selecting the middle section of the second youngest leaf in a shoot, and wiping the entire leaf clean of epiphytes. To test similar leaf surface area for both species, a 2 cm leaf segment for *Z. marina* and 4 cm leaf segment for *Z. japonica* was excised. While the middle section of the leaf was selected for, variation among shoot length of the second youngest leaf existed. Each leaf segment was then cut in two, and one half randomly frozen for chlorophyll extraction and the other placed into the incubation vial filled with 5 ml of filtered and sterilized seawater. Leaf segment stock vials were then held in a low light incubator at ~12°C for 16–20 h until experimentation.

Immediately before each experiment, stocked vials were emptied of filtered seawater, rinsed and filled with treatment seawater. Vials were overflowed to eliminate headspace, and a 0.5-mm glass bead was inserted as a stirring mechanism. Mesh coverings (no mesh, 1 layer, 3 layer, 6 layer, and black electrical tape for opaque) were placed around vials to achieve varied levels of PFD. Vials were haphazardly placed into water bath trays, and incubated for 90 min until termination of the experiment. Vials were physically inverted by hand, three times, every 5 min to stir water inside and minimize the development of a TCO\(_2\) poor boundary layer around leaf segments. Temperature in water bath trays was continuously monitored and recorded every 20 min with a Fluke 1523 reference thermometer from the beginning of the experiment until all vials were terminated. PFD was measured at each vial location with a QSL-101 PAR irradiance sensor (Biospherical Instruments Inc.) by setting vials with mesh coverings in their respective locations after the conclusion of the experiment. Due to the discontinuity of PFD along a bulb (i.e., vials positioned at the center of a bulb received more light than those at either end), light measurements were treated as continuous data for all statistical analyses.

### Leaf Segment and Carbonate Chemistry Analysis

Photosynthetic rates were determined from leaf segment incubations of *Z. marina* and *Z. japonica* by measuring treatment TCO\(_2\) concentration before and after incubation. While photosynthetic rates are normalized to TCO\(_2\), the O\(_2\) convention is followed. That is, positive photosynthetic rates are a negative flux of TCO\(_2\) from the medium, while respiration is a positive flux of TCO\(_2\) to the medium: this is opposite when O\(_2\) normalized. Initial TCO\(_2\) samples were collected in triplicate 20 ml scintillation vials, poisoned with 10 µl of saturated HgCl\(_2\), capped, wrapped with parafilm to minimize any potential gas leakage, and refrigerated at 2°C until analysis. Initial total alkalinity (TA) samples were collected in triplicate 350 ml amber glass bottles with polyurethane-lined crimp-sealed metal caps and poisoned with 30 µl of saturated HgCl\(_2\). Salinity was measured from each TA sample with a refractometer before poisoning occurred, and again for each treatment vial when samples were processed.

Incubations were terminated by removing vials, one at a time haphazardly across treatments, but ordinarily by replicate assignment—this allowed for an equal experimental termination time across treatments. Leaf segments were removed from the vial, marked for incubation time, and stored in an empty vial for dry-weight measurement. The experimental vial was immediately poisoned and stored in the same manner as initial condition samples. TCO\(_2\) samples were analyzed within 5 days of each experiment using an Apollo SciTech AS-C3 dissolved inorganic carbon analyzer. TA samples were titrated within 30 days of the experiment using the open-cell method as in Dickson et al. (2007) with a Metrohm 888 Titrand. Certified reference material was used to construct a five-point standardization curve for TCO\(_2\) and to verify accuracy of TA open-cell titration (Batch 144, A.G., Dickson, Scripps Institute of Oceanography). All other carbonate chemistry parameters were calculated using CO\(_2\)SYS (Pierrot et al., 2006) with K\(_1\) and K\(_2\) equilibrium constants from Mehrbach et al. (1973) and refit by Dickson and Millero (1987). Dry weight was recorded after rinsing leaf segments 3x with deionized water and drying at 55°C for at least 24 h. Frozen leaf segments were prepped for chlorophyll extraction by sonication for 30 s in a 10 ml 90% acetone solution. Segments were then refrozen at −20°C for 24 h and centrifuged for 5 min directly before chlorophyll measurement. Extract was measured with a Trilogy fluorometer (Turner designs), acidified with 0.1 N HCl, and measured again. Chlorophyll and phaeopigment concentrations were calculated following the methods described by Lorenzen (1966).

### Statistical Methods and Photosynthetic Response

Predicted photosynthetic rates for both species were determined by modeling the empirical data separately for each experiment as a function of continuous PFD, and a combined effect of
continuous PFD and TCO$_2$ in one integrated, iterative (600 iterations) model, using the non-linear and linear curving fitting functions and curve fitting tools in the MathWorks software Matlab (V. 2015b). Species comparison was determined from the robustness of the model fit and whether or not 95% confidence intervals overlapped for each predicted photosynthetic parameter. Photosynthetic rates were normalized to chlorophyll (a/b) rather than dry-weight in order to account for variability of pigment concentration that occurs between and along leaves (Enríquez et al., 2002). Following the methods described in Jassby and Platt (1976), the net photosynthetic rate ($P_{\text{net}}$) was calculated as:

$$P_{\text{net}} = P_{\text{max}} \tanh \left( \frac{\alpha E}{P_{\text{max}}} \right) + R_d$$

(1)

where $P_{\text{max}}$ is the maximum photosynthetic rate ($\mu$mol TCO$_2$ mg chl$^{-1}$ h$^{-1}$), $E$ is the PFD ($\mu$mol photons m$^{-2}$ s$^{-1}$), $R_d$ is the dark respiration rate ($\mu$mol TCO$_2$ mg chl$^{-1}$ h$^{-1}$), and $\alpha$ (photosynthetic efficiency) is the initial slope of the photosynthetic-irradiance curve ($\mu$mol TCO$_2$ mg chl$^{-1}$ h$^{-1}$ ($\mu$mol photons m$^{-2}$ s$^{-1}$)$^{-1}$). In order to integrate the empirical instantaneous photosynthetic rates (i.e., carbon uptake) to hourly changes in the carbonate system, TCO$_2$ was integrated into the standard photosynthesis-irradiance curve (Equation 1) as a linear effect on $P_{\text{max}}$. A vector of initial TCO$_2$ concentration was applied to the $P_{\text{max}}$ term, thus allowing $P_{\text{max}}$ to vary with TCO$_2$:

$$P_{\text{TC}}^{\text{max}} = P_{\text{base}} + \beta \ast \text{T}_i \text{CO}_2$$

(2)

where $P_{\text{TC}}^{\text{max}}$ is the TCO$_2$ dependent $P_{\text{max}}$, $\beta$ is the slope of the $P_{\text{max}}$ response to TCO$_2$, $P_{\text{base}}$ is the intercept, and $\text{T}_i \text{CO}_2$ is the initial TCO$_2$ concentration. The integrated TCO$_2$-irradiance model was, thus, a combination of the above equations:

$$P_{\text{net}} = P_{\text{TC}}^{\text{max}} \tanh \left( \frac{\alpha E}{P_{\text{TC}}^{\text{max}}} \right) + R_d .$$

(3)

where $P_{\text{net}}$ is now a function of irradiance and TCO$_2$. The estimated mean $P_{\text{max}}$ and photosynthetic efficiency ($\alpha$) from the TCO$_2$-irradiance model (Equation 3) output were used to calculate the saturation irradiance:

$$E_k = \frac{p_{\text{TC}}^{\text{max}}}{\alpha}$$

(4)

where $E_k$ is the saturation irradiance and $p_{\text{TC}}^{\text{max}}$ and $\alpha$ the outputs from the TCO$_2$-irradiance model (Equation 3). Since all TCO$_2$-irradiance model predictions of $P_{\text{max}}$ are TCO$_2$ dependent ($P_{\text{TC}}^{\text{max}}$), for simplicity, we will simply refer to them as $P_{\text{max}}$ rather than $P_{\text{TC}}^{\text{max}}$. $P_{\text{max}}$-values for both species at every initial TCO$_2$ treatment were statistically compared based on the predicted standard error and confidence bounds from the model output. In addition, $P_{\text{max}}$-values as a function of initial CO$_2$ and HCO$_3^-$ were observed as both carbonate species are generally utilized for photosynthesis.

**Model Evaluation: Photosynthesis and Carbon Uptake**

The TCO$_2$-irradiance model was conceived by applying realistic PFD values representing a diurnal cycle and the experimental TCO$_2$ treatment values as model input parameters (Equation 3), producing hourly photosynthesis estimates and the concomitant uptake of TCO$_2$. A Gaussian function was fit to field collected PFD measurements resulting in a generalized light cycle. The TCO$_2$ values for the ambient treatment from both experiments were averaged (1,964 $\mu$mol kg$^{-1}$) and utilized as a baseline concentration from which hourly photosynthetic carbon uptake could be used to calculate changes in TCO$_2$ at each hourly step over a diurnal period. Assuming TA constant (also averaged from both experiments—2,134 $\mu$mol kg$^{-1}$), the relative changes in pH and $\Omega_{ar}$ could then be calculated based on the change in TCO$_2$ as driven by the estimated photosynthetic carbon uptake of each species. The calculated differences were always determined from the baseline TCO$_2$ for each time step. This is in accordance with the initial condition of each of our experimental TCO$_2$ and light conditions, and is analogous to the situation when residence time in a seagrass bed is small. In the scenario where reduction in TCO$_2$ is compounded, concentrations in the conceived model would be reduced to minimal values rapidly upon initiation of photosynthesis and the effects of both species-specific rates and initial TCO$_2$ would disappear under the general carbon limitation.

**Estimated Changes in Carbonate Chemistry**

The experimental results were determined at a fixed ratio of biomass to water volume, constraining the ability to compare the laboratory results to other studies. Therefore, the model predicted results were examined at different biomass to water volume ratios, which would be similar to variation in seagrass density and water depth in the field. Predicted $P_{\text{max}}$ results were applied to a simple box model consisting of a 1 m$^2$ area, with seagrass at a moderate density, and carbonate chemistry predictions driven by saturating irradiance, TCO$_2$ and water depth. The specific changes induced on the carbonate system by both species’ maximum photosynthetic potential were extrapolated from vial volume (20 ml) to different volumes of static water corresponding to various depths over a 1 m$^2$ patch of seagrass with a biomass of 100 g DW m$^{-2}$. It should be noted that we don’t take into account light attenuation at these depths, but at mid-day, irradiance should still be saturating. Estimates for both species from various locations in Padilla Bay reported in Bulthuis (2013), show a range from ~60–200 g DW m$^{-2}$ with a mean of ~100 for both species. The change in TCO$_2$ was calculated assuming a well-mixed water column, the average mg chl gDW$^{-1}$ (1.88 ± 0.89 and 0.67 ± 0.33 for Z. marina and Z. japonica, respectively) for each species, and using projected $P_{\text{max}}$ values, as:

$$\Delta\text{TCO}_2 = \text{TCO}_2i - \left( C_b \ast \left( \frac{b_i}{a} \right) \ast \left( \frac{1}{d} \right) \ast r \right)$$

(5)

where $\text{TCO}_2i$ is the initial TCO$_2$, $C_b$ is mg chl gDW$^{-1}$, $b_i$ is the g DW over a m$^2$ ($a$), $d$ is depth (m), $r$ is $P_{\text{max}}$ ($\mu$mol TCO$_2$ mg
chlorophyll (chl) h⁻¹), and f is the unit conversion m²·1,000 L⁻¹. Changes in TCO₂ from photosynthetic carbon uptake by each species were then used to determine the changes in carbonate chemistry parameters while assuming TA constant (averaged from both experiments—2,134 µmol kg⁻¹).

RESULTS

Incubation Conditions
Experimental conditions for the leaf segment incubations were similar between experiments (Table 1), which were run at nearly the exact same time of day in the mid-morning (<2 min apart). Temperature averaged 13.2 ± 0.4°C, for the Z. marina experiment, and 13.4 ± 0.1°C for the Z. japonica experiment. Photon flux density (PFD) had a greater variance for the high light treatments than for the lower, more heavily shaded treatments. Salinity ranged from 30.5 to 32 ppt for the Z. japonica experiment (Table 1). This variation was likely a result of unequal effectiveness by the humidifiers during bubbling, or from differential evaporation in the autoclaved bottles used to fill carboys. The pCO₂ of the seawater treatments did not indicate complete equilibration with the gas mixtures, which ranged from 100 to 1,200 µatm whereas seawater treatment conditions only ranged from 138 to 918 µatm (Table 1). However, the treatment values did produce a large range in TCO₂ and were similar across both experiments. The high TCO₂ treatment had the greatest difference between experiments, which was 55 µmol kg⁻¹ higher for the Z. marina experiment than for the Z. japonica experiment (Table 1). It should be noted that sample size by treatment TCO₂ varied from 20 to 17 due to either the malfunction of the dissolved inorganic carbon analyzer or loss of treatment seawater stored in the incubation vial.

Species-Specific Photosynthetic Response to Irradiance and TCO₂

All photosynthetic parameters for Z. japonica were best predicted by the TCO₂-irradiance model (Equation 3), which had a more robust fit (RMSE = 150 µmol TCO₂ mg chl⁻¹ h⁻¹) than the standard photosynthesis-irradiance model (Equation 1), RMSE = 161 µmol TCO₂ mg chl⁻¹ h⁻¹ (Table 2). There was no difference in the predictive power between the TCO₂-irradiance and the standard photosynthesis-irradiance models for Z. marina, both had an RMSE of 43 µmol TCO₂ mg chl⁻¹ h⁻¹. Based on the TCO₂-irradiance model relating the photosynthesis-irradiance curve to TCO₂, the mean maximum photosynthetic rate (Pmax) for Z. japonica was 3x greater than Z. marina, and photosynthetic efficiency (a) was 4.5x greater on a per chlorophyll basis (Table 2, Figure 1). The non-linear fit for both seagrasses in Figure 1 is the mean fit over all initial TCO₂, which displays higher variability for Z. japonica compared to Irradiance and TCO₂

### Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pCO₂ (µatm) treatment</th>
<th>TCO₂ (µmol kg⁻¹)</th>
<th>TA (µmol kg⁻¹)</th>
<th>pCO₂ (µatm)</th>
<th>pH (total)</th>
<th>Salinity</th>
<th>Temp. °C</th>
<th>% Sal. ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z. marina</td>
<td>140</td>
<td>1779 ± 2.7</td>
<td>2157 ± 15</td>
<td>140 ± 4.4</td>
<td>8.40 ± 0.01</td>
<td>4.0 ± 0.0</td>
<td>14.2 ± 0.8</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1873 ± 4.5</td>
<td>2157 ± 6.3</td>
<td>225 ± 4.0</td>
<td>8.24 ± 0.01</td>
<td>3.0 ± 0.1</td>
<td>14.4 ± 0.6</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>1972 ± 1.8</td>
<td>2141 ± 7.4</td>
<td>421 ± 6.6</td>
<td>8.01 ± 0.01</td>
<td>1.9 ± 0.0</td>
<td>14.0 ± 0.2</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>650</td>
<td>2083 ± 2.1</td>
<td>2197 ± 5.5</td>
<td>652 ± 18</td>
<td>7.85 ± 0.01</td>
<td>1.4 ± 0.0</td>
<td>14.4 ± 0.3</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>900</td>
<td>2106 ± 1.5</td>
<td>2180 ± 1.2</td>
<td>863 ± 15</td>
<td>7.73 ± 0.01</td>
<td>1.1 ± 0.0</td>
<td>14.1 ± 0.3</td>
<td>32</td>
</tr>
<tr>
<td>Z. japonica</td>
<td>140</td>
<td>1770 ± 6.1</td>
<td>2147 ± 6.2</td>
<td>138 ± 1.8</td>
<td>8.41 ± 0.00</td>
<td>3.9 ± 0.1</td>
<td>14.0 ± 0.5</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1868 ± 1.1</td>
<td>2147 ± 2.1</td>
<td>214 ± 4.8</td>
<td>8.26 ± 0.01</td>
<td>3.0 ± 0.0</td>
<td>13.8 ± 0.4</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>1958 ± 1.5</td>
<td>2127 ± 1.9</td>
<td>393 ± 8.4</td>
<td>8.03 ± 0.01</td>
<td>1.9 ± 0.0</td>
<td>13.6 ± 0.4</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>650</td>
<td>2018 ± 0.8</td>
<td>2113 ± 6.2</td>
<td>684 ± 9.6</td>
<td>7.81 ± 0.01</td>
<td>1.2 ± 0.0</td>
<td>13.5 ± 0.3</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>900</td>
<td>2051 ± 1.7</td>
<td>2103 ± 3.2</td>
<td>918 ± 15</td>
<td>7.70 ± 0.01</td>
<td>0.9 ± 0.1</td>
<td>13.2 ± 0.1</td>
<td>30.5</td>
</tr>
</tbody>
</table>

*Measured mean values and standard deviation of TA, TCO₂, temperature, salinity, and calculated values pH (total), and aragonite saturation state (Ωₐ).*

**Table 2** Predicted photosynthetic parameters from the TCO₂-irradiance (TCO₂-IRR) model (Equation 3) and the standard photosynthesis-irradiance (P vs. E) model (Equation 1).

<table>
<thead>
<tr>
<th>Species</th>
<th>Model</th>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>tstat</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z. marina</td>
<td>TCO₂-IRR</td>
<td>Pmax</td>
<td>113</td>
<td>9.91</td>
<td>2.02</td>
<td>0.0466</td>
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<tr>
<td></td>
<td></td>
<td>α</td>
<td>0.819</td>
<td>0.211</td>
<td>3.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R_d</td>
<td>2.92</td>
<td>69.68</td>
<td>0.337</td>
<td>0.737</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>-0.054</td>
<td>0.055</td>
<td>0.986</td>
<td>0.327</td>
</tr>
<tr>
<td>Z. japonica</td>
<td>P vs. E</td>
<td>Pmax</td>
<td>113</td>
<td>10.7</td>
<td>10.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α</td>
<td>0.842</td>
<td>0.211</td>
<td>3.99</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R_d</td>
<td>2.13</td>
<td>8.77</td>
<td>0.242</td>
<td>0.809</td>
</tr>
<tr>
<td>Z. japonica</td>
<td>TCO₂-IRR</td>
<td>Pmax</td>
<td>360</td>
<td>33.0</td>
<td>2.98</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α</td>
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<td>1.14</td>
<td>3.24</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R_d</td>
<td>0.180</td>
<td>31.2</td>
<td>0.005</td>
<td>0.995</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β</td>
<td>0.841</td>
<td>0.219</td>
<td>3.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Z. japonica</td>
<td>P vs. E</td>
<td>Pmax</td>
<td>360</td>
<td>40.3</td>
<td>8.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α</td>
<td>3.47</td>
<td>1.11</td>
<td>3.13</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R_d</td>
<td>1.42</td>
<td>33.7</td>
<td>0.042</td>
<td>0.966</td>
</tr>
</tbody>
</table>

Pmax is the maximum photosynthetic rate, a (photosynthetic efficiency) is the initial slope of the photosynthetic-irradiance curve, and β is the slope of the linear relationship between Pmax and initial TCO₂ concentration. Total sample size for Z. marina and Z. japonica: n = 98 and 93, respectively. The TCO₂-irradiance model fit for Z. marina produced a RMSE of 43 µmol TCO₂ mg chl⁻¹ h⁻¹ and 150 for Z. japonica. Units: Pmax and R_d = µmol TCO₂ mg chl⁻¹ h⁻¹; α = µmol TCO₂ mg chl⁻¹ h⁻¹ (µmol photons m⁻² s⁻¹); β = µmol TCO₂ mg chl⁻¹ h⁻¹ (µmol TCO₂ kg⁻¹).
to *Z. marina* indicating a greater response by *Z. japonica* to 

TACO. The model produced a good fit for *Z. japonica* and for 

*Z. marina* for two out of the three photosynthetic parameters, 

however, the mix of positive and negative TACO fluxes in the 

respiration vials resulted in a positive value rather than a negative 

value predicted by the model for *Z. marina* and *Z. japonica* 

respiration (intercept) ([Figure 1, Table 2]). Across all treatments, 

the mean leaf segment mg chlDW$^{-1}$ ratio—used to normalize 

the model results—was $\sim3x$ greater for *Z. marina* (1.88 $\pm$ 0.89) than for *Z. japonica* (0.67 $\pm$ 0.33). Model predicted mean 

$P_{\text{max}}$ for *Z. japonica* was 360 $\pm$ 33 $\mu$mol TACO mg chl$^{-1}$ h$^{-1}$ 

with an $\alpha$ of 3.70 $\pm$ 1.1 $\mu$mol TACO mg chl$^{-1}$ h$^{-1}$ ($\mu$mol photons m$^{-2}$ s$^{-1}$)$^{-1}$, and where considered significant with 

standard error (SE) ([Table 2]). Model prediction for *Z. marina* 

$P_{\text{max}}$ was 113 $\pm$ 10 $\mu$mol TACO mg chl$^{-1}$ h$^{-1}$ with an $\alpha$ of 0.819 $\pm$ 0.21 $\mu$mol TACO mg chl$^{-1}$ h$^{-1}$ ($\mu$mol photons 

m$^{-2}$ s$^{-1}$)$^{-1}$, and were also predicted as significant ([Table 2]). 

In addition, the point at which each species experiences a 

saturating irradiance ($E_k$) was also different. *Z. japonica* displayed 

a lower $E_k$ at 97 (propagated SE = 31) $\mu$mol photons m$^{-2}$ 

s$^{-1}$, which was $\sim70\%$ of *Z. marina’s $E_k$ of 138 (propagated SE 

= 37) $\mu$mol photons m$^{-2}$ s$^{-1}$. The maximum photosynthetic 

rates—which were used to fit the $P_{\text{max}}$ values—for *Z. japonica* 

appeared to respond positively with increasing TACO ([Figure 2]). 

*Z. marina*, however, did not display any positive increase in 

photosynthetic rate with increasing TACO. It is important to note 

that [Figure 2A] strictly shows one half of the TACO-irradiance 

model (photosynthetic rate to TACO) and does not directly reflect 

the interaction between TACO and light, as is really described by 

the model.

The TACO-irradiance model predicted a positive increase in 

$P_{\text{max}}$ to increasing carbon availability by *Z. japonica* over the 

entire range of TACO tested ([Figure 3]). The TACO-irradiance 

model predicted a significant and positive $\beta$ term for *Z. japonica*, 

whereas $\beta$ for *Z. marina* was non-significant (where $\beta$ is 

the slope of the $P_{\text{max}}$ to TACO relationship). Due to the variance 

of maximum photosynthetic rates and the resulting non-significant 

effect of TACO on *Z. marina*, we are unable to properly analyze 

any trends in *Z. marina* $P_{\text{max}}$ with TACO, HCO$_3^-$, and CO$_2$. 

According to the model, *Z. japonica* $P_{\text{max}}$ increased by $\sim50\%$ 

from the lowest to highest initial TACO treatment ([Figure 3]). 

Across all initial TACO treatments, *Z. japonica* had a significantly 

higher $P_{\text{max}}$ than *Z. marina* (i.e., non-overlapping 95% CI) 

([Table 3]).

**Model Predictions for Diurnal Variation of the Carbonate System**

Under realistic natural light conditions, both species are at 
saturating irradiances for about 6 h during a typical summer day. 
The resulting maximum predicted photosynthetic rates for 

*Z. japonica*, are 335 $\mu$mol TACO mg chl$^{-1}$ h$^{-1}$ when initial 

TACO is set to 2051 $\mu$mol kg$^{-1}$ (highest TACO treatment), 387 

$\mu$mol TACO mg chl$^{-1}$ h$^{-1}$ when TACO is set at 1964 $\mu$mol kg$^{-1}$ 

(ambient treatment) and only 145 $\mu$mol TACO mg chl$^{-1}$ h$^{-1}$ 

when TACO is set at 1770 $\mu$mol kg$^{-1}$ (lowest TACO treatment) 

([Figure 4]). The predicted maximum photosynthetic rate for *Z. marina* is 116 $\mu$mol TACO mg chl$^{-1}$ h$^{-1}$ under these saturating 

light conditions, and no sensitivity to TACO was detected. Due to 

the poor respiration data, estimates of respiration were excluded 

during times of zero irradiance. When light was saturating, model 

estimates predict that the carbon drawdown based on a $P_{\text{max}}$ 

of 387 TACO mg chl$^{-1}$ h$^{-1}$ for *Z. japonica* would result in a 

maximum increased change in pH and omega of 0.65 and 3.8 

units mg chl$^{-1}$ h$^{-1}$ compared to the 0.25 and 1.1 unit increase 

by *Z. marina*. The estimated changes are, however, based on the 
total carbon draw down by the leaf segment in a 20 ml vial and in 

isolation from *in situ* bed conditions, which would be driven by 

additional considerations.

Model ([Equation 5]) estimated hourly changes in pH, $\Omega_{ar}$, and 

the substrate-to-inhibitor ratio ([HCO$_3^-$]/[H$^+$]) for biomass 

to water volume ratios similar to a typical seagrass bed under 1 m 
of water were greater when mediated by *Z. marina* than for 

*Z. marina*, with identical initial TACO ([Figure 5]). Under these 

differences, *Z. japonica* induced an hourly rate of change for pH 

and $\Omega_{ar}$ that was approximately 20% greater than *Z. marina* 

([Figure 5]). Both seagrass species induced positive changes in 

carbonate chemistry parameters. The greatest change in pH 

(0.49 h$^{-1}$) and $\Omega_{ar}$ (1.61 h$^{-1}$) occurred at a shallow depth of 

0.2 m with high TACO (2051 $\mu$mol kg$^{-1}$) and was mediated by 

*Z. japonica* ([Figure 5]). The greatest change in the substrate-to- 
inhibitor ratio was not determined by high carbon uptake rates 

via photosynthesis, but rather by the low concentration of H$^+$ at 

low TACO.

**DISCUSSION**

Species-specific difference in TACO-dependent photosynthetic 

carbon uptake is a critical component that determines the 

capacity of seagrass to remove dissolved CO$_2$ from seawater and
potentially create OA refugia on short timescales. Our results indicate that, on a per chlorophyll basis, *Z. japonica* is able to take up more TCO₂ and more efficiently utilize available irradiance compared to *Z. marina* occurring in the same intertidal zone under similar TCO₂ and irradiance conditions (Figures 1, 2, Table 2). Because of this, *Z. japonica* may be more effective
at mitigating OA (increasing pH and $\Omega_{ar}$) for more hours of the day than $Z$. marina on a per chlorophyll basis (Figure 4). The maximum potential drawdown of TCO$_2$ over the 6 h of saturating irradiance induces a positive change in pH (from 8.03 to 8.33 and 8.40) and $\Omega_{ar}$ (from 1.94 to 3.1 and 3.4) by $Z$. marina and $Z$. japonica, respectively, in our model. Even though these estimates of carbonate chemistry modification by $Z$. marina and $Z$. japonica are specific to our experimental conditions, we are able to detect the differential response of TCO$_2$-dependent carbon uptake between the two species, and provide insight for potential OA mitigation by two PNW seagrasses.

**Extrapolated Changes in Carbonate Chemistry**

One focus of this study was to better understand the OA mitigation potential of seagrass clippings in isolation, and extend those results to various biomass to water volume ratios in an attempt to conceptualize how these attributes of seagrass beds drive carbonate chemistry. While these results are not representative of in situ conditions, by incorporating realistic biomass values, the box model results provide a point of comparison to field based studies and information about photosynthetic effects measured under controlled conditions to those quantifying potential seagrass contribution to a dynamic and complex system. The estimates presented here are considered the maximum potential possible by each species, as photosynthetic rates calculated from leaf segments are likely an overestimate of whole plant photosynthetic rates (Herzka and Dunton, 1997); however, previous studies have shown that empirically derived photosynthetic rates can be similar between leaf segments and whole plants (see Table 2 in Lee et al., 2007; Table 6 in Shafer and Kaldy, 2014). It is important to note that the predicted changes in carbonate chemistry are derived from our measured mg chl:gDW$^{-1}$ ratio (Equation 5), however, we do not use gDW normalized photosynthetic rates as the variance was extremely high within treatments and resulted in non-significant differences (see Miller, 2016).

When extending our laboratory results of seagrass photosynthetic carbon uptake to changes in carbonate chemistry given specific biomass to water volume ratios, the expected potential change in pH, $\Omega_{ar}$, and the substrate-to-inhibitor ratio—[HCO$_3^-$]/[$H^+$]—would be positively affected by seagrass carbon uptake (Figure 5). Estimated increases in pH per hour from this model at a volume corresponding to 1 m depth over 6 h result in maximum pH changes from 0.09 to 0.73 under low and high TCO$_2$ conditions for $Z$. japonica (Figure 5). Since $Z$. japonica photosynthesis is carbon sensitive, the more realistic pH change is likely somewhere in the middle and more accurately predicted by the mean TCO$_2$ treatment, which would result in a pH change of 0.37 over 6 h. This would not be a concern for $Z$. marina, however, as we did not find sensitivity to TCO$_2$ over the range tested in this study. These rates are based on maximum photosynthetic rates, but also on conservative assumptions about short residence time in seagrass
whereas field based calculations of diurnal changes ranged from 1.38 to 1.67 (Hendriks et al., 2014). We estimated diurnal increases in \( \Omega_{ar} \) from 0.59 to 1.6 at a 1 m depth over 6 h under low and high TCO\(_2\) conditions for \( Z. \) japonica (Figure 5). Model predictions from an extensive data set of Indo-Pacific seagrass community metabolism estimated maximum diurnal changes in \( \Omega_{ar} \) to be 2.9 (Unsworth et al., 2012), whereas field based calculations of diurnal changes ranged from 1.38 to 1.67 (Hendriks et al., 2014). At shallower water depths, the higher Indo-Pacific change in \( \Omega_{ar} \) of 2.9 is easily reached under our box model conditions (Figure 5). The congruence of these estimates illustrates how the laboratory studies presented here corroborate the idea that seagrass meadows can be drivers of locally important changes in carbonate chemistry. We note that the results of our box model should be viewed as context for field results and not as estimates of in situ processes. In order to make accurate predictions of carbonate chemistry variability in seagrass beds, higher resolution in situ studies and whole plant laboratory studies need to be performed.

### Photosynthetic Response to TCO\(_2\)

Based on our results from the TCO\(_2\)-irradiance model (Equation 3), \( Z. \) japonica increased its \( P_{max} \) proportionally across TCO\(_2\) treatments—where the slope (\( \beta \)) of the linear TCO\(_2\) response was highly significant (\( p < 0.001 \)). Conversely, we were unable to detect any positive response of \( Z. \) marina \( P_{max} \) with increasing TCO\(_2\). That is according to the TCO\(_2\)-irradiance model, the slope of the linear TCO\(_2\) response was non-significant and slightly negative (\( p = 0.327, \beta = -0.054 \)). Due to the lack of any significant trend and high variance within treatments, we suggest that \( Z. \) marina \( P_{max} \) was not positively affected by increasing TCO\(_2\) under our study conditions. Our findings here are somewhat contradictory as most studies have reported \( Z. \) marina to have enhanced photosynthetic rates with increasing TCO\(_2\) (Beer and Koch, 1996; Thom, 1996; Zimmerman et al., 1997), however, a recent study also found there to be no positive effect of TCO\(_2\) on \( Z. \) marina photosynthesis up to pCO\(_2\) levels of 2000 \( \mu \)atm (Pajusalu et al., 2016).

One reason for the different result in this study regarding \( Z. \) marina response to increasing TCO\(_2\) may be due to the range of concentrations tested. The lowest enriched TCO\(_2\) treatment in previous studies was \( \sim \)25% higher than our highest TCO\(_2\) treatment (Thom, 1996 does not give exact numbers, but minimum CO\(_2\) increase was \( \sim \)25% higher than ambient), ranging up to 230% times greater than our highest treatment (Beer and
Koch, 1996). The first reason for the relatively low TCO$_2$ tested in this study is that ambient seawater from the Salish Sea has lower salinity and, therefore, a lower TCO$_2$ than open ocean values. In addition, the range of TCO$_2$ tested in this study was representative of conditions currently observed in Padilla Bay, not the much greater range in other studies, which were designed to highlight potential physiological responses, not necessarily in situ conditions. The result showing that Z. marina $P_{max}$ does not respond to TCO$_2$ in this study may indicate that northern Salish Sea populations are not sensitive to TCO$_2$ at these lower concentrations.

Additionally, the high degree of variability within our treatments may have obscured a positive response. Zimmerman et al. (1997) showed that Z. marina increased its $P_{max}$ 225% with an exposure to TCO$_2$ 77% higher than their ambient treatment (from 2,074 to 3,673 µmol kg$^{-1}$). Over this range of TCO$_2$, a linear relationship has been observed between $P_{max}$ and TCO$_2$ (Beer and Koch, 1996). The linear equation derived from Zimmerman et al. (1997) can be extrapolated down to the range in this study (1,770–2,051 µmol kg$^{-1}$), which would therefore predict that the 18% increase in TCO$_2$ between our lowest and highest TCO$_2$ treatments would induce a 55% increase in Z. marina $P_{max}$. Given that the coefficient of variation for Z. marina $P_{net}$ at, or above, $E_k$ ranged from 30 to 60% over all initial TCO$_2$ treatments, any small positive responses to TCO$_2$ by Z. marina may likely be concealed by the variance—assuming an extrapolation of the Zimmerman et al. (1997) data is accurate given this is outside of the bounds tested. Physiological and methodological factors contributing to the large variance within treatments may be a result of, insufficient incubation period, variance in illumination, shoot to shoot variability of the photosynthetic apparatus, inefficiencies in chlorophyll extraction from leaf segments, or propagation of a diffusive boundary layer due to periodic rather than continuous stirring over the 90+ min incubation time, which would increase the variability of the relative TCO$_2$ conditions experienced by the leaf segment compared to the entire vial.

Interestingly, the increase in Z. japonica $P_{max}$ was ~50% over an 18% increase in TCO$_2$, which coincidentally is very close to the predicted increase from the linear equation derived from Zimmerman et al. (1997). This robust response by Z. japonica was observed despite a coefficient of variation in $P_{net}$ at, or above, $E_k$ identical to that of Z. marina (30–60%). This is the first time that a response in the photosynthetic rate of Z. japonica to TCO$_2$ has been documented, to our knowledge. Previous research has shown that Z. japonica photosynthesis is more sensitive to light and salinity (Shafer et al., 2011; Shafer and Kaldy, 2014) than is Z. marina photosynthesis (Hellblom and Björk, 1999; Shafer and Kaldy, 2014). Given that the two species appear to express different physiological response to environmental factors, it is likely that the utilization of TCO$_2$ would also be inherently different. The differential response of $P_{max}$ to TCO$_2$ between species may be due to the mechanism by which TCO$_2$ is utilized for photosynthesis. Previous research suggests that Z. marina HCO$_3^-$ utilization is saturated at pH ranging from 7.5 to 8.5 (Invers et al., 2001), which was the range of pH in our study (Table 1). Although we did not specifically measure independent HCO$_3^-$- and CO$_2$-uptake, an increase in HCO$_3^-$ in addition to CO$_2$ may be one mechanism that explains such a robust response by Z. japonica to TCO$_2$; however, further studies are needed to examine Z. japonica’s photosynthetic response to HCO$_3^-$ and CO$_2$ independently.

**Species-Specific Photosynthetic Rates**

The relative difference between Z. marina and Z. japonica photosynthetic rates reported in our study are similar to previous findings, but differ in magnitude (Figure 1). The large degree of variability around the mean estimated model fit for Z. japonica photosynthesis can be partially explained by the increasing response of photosynthesis to TCO$_2$ (Figure 2). We reiterate, however, that the variability in our respiration measurements may somewhat lessen the predictive power of model estimates, particularly the photosynthetic efficiency, as the initial slope of the photosynthetic-irradiance curve is dependent on robust respiration values. For this reason, we have focused specifically on only our $P_{max}$ results when interpreting photosynthetic carbon uptake induced changes on carbonate chemistry. A likely reason for the mix of positive and negative TCO$_2$ fluxes by our leaf segments in dark vials may be due to a short incubation time, volume of media to leaf segment ratio, or internal seagrass biorhythms of photosynthetic and respiration cycles. Similar to our findings, Shafer and Kaldy (2014) found that central Oregon populations of Z. japonica have a $P_{max}$ that is ~3x greater than Z. marina when exposed to the same light conditions; however, the chlorophyll standardized rates reported in that study were considerably lower than the rates found in this study. The differing magnitude of rates suggest that other factors such as organization of the photosynthetic package within seagrass tissue may have a significant impact on photosynthetic rates rather than chlorophyll pigment concentration alone. A multitude of environmental factors such as intertidal location, canopy density, age of shoot and leaf, time of year, and acclimatization to epiphytic growth can affect chlorophyll pigment concentration and the photoacclimation amongst shoots and along leaves, thus leading to drastically different mg chl:gDW$^{-1}$ ratios (Dennison and Alberte, 1986; Durako and Kunzelman, 2002; Enríquez et al., 2002; Major and Dunton, 2002; Cummings and Zimmerman, 2003; Drake et al., 2003; Larkum et al., 2006 and references therein). Any of these factors may be responsible for the lower mg chl:gDW$^{-1}$ ratio and dissimilar photosynthetic rates in our study. In addition, the Z. marina samples were collected at the upper limit of their distribution, potentially affecting the physiology and phenotypic expression of Z. marina at this location. Local acclimatization to in situ temperature and salinity can also impact photosynthetic rates via osmotic stress and changes in the photosynthesis-respiration ratio (Kenneth and Short, 2006 and references therein). In addition, our study examined photosynthetic rates by measuring the change in TCO$_2$, which to our knowledge, has not been done before in the lab and rarely done in the field despite the robustness of the method (Silva et al., 2009). The photosynthetic quotient (O$_2$/CO$_2$), while assumed to be unity or close to in many studies, is based on community metabolism in seagrass beds or meadows (Oviatt et al., 1986; Leuschner and Rees, 1993; Mateo et al., 2001; Martin et al., 2005). Measuring TCO$_2$ rather than O$_2$ may result in photosynthetic
rates that are dissimilar if the photosynthetic quotient is not close to unity under these conditions. That is, given the higher photosynthetic rates found in this study, the change in CO$_2$ per O$_2$ would have to be much lower in seagrass communities than what was measured in our vials. Alternatively, differences in chlorophyll extraction methods, or inefficient extraction may have resulted in the higher photosynthetic rates found in our study. Additionally, recycling and movement of gases within the lacunal system are not well-understood (Mateo et al., 2001), further convoluting the comparison of instantaneous O$_2$ production and CO$_2$ uptake. It may be that deriving photosynthesis by measuring TCO$_2$ is not as robust as the O$_2$ normative, but due to the lack of studies measuring seagrass photosynthesis with TCO$_2$, this remains uncertain. Studies which capture O$_2$ production and CO$_2$ uptake would be best suited to determine the relative advantage of each in determining the interactions between carbonate chemistry and seagrass photosynthesis.

Variations of Carbonate Chemistry in Seagrass Beds

While this study provides a point of reference for how seagrass beds can modify carbonate chemistry, determining the effects of macrophytes on acidification is challenging due to the extreme spatial and temporal variability of carbonate chemistry in these zones (Hendriks et al., 2014, 2015; Krause-Jensen et al., 2015; Challener et al., 2016). A multitude of factors such as seagrass epiphyte communities, heterotrophic respiration, tidal exchange, groundwater flux, and riverine input all contribute in modifying carbonate chemistry on various spatial and temporal scales. For example, differences in seagrass shoot density over a given area result in different mixing rates and flow regimes, which will further shift the carbonate chemistry of water parcels that have been modified by photosynthetic carbon uptake (Peterson et al., 2004; Koch et al., 2006; Marbá et al., 2006; Hendriks et al., 2014). In addition, variations in chlorophyll content along the leaf and amongst shoots results in differential photosynthetic rates and carbon uptake within the seagrass canopy, thereby creating disparate water parcels with respect to TCO$_2$ concentration on various spatial scales. These various interactions between water flow within a seagrass bed, irradiance, and TCO$_2$ are some of the determining factors of carbon assimilation and uptake (McPherson et al., 2015) and, therefore, directly affect the scale of OA mitigation by seagrasses via the formation of microzones with differential carbonate chemistry. Because organisms vulnerable to OA are more sensitive at particular life-stages and over hourly durations, the scale of carbonate chemistry variability (i.e., magnitude and duration of low saturation state and pH) is critical to understand when determining organismal resilience to OA (Kurihara, 2008; Barton et al., 2012; Hettenger et al., 2012; Onitsuka et al., 2014; Waldbusser et al., 2015; Miller and Waldbusser, 2016). Therefore, in order to elucidate a more nuanced amelioration of acidification by seagrasses, it will be necessary to conduct in situ studies that can effectively capture all the drivers of the carbonate system in spatiotemporal context.

CONCLUSION

A comparison of the photosynthetic potential between Z. marina and Z. japonica has implications for elucidating the contribution each species has on the carbonate system. Z. japonica exhibits a strong increase in photosynthetic rate in response to increasing TCO$_2$, while a similar response from Z. marina could not be identified. In the intertidal zone where species overlap occurs, our results indicate that Z. japonica also has a 3-fold greater photosynthetic potential than Z. marina when normalized to chlorophyll. By measuring photosynthetic potential as a change in TCO$_2$ and utilizing measured mg chl:gDW$^{-1}$ in addition to estimated m$^2$ biomass of each species, our results were extended to conceptualize how these additional attributes of biomass to depth ratios in seagrass beds affect hourly changes in carbonate chemistry. Based on our findings, Z. japonica appears to be better suited to mitigate OA on a per chlorophyll basis given its higher photosynthetic rate and efficiency and enhanced response to increases in TCO$_2$. Our study illuminates the potential of PNW populations of Z. marina and Z. japonica to modify the carbonate system, and provides a direct comparison of photosynthetic potential when exposed to varying levels of TCO$_2$. This is an initial step in attempting to determine the OA mitigation potential of seagrass systems in the PNW, where quantitative estimates can aid management practices and provide a better understanding for the local initiatives that aim to protect Washington state's aquaculture resources (Blue ribbon panel on OA 2012).

AUTHOR CONTRIBUTIONS

CM conceptualized and designed the experiment with modifications directed by BL and SY. CM led the writing of the paper with contributions by BL and SY. CM performed all data analysis, with statistical analysis supported by SY. BL provided substantial support for utilization of model projections. All authors reviewed and contributed to the writing of the final manuscript.

FUNDING

This work was supported by the Padilla Bay National Estuarine Research Reserve assistantship. Funding granted by the Padilla Bay foundation, Jude Apple and Sharon Riggs: Contract/Grant # Borman-01-2015.

ACKNOWLEDGMENTS

The authors would like to thank David Shull for thoughtful interpretation of our results. CM would like to thank Katherine L. Schoo for assistance with the ocean acidification experimental system. CM would also like to thank Rachel Blyth, Melissa Ciesielski, Taylor Clement, Rosie Gradoville, and Anne Harmann who were integral for carrying out the experiments.
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Frontiers in Marine Science | www.frontiersin.org 14  
July 2017 | Volume 4 | Article 228

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July 2017 | Volume 4 | Article 228
Species-Specific Seagrass Ocean Acidification Mitigation


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