Seagrasses (Zostera marina) and (Zostera japonica) Display a Differential Photosynthetic Response to TCO2: Implications for Acidification Mitigation

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SEAGRASSES \textit{(Zostera marina)} AND \textit{(Zostera japonica)}

DISPLAY A DIFFERENTIAL PHOTOSYNTHETIC RESPONSE TO 
\textit{TCO}_2: IMPLICATIONS FOR ACIDIFICATION MITIGATION

By

Cale A. Miller

Accepted in Partial Completion
Of the Requirements for the Degree
Master of Science

Kathleen Kitto, Dean of the Graduate School

ADVISORY COMMITTEE

Chair, Dr. Brooke Love

Dr. Sylvia Yang

Dr. David Shull
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Cale Miller
July 15, 2016
SEAGRASSES (ZOSTERA MARINA) AND (ZOSTERA JAPONICA) DISPLAY A DIFFERENTIAL PHOTOSYNTHETIC RESPONSE TO TCO₂: IMPLICATIONS FOR ACIDIFICATION MITIGATION

A thesis Presented to
The Faculty of
Western Washington University

Accepted in Partial Completion
Of the Requirements for the Degree
Master of Science

by
Cale A. Miller

August 2015
Abstract

Excess atmospheric CO₂ is being absorbed at an unprecedented rate by the global and coastal oceans, shifting the baseline pCO₂ and altering seawater carbonate chemistry in a process known as ocean acidification (OA). Recent attention has been given to near-shore vegetated habitats, such as seagrass beds, which may have the potential to mitigate the effects of acidification on vulnerable calcifying organisms via photosynthesis. Seagrasses are capable of raising seawater pH and calcium carbonate saturation state during times of high photosynthetic activity. To better understand the photosynthetic potential of seagrass OA mitigation, we exposed Pacific Northwest populations of native *Zostera marina* and non-native *Zostera japonica* seagrasses from Padilla Bay, WA, to various irradiance and total CO₂ (TCO₂) concentrations ranging from ~1770 – 2100 µmol TCO₂ kg⁻¹.

Our results indicate that the maximum net photosynthetic rate ($P_{\text{max}}$) for *Z. japonica* as a function of irradiance and TCO₂ was 3x greater than *Z. marina* when standardized to chlorophyll (360 ± 74 µmol TCO₂ mg chl⁻¹ hr⁻¹ and 113 ± 21 µmol TCO₂ mg chl⁻¹ hr⁻¹, respectively). In addition, *Z. japonica* increased its $P_{\text{max}}$ 77% (± 56%) when TCO₂ increased from ~1770 to 2050 µmol TCO₂ kg⁻¹, whereas *Z. marina* did not display an increase in $P_{\text{max}}$ with higher TCO₂. The lack of response by *Z. marina* to TCO₂ is a departure from previous findings; however, it is likely that the variance within our treatments (coefficient of variation: 30 – 60%) obscured any positive effect of TCO₂ on *Z. marina* given the range of concentrations tested. Because previous findings have shown that *Z. marina* is saturated with respect to HCO₃⁻ at low pH (≥ 7.5) we, therefore, suggest that the unequivocal positive response of *Z. japonica* to TCO₂ is a result of increased HCO₃⁻ utilization in addition to increased CO₂ uptake.
Considering that *Z. japonica* displays a greater photosynthetic rate than *Z. marina* when normalized to chlorophyll, particularly under enhanced TCO₂ conditions, the ability of *Z. japonica* to mitigate OA may also increase relative to *Z. marina* in the future ocean. Higher photosynthetic rates by *Z. japonica* result in a greater potential, on a per chlorophyll basis, to increase pH and calcium carbonate saturation state—both of which affect acid-base regulation and calcification of calcifying organisms vulnerable to acidification. While it is important to consider genotypic differences throughout *Z. marina* and *Z. japonica*’s biogeographical distribution, our findings help elucidate the potential contribution both seagrasses have on variations in carbonate chemistry. Further, our results could be applied to ecosystem service models aimed at determining how specific seagrass species can be grown in a controlled setting to help mitigate OA hotspots that affect commercial shellfish aquaculture.
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Introduction

The rate at which anthropogenic fossil fuel emissions are taken up by the global oceans is shifting the acid-base balance of the oceanic carbonate system, leading to a decrease in carbonate mineral saturation state ($\Omega_{\text{ar/cal}}$) and pH, in a process known as ocean acidification (OA) (Sabine et al. 2004, Orr et al. 2005, Hönisch et al. 2012). While an increasing baseline of CO$_2$ will affect the entirety seawater carbonate chemistry, coastal ocean carbonate chemistry is predominately driven by biological metabolism, riverine discharge and associated organic matter composition, tidal pumping, upwelling, nutrient input, and eutrophication (Feely et al. 2008, Cai 2011, Duarte et al. 2013, Waldbusser and Salisbury 2014, Wallace et al. 2014). These myriad factors driving coastal ocean carbonate chemistry induce high variability to the system, and result in periodic and episodic pH and saturation state decreases that are more extreme than the $\sim$0.4 pH and $\sim$1.5 $\Omega_{\text{ar}}$ decreases that are predicted for in open ocean surface waters by the end of the century (Duarte et al. 2013, Ciais et al. 2014, Waldbusser and Salisbury 2014). The high variability of coastal ocean carbonate chemistry is expected to be superimposed on the long term global trend of increasing seawater CO$_2$, potentially leading to even more extreme conditions for near-shore coastal waters (Harris et al. 2013, Hauri et al. 2013, Waldbusser and Salisbury 2014). It is possible, however, that biological photosynthetic and respiration cycles, which in large part control coastal ocean carbonate chemistry, may respond in a way that could dampen or exacerbate the magnitude of extreme carbonate chemistry events. Due to the economic and social vulnerability of human coastal communities to near-shore acidification (Ekstrom et al. 2015), it is imperative to understand how coastal acidification may be mitigated on a local scale in order to prevent the negative effects on ecologically important organisms and ecosystems.
A broad range of organisms will exhibit impaired survival, growth, and calcification from the effects of acidification—increasing the potential for shifts in ecosystem dynamics by altering species distribution, diversity, and organismal competitive interactions (Doney et al. 2009, Kroeker et al. 2010, 2013, Gaylord et al. 2015). Due to the natural variability of the carbonate system in near-shore coastal waters, however, it has been suggested that resident calcifying organisms vulnerable to the effects of acidification may be more physiologically resilient if they demonstrate plasticity and are pre-adapted to highly variable carbonate systems (Pörtner 2008, Hofmann et al. 2011, Bernhardt and Leslie 2013). While some organisms may indeed demonstrate an increased tolerance to highly variably carbonate chemistry, it is important to recognize species-specific resilience to acidification. For example, a recent study comparing the effects of acidification on larval mussels found there to be no difference in development and growth between mussels pre-adapted to areas that experience season upwelling—and therefore high spatial and temporal carbonate chemistry variability—and those local to an area without upwelling (Waldbusser et al. 2015a).

While many calcifying organisms will be negatively affected by acidification, some autotrophs may benefit from rising concentrations of seawater CO$_2$ by increasing photosynthesis and growth (Kroeker et al. 2010, 2013, Koch et al. 2013). The positive effects of increasing CO$_2$ on organisms such as macrophytes have spurred research investigating the potential mitigating effects of seagrass photosynthesis on acidification (Unsworth et al. 2012, Manzello et al. 2012, Hendriks et al. 2014). OA mitigation by seagrass specifically refers to the removal of CO$_2$ from seawater, which raises the pH and reduces the TCO$_2$ (i.e., sum of all forms of carbonic acid and its conjugate bases), minimizing unfavorable carbonate chemistry prevalence (Marbà 2006, Unsworth et al. 2012). The premise of seagrass as OA refuge originates from evidence indicating
that in near-shore, shallow coastal waters, most seagrass systems tend to be net autotrophic, sequestering large amounts carbon (Duarte et al. 2010, 2011, Unsworth et al. 2012). Net autotrophic seagrass systems, however, foster enhanced rates of respiration, due to the accumulation of allochthonous organic matter within their canopies, and from dark respiration by seagrass themselves; this results in a biologically dominated carbonate system with high variability on a diurnal scale (Koch et al. 2006, Duarte et al. 2013, Hendriks et al. 2014, Waldbusser and Salisbury 2014, Wallace et al. 2015).

Three different mechanisms, or a combination of, appear to act as the primary means for TCO₂ uptake: direct CO₂ uptake from the DBL, carbonic anhydrase (CA) secretion into the cell wall which dehydrates bicarbonate (HCO₃⁻ + CA → CO₂), and symport of HCO₃⁻ via proton pumping (Beer et al. 2002, Larkum 2006, Koch et al. 2013). In addition, studies have found that increases in seawater TCO₂ can enhance seagrass photosynthesis, growth, biomass, and tissue thickness (Beer and Koch 1996, Thom 1996, Zimmerman 1997, Kroeker et al. 2013, Koch et al. 2013, Cox et al. 2016). These findings are not universal, however, and the capacity of TCO₂ uptake by seagrass varies by species, geographical location, distribution depth, and the process by which TCO₂ moves across the diffusive boundary layer (DBL) and cell wall (Larkum 2006, Lee et al. 2007, Koch et al. 2013). Due to species-specific physiology, the full potential and ability to uptake TCO₂ varies, as evidence shows mixed results of HCO₃⁻ saturation points and changes in photosynthetic rate when exposed to high TCO₂ levels under replete and limited light intensities (Beer and Koch 1996, Zimmerman et al. 1997, Palacios and Zimmerman 2007, Invers et al. 2001, Ow et al. 2016, Cox et al. 2016). Even though ambiguity remains regarding the mechanisms and degree of TCO₂ uptake, studies have determined that most seagrass species are carbon limited at present day TCO₂ levels (Larkum 2006 and references therein, Koch et al.)
The apparent TCO$_2$ limitation of seagrasses, therefore, has implications for how individual species will respond to a globally increasing TCO$_2$ baseline, which will likely alter the photosynthetic potential, and affect the amelioration of OA by seagrasses.

Numerous studies have examined the long-term carbon sequestration potential of seagrass beds (Duarte et al. 2005, 2011, Chung et al. 2011, McLeod et al. 2011, Fourquarean et al. 2012, Marbà et al. 2015, Poppe 2015). While burial determines long-term effects of seagrass on carbon cycling, it does not drive OA-mitigation potential on short timescales; rather, instantaneous photosynthetic rates are more relevant. Short-term carbon drawdown on the hourly scale coincides with timescales of rapid development for calcifiers, for whom sensitivity to OA is driven by the duration and intensity of exposure (Kurihara 2008, Talmage and Gobler 2009, Hettinger et al. 2012, Waldbusser et al. 2015b). The continued uptake of CO$_2$ by surface waters will increase the baseline CO$_2$ affecting the frequency, duration, and magnitude of acidification events in coastal waters (Harris et al. 2013, Hauri et al. 2013, Waldbusser and Salisbury 2014). That is, increasing the duration and magnitude of carbonate chemistry variability will likely result in conditions that periodically surpass physiological thresholds of resident organisms that already exist at their tolerance limits (Grantham et al. 2004, Waldbusser and Salisbury 2014). Seagrasses, however, may be able to improve times of favorable carbonate chemistry, or dampen episodic extremes of unfavorable carbonate chemistry on hourly timescales, thus lessening the duration and magnitude of exposure to extreme acidification.

In the U.S. Pacific Northwest (PNW) where OA mitigation initiatives have been established for the Salish Sea, seagrasses have been specifically identified as a biological means to offset the effects of acidification (Blue Ribbon Panel on Ocean Acidification 2012). Two
seagrass species found in the Salish Sea: native *Zostera marina* L. and non-native *Zostera japonica* Ascher. & Graeb. have strong habitat-association with organisms vulnerable to OA (Harrison 1982a, Ferraro and Cole. 2012, Mach et al. 2014, Dumbauld and McCoy 2015). Whereas *Z. marina* has been the focus of many studies examining its high photosynthetic rate (Lee et al. 2007) and response to TCO$_2$ increase (Beer and Koch 1996, Thom 1996, Zimmerman et al. 1997, Koch et al. 2013), little work has been done investigating *Z. japonica* photosynthesis and physiology for established PNW populations (Shafer et al. 2011, Shafer and Kaldy 2014, Kaldy et al. 2015). In the Salish Sea, *Z. japonica* occurs in the mid to upper intertidal zone where it has transformed previously unvegetated mudflats via continued expansion of its distribution, and has thus been labeled as a noxious weed in Washington state (NWCB 2012, Thom et al. 2014). Conversely, *Z. marina* is a protected species with a distribution extending from the lower intertidal to shallow subtidal region: species overlap with *Z. japonica* can occur in the lower intertidal zone on flat shorelines (Harrison 1982a, Thom 1990, Ruesink et al. 2010, Thom et al. 2014). Despite *Z. japonica*’s designation as a noxious weed in Washington state, studies have highlighted that the non-native seagrass provides many of the benefits associated with other seagrasses, including predator protection, sediment stability, and enhanced community productivity and diversity (Orth et al. 1984, Duffy 2006, Wonham and Carlton 2005, Mach et al. 2014). Given the potential of *Z. japonica* to colonize unvegetated habitat and, thus, increase the expanse of seagrass meadows, it is relevant to consider how the photosynthetic potential of *Z. japonica*—in addition to *Z. marina*—impacts and interacts with the carbonate system, given that seagrass OA mitigation is a focus in the Salish Sea (Blue ribbon panel on ocean acidification 2012)
To better understand how *Z. marina* and *Z. japonica* may mitigate the effects of acidification, studies investigating the photosynthetic potential and efficiency in response to TCO\(_2\) increase are needed. A recent study comparing *Z. marina* and *Z. japonica* photosynthesis found that *Z. japonica* has a higher photosynthetic rate; however, the results are specific to populations on the central Oregon coast (Shafer and Kaldy 2014), and geographical location most be considered as local adaptations exists throughout a species’ distribution (Backman 1991 and references therein). Given the importance of site-specific physiology, we investigated the differential responses of photosynthetic rates over a spectrum of irradiance and seawater TCO\(_2\). Specifically, we conducted one experiment per species to examine the (1) potential differences in photosynthetic rates between *Z. marina* and *Z. japonica*, (2) how changes in TCO\(_2\) affect photosynthetic rates, and (3) how the photosynthetic potential is likely to induce hourly changes on the carbonate system.

**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* beds in Padilla Bay are considered to be one of the most contiguous stands on the North American Pacific Coast, constituting a submerged and emergent total area of ~4,000 ha, where *Z. marina* accounts for ~3,000 of the total area (Bulthuis 2013). In the lower intertidal region where *Z. japonica* distribution diminishes and transitions to *Z. marina* moving offshore, the morphological difference of *Z. japonica* is conspicuous, with a surface area and mass that is ~5x less than that of *Z. marina* when comparing equal length segments. In the lower intertidal where species overlap occurs, a 250 m
transect was marked for shoot collection two weeks prior to sampling. (48° 29' 36.6” N, 122° 29' 8.5” W). Two HOBO Pendant Temperature/Light 64k data loggers were attached ~1 m above the sediment on transect marking poles for the purpose of obtaining irradiance data at the elevations where Z. japonica and Z. marina co-occur (average tidal height of two consecutive sunny days used in analysis ranged from -0.55 – 3.0 m MLLW). Light sensors logged irradiance for ~12 days before they were collected from the field.

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 along the entire 250 m transect. Ten days later (2nd experiment) on August 26, 2015, adult Z. japonica shoots with intact rhizomes were collected in the same manner at low tide (~0.0 MLLW). Approximately 200 shoots of each species were collected, placed in a cooler, and transported to Shannon Point Marine Center in Anacortes, Washington, within 1 hour of collection. One hundred of the most healthy looking 200 shoots (i.e., shoots without visible damage and well preserved rhizomes) were then haphazardly selected, rinsed with seawater, and dispersed among four separate 40-liter acrylic flow through tanks under low irradiance (~50 umol photons m\(^{-2}\) s\(^{-1}\)) on a 12:12 L:D cycle for ~48 hours before experimentation. The 48 hour holding period also served the purpose of acclimating shoots to a slightly higher salinity than found at collection site: ~28 – 29 PSU.

*Experimental system.* A light fixture housing five T5 high output 54W 6500K Spectralux bulbs was mounted to the bottom of a box with one open side, and was used as the experimental chamber for both experiments. A 5/8” clear acrylic sheet covered the top of the light fixture. Five, shallow, three-sided clear acrylic boxes (56.5 x 7.62 x 3.81 cm) fitted with flow through seawater taps were mounted to the acrylic sheet and placed directly above an individual bulb in
the fixture. Shallow acrylic boxes served as water baths to stabilize temperature, and as position holders for incubating vials (Fig. 1). 20 ml borosilicate scintillation vials with a polyethylene cone-shaped liner were used as incubation vessels, and were nearly fully submerged (water line stopped at cap) when placed in acrylic water baths. Vinyl mesh wraps were constructed and fitted to scintillation vials to attenuate light. Mesh covers either had one, three, or six layers, which provided an irradiance range from ~40 – 500 µmol photons m⁻² s⁻¹.

Seawater pumped from Guemes Channel was collected, 0.2 µm filtered, sterilized via autoclave, and distributed into five, 20 L polycarbonate carboys. To manipulate CO₂, carboys were fitted with custom plumbed lids for gas exchange, and bubbled with a controlled (Sierra SmartTrak mass flow controller) mixture of compressed ambient air stripped of CO₂ using a regenerative molecular sieve adsorber (Twin Tower Engineering, CAS2-11), and research grade-5 pure CO₂ gas for ~72 hours before use. Carboys were held in an incubator which maintained water temperature at approximately ambient in Guemes Channel: 12.5°C. One liter Nalgene bottles half-way filled with tap water acted as humidifiers, which were plumbed in between gas lines and carboys to minimize the evaporation that occurs when the compressed, dry, and stripped CO₂ air is streamed through the system.
Figure 1. Experimental setup. Light bulbs illuminate from under clear acrylic sheet. Five clear acrylic trays with flow through seawater taps served as water baths that housed experimental vials. Total of 150 vials per experiment: 5 light and 5 pCO₂ conditions yield 25 treatments.
Experimental Design. Leaf segments harvested from shoots served as the experimental unit for both experiments. Approximately 20 hours before experimentation, the middle section of the second youngest leaf in a shoot was selected, wiped clean of epiphytes, and a 2 cm segment for *Z. marina* and 4 cm segment for *Z. japonica* was excised. It should be noted that while the middle section of the leaf was selected for, variation among shoots regarding the overall length of the 2nd youngest leaf existed. Each segment was then cut in half with one segment frozen for chlorophyll extraction, and the other placed into a 20 ml scintillation vial filled with 5 ml of filtered and sterilized seawater. Leaf segment stocked vials were then held in an incubator at 12°C until experimentation.

We used a 5 x 5 factorial design that targeted 25 treatment combinations with an estimated irradiance and $pCO_2$ range of ~750, 500, 200, 40, 0 µmol photons m$^{-2}$ s$^{-1}$ and ~100, 250, 400, 650, and 900 µatm, respectively. Experimental design consisted of quadruplicate replication and duplicate blanks. Blanks mimicked experimental conditions but lacked leaf segments, and were used to account for any changes in seawater chemistry induced by microbial activity that may have remained after filtration and sterilization. The design resulted in a total of 100 leaf segments and 50 blanks per experiment. Light intensities for treatment values were based on deployed HOBO light pendants at marked transect, and $pCO_2$ values were selected given the *in situ* variability of the carbonate system in Padilla Bay (Love et al. 2016).

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 placed in each vial for the purpose of stirring the seawater. Filled and stocked vials were pre-assigned mesh coverings (no mesh, 1 layer, 3 layer, 6 layer, or opaque) to achieve desired irradiance levels for each leaf segment. Vials were haphazardly placed into water
baths inside lit experimental chamber and incubated for 90 minutes until termination of experiment began. Vials were physically inverted by hand, three times, every five minutes to stir water inside and prevent an oxygen rich and TCO$_2$ poor boundary layer around leaf segments. Photographs of all vials during the experiment were taken in order to identify the exact position and irradiance emitted at a given spot—irradiance measurements were then taken after the experiment by placing treatment vials back in water baths at their respective locations. Irradiance was measured at each vial location with a QSL-101 PAR irradiance sensor (Biospherical Instruments Inc.).

*Photosynthetic measurements.* Photosynthetic rates as a function of irradiance and seawater $p$CO$_2$ were determined by incubating excised leaf segments of *Z. marina* and *Z. japonica* and measuring seawater TCO$_2$ before and after incubation. During incubation, temperature in water baths was continuously monitored and recorded every 20 minutes with a Fluke 1523 reference thermometer and probe. Initial conditions of carbonate chemistry were determined for each $p$CO$_2$ treatment by collecting triplicate 350 ml total alkalinity (TA) samples in amber glass bottles with polyurethane-lined crimp-sealed metal caps, and triplicate 20 ml samples in scintillation vials for TCO$_2$ concentration from treatment seawater held in the 20 L carboys (Table 1). All initial condition TCO$_2$ and TA samples were measured for temperature and salinity (measured with refractometer), poisoned with either 30 or 10 $\mu$l of HgCl$_2$ (TA or TCO$_2$), capped, wrapped with parafilm to minimize any potential gas leakage, and refrigerated at $2^\circ$C until analysis. Salinity ranged from 30.5 – 32 $^\circ$/oo for the *Z. japonica* experiment (Table 1). The variation was likely a result of unequal effectiveness by the humidifiers during bubbling, or
from differential evaporation in autoclaved bottles used to fill carboys, thus resulting in dissimilar evaporation rates in each of the carboys.

To terminate the experiment, vials were removed one at time haphazardly across treatments, but ordinarily by replicate assignment—this allowed for an equal experimental termination time across treatments. Each leaf segment was removed from a vial, marked for incubation time, and stored in an empty vial for later dry-weight measurement. The experimental vial was immediately poisoned with 10 µl of a saturated HgCl₂ solvent, capped, wrapped with parafilm, and refrigerated at 2°C.

All TCO₂ samples were analyzed within 5 days of each experiment. TCO₂ for each sample was quantified using an Apollo SciTech AS-C3 dissolved inorganic carbon (DIC) analyzer by drawing 0.75 ml samples from the 20 ml scintillation vial 3 – 5 times until two consecutive measurements with a difference no greater than 5 µmol kg⁻¹ was measured. TA samples were titrated within 30 days of experiment using the open-cell method as in Dickson et al. (2007) with a Metrohm 888 Titrando. Certified reference material for both TCO₂ and TA analyses was used to construct a five-point standardization curve and verify accuracy of open-cell titration, respectively (Batch 144, A.G., Dickson, Scripps Institute of Oceanography). The partial pressure of CO₂ (µatm) and associated carbonate system parameters pH (total) and aragonite saturation state (Ωₘₐ) were calculated using CO2SYS (Pierrot et al. 2006) with K₁ and K₂ equilibrium constants from Mehrbach et al. (1973) and refit by Dickson and Millero (1987).

Leaf segments from the experiment were rinsed 3x with deionized water and dried at 55°C for at least 24 hours before dry mass was recorded. Frozen leaf segments were prepped for chlorophyll extraction by sonicating for 30 seconds in a 10 ml 90% acetone solution. Segments were then refrozen at -20°C for 24 hrs. and centrifuged for five minutes 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. Photosynthetic rate as a function of irradiance ($P$ vs. $E$) was normalized to chlorophyll ($a/b$) to minimize variance in photosynthetic pigment content between shoots. $P$ vs. $E$ curves were fit and photosynthetic parameters estimated using the non-linear hyperbolic tangent function described by Jassby and Platt (1976):

\[
P = P_{\text{max}} \tanh \left( \frac{aI}{P_{\text{max}}} \right) + R_d
\]

where $P_{\text{max}}$ is the maximum photosynthetic rate (µmol TCO$_2$ mg chl$^{-1}$ hr$^{-1}$), $I$ is the irradiance (µmol photons m$^{-2}$ s$^{-1}$), $R_d$ is the dark respiration rate, and $\alpha$ (photosynthetic efficiency) is the initial slope of the photosynthetic curve (µmol TCO$_2$ mg chl$^{-1}$ hr$^{-1}$ (µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$).

A multi-dimensional model was fit to determine the combined effects of irradiance and TCO$_2$ on photosynthetic rate using the function:

\[
P = P_{\text{max}} \tanh \left( \frac{aI}{P_{\text{max}}} \right) + R_d + \beta [TCO_2]
\]

where $TCO_2$ is the initial concentration of DIC (µmol TCO$_2$ kg$^{-1}$) for each $p$CO$_2$ treatment, and $\beta$ is the slope corresponding to the observed linear response of photosynthetic rate to the measured TCO$_2$ concentration. A linear response of photosynthetic rate to TCO$_2$ concentration was a more accurate predictor than a non-linear function given the range of our experimental TCO$_2$ concentrations. The estimated maximum photosynthetic rate ($P_{\text{max}}$) and photosynthetic efficiency ($\alpha$) from the model output was used to calculate the saturation irradiance:

\[
I_k = \frac{P_{\text{max}}}{\alpha}
\]
where $I_k$ is the saturation irradiance and $P_{\text{max}}$ and $\alpha$ the outputs from the two-parameter model (eq. 2). $P_{\text{max}}$ values across all treatments were then plotted as a function of initial TCO$_2$ concentration and statistically analyzed using an ANCOVA to determine any significant differences in species’ response. In addition, the constructed two-parameter non-linear model (equation 2) was used to predict the hourly photosynthetic potential of each species in Padilla Bay by inputting the in situ irradiance data from the field and the initial TCO$_2$ concentrations from the experiments. The predicted photosynthetic rate ($\mu$mol TCO$_2$ mg chl$^{-1}$ hr$^{-1}$) from the model output was then used to associate the change in TCO$_2$ to the potential change in pH and $\Omega_{ar}$ over a diurnal light period.
Table 1. Initial conditions for both *Z. marina* and *Z. japonica* experiments. Measured mean values and standard deviation of TA, TCO$_2$, temperature, salinity, and calculated values pH (total), and aragonite saturation state ($\Omega_{ar}$).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>$p$CO$_2$ treatment (µmol kg$^{-1}$)</th>
<th>TCO$_2$ (µmol kg$^{-1}$)</th>
<th>TA (µmol kg$^{-1}$)</th>
<th>$p$CO$_2$ (µatm)</th>
<th>pH (total)</th>
<th>$\Omega_{ar}$</th>
<th>Temp. °C</th>
<th>Sal. °/oo</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Z. marina</em></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><em>Z. japonica</em></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>13.6 ± 0.4</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>1956 ± 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>
Results

Incubation conditions. Experimental conditions for the leaf segment incubations were similar between experiments (Table 1). Temperature averaged 13.2 ± 0.4°C, increasing slightly over the duration of the Z. marina experiment, but remained stable at 13.4 ± 0.1°C for the Z. japonica experiment. Any possible confounding effect of temperature on photosynthetic rate was likely captured within the treatment variance due to the ordinal manner in which replicate vials were terminated. The pCO₂ of the treatments did not match that of the equilibrating gas, which ranged from 100 – 1200 µatm (see Table 1 for actual pCO₂), however, the treatment values did produce a large range in TCO₂ and were similar across both experiments. Actual pCO₂ values will be referred to henceforth as treatment values and for all calculations. The high pCO₂ treatment had the greatest difference between experiments, resulting in a pCO₂ that was 55 µatm higher for the Z. japonica experiment than for the Z. marina experiment (Table 1). It should be noted that sample size by treatment varied from 20 – 17 (Table 2) due to either loss of a sample or dissolved inorganic carbon analyzer malfunction.

P vs. E curves. The net photosynthetic rates for Z. marina and Z. japonica were characteristic of autotrophs (Fig. 2a-e), increasing photosynthetic rate with irradiance, and reaching an asymptote once light was saturating. The hyperbolic tangent function described by Jasspy and Platt (1976) fit the general trend of the P vs. E curves (eq. 1), but the photosynthetic parameters P_max and α predicted from the model were not well constrained due to the high variance within pCO₂ treatments (Table 2). The robustness of the overall model fit for all treatments was low as indicated by a high RMSE (Fig. 2a-e, Table 2). A conspicuous trend of increasing photosynthetic rate by Z. japonica with increasing TCO₂ was observable. The
estimated \( P_{\text{max}} \) for \textit{Z. japonica} was greater than \textit{Z. marina} when normalized to chlorophyll, with a difference that increased from 0 – 62 \( \mu \text{mol TCO}_2 \) mg chl\(^{-1}\) hr\(^{-1}\) as target treatments increased from 140 – 650 \( \mu \text{atm} \) (Fig. 2a-e). When normalized to dry-weight, however, there was no observable difference in \( P_{\text{max}} \) or \( \alpha \) between species, and variance was considerably larger than when normalized to chlorophyll (Appendix A). While we acknowledge that biomass-normalized results may suggest different conclusions than data normalized to chlorophyll, we choose to focus our discussion on the data with chlorophyll standardization because of the microscale changes that are present in photosynthetic pigment content along a leaf (Enríquez et al. 2002).

Respiration rates determined from the darkened and opaque vials were not statistically different across \( p\text{CO}_2 \) treatments (Appendix B). There was high variance and net photosynthesis occurring within the darkened treatment replicates for both species (Fig. 2a-e, Appendix C). We were, therefore, not able to quantitatively predict respiration rates from our model fit.
Table 2. Predicted photosynthetic parameters from hyperbolic tangent model (Eq. 1) where \( P_{\text{max}} \) is the maximum photosynthetic rate, \( \alpha \) is the photosynthetic efficiency, and \( R_d \) is the respiration rate normalized to mg chl. Errors are 95% CI for individual parameters, and the root mean square error (RMSE) of the model fit.

<table>
<thead>
<tr>
<th>Species</th>
<th>( p_{\text{CO}_2} ) treatment</th>
<th>( P_{\text{max}} )</th>
<th>( \alpha )</th>
<th>( R_d )</th>
<th>n</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>( Z. \text{ marina} )</td>
<td>140</td>
<td>137 ± 59</td>
<td>0.651 ± 0.60</td>
<td>11.9 ± 43</td>
<td>20</td>
<td>48.3</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>142 ± 103</td>
<td>0.324 ± 0.48</td>
<td>1.42 ± 32</td>
<td>18</td>
<td>34.2</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>115 ± 39</td>
<td>2.18 ± 1.8</td>
<td>−12.0 ± 34</td>
<td>20</td>
<td>32.1</td>
</tr>
<tr>
<td></td>
<td>650</td>
<td>94.8 ± 37</td>
<td>0.469 ± 0.45</td>
<td>4.53 ± 27</td>
<td>20</td>
<td>30.8</td>
</tr>
<tr>
<td></td>
<td>900</td>
<td>138 ± 60</td>
<td>2.78 ± 3.4</td>
<td>−3.41 ± 52</td>
<td>20</td>
<td>51.3</td>
</tr>
<tr>
<td>( Z. \text{ japonica} )</td>
<td>140</td>
<td>283 ± 88</td>
<td>3.01 ± 2.2</td>
<td>−50.5 ± 73</td>
<td>20</td>
<td>74.2</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>406 ± 181</td>
<td>10.3 ± 11</td>
<td>−96.2 ± 153</td>
<td>17</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>368 ± 175</td>
<td>4.06 ± 7.6</td>
<td>60.5 ± 141</td>
<td>18</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>650</td>
<td>448 ± 254</td>
<td>4.41 ± 7.3</td>
<td>69.8 ± 210</td>
<td>19</td>
<td>216</td>
</tr>
<tr>
<td></td>
<td>900</td>
<td>337 ± 151</td>
<td>4.91 ± 5.2</td>
<td>21.2 ± 130</td>
<td>19</td>
<td>123</td>
</tr>
</tbody>
</table>

Units: \( P_{\text{max}} \) and \( R_d = \mu\text{mol TCO}_2 \text{ mg chl}^{-1} \text{ hr}^{-1} \); \( \alpha = \mu\text{mol TCO}_2 \text{ mg chl}^{-1} \text{ hr}^{-1} (\mu\text{mol photons m}^{-2} \text{ s}^{-1})^{-1} \).
Figure 2. $P$ vs. $E$ curves at each mean initial $p$CO$_2$ (a) 139 µatm, (b) 219 µatm, (c) 407 µatm, (d) 668 µatm, and (e) 890 µatm (i.e., initial $p$CO$_2$ for both experiments were averaged to obtain mean initial $p$CO$_2$). Open circles are Z. japonica and closed circles are Z. marina photosynthetic rates (µmol TCO$_2$ mg chl$^{-1}$ hr$^{-1}$). Dashed black line is irradiance compensation. Negative values are respiration.
Two-parameter photosynthesis model. Net photosynthetic rates were collated across treatments for both species and fit to the combined hyperbolic tangent and linear functions describing the observed relationship of photosynthesis to irradiance and TCO₂, respectively (Fig. 3, Eq. 2). Using the entire data set and incorporating irradiance and TCO₂ resulted in a more robust prediction of the photosynthetic parameters, and a clear delineation of the species’ differential response (Fig. 4). *Z. japonica* displayed a \( P_{\text{max}} \) of 360 ± 74 µmol TCO₂ mg chl⁻¹ hr⁻¹ and an \( \alpha \) of 3.62 ± 2.1 µmol TCO₂ mg chl⁻¹ hr⁻¹ (µmol photons m⁻² s⁻¹)⁻¹ where the error is the 95% CI (Table 3). Both \( P_{\text{max}} \) and \( \alpha \) were significantly higher for *Z. japonica* than for *Z. marina*, which had a \( P_{\text{max}} \) of 113 ± 21 µmol TCO₂ mg chl⁻¹ hr⁻¹ and an \( \alpha \) of 0.840 ± 0.42 µmol TCO₂ mg chl⁻¹ hr⁻¹ (µmol photons m⁻² s⁻¹)⁻¹ where the error is the 95% CI (Table 3, Fig. 4). \( P_{\text{max}} \) for *Z. japonica* was 3x greater than *Z. marina* \( P_{\text{max}} \), and \( \alpha \) was 4x greater, indicating an overall higher maximum photosynthetic rate and efficiency for *Z. japonica* on a per chlorophyll basis. In addition, *Z. japonica* displayed a lower \( I_k \) at 99 (propagated SE = 31) µmol photons m⁻² s⁻¹, which was ~ 75% of *Z. marina*’s \( I_k \) of 131 (propagated SE = 35) µmol photons m⁻² s⁻¹. Error for both \( I_k \) values is the propagated standard error from model predicted \( P_{\text{max}} \) and \( \alpha \) (Eq. 3).

TCO₂ concentration and the TCO₂ species interaction were both significant predictors of \( P_{\text{max}} \) (Table 4). The ordinal interaction between TCO₂ and species indicates a differential species’ response of \( P_{\text{max}} \) to increasing TCO₂. The mean \( P_{\text{max}} \) of *Z. japonica* increased linearly with TCO₂ across all treatments, whereas *Z. marina* \( P_{\text{max}} \) appeared constant with increasing TCO₂ (Fig. 5). Since there was an interaction effect between the main effects TCO₂ and species, and no observable response of *Z. marina* to increasing TCO₂, further analysis was warranted. A one-way ANOVA examining the effect of initial TCO₂ on \( P_{\text{max}} \) determined there was a significant difference in mean \( P_{\text{max}} \) between initial TCO₂ concentrations for *Z. marina*: \( F_{4,53} = 3.01, p = \)
0.0258). Given the high degree of variability and low F-ratio, however, *Z. marina*’s $P_{\text{max}}$ response to TCO$_2$ remains unclear based on our findings. *Z. japonica*’s $P_{\text{max}}$ increased by ~150 µmol TCO$_2$ mg chl$^{-1}$ hr$^{-1}$ over the range of initial TCO$_2$ concentrations (Fig. 5). The increase in *Z. japonica* $P_{\text{max}}$ to increasing TCO$_2$ resulted in a broadening separation of the maximum photosynthetic rate potential between the two species at higher TCO$_2$ conditions (Fig. 5).
**Figure 3.** Net photosynthetic rate across all $p$CO$_2$ treatments for (a) *Z. marina* and (b) *Z. japonica* as a function of irradiance and TCO$_2$. TCO$_2$ values are initial concentrations corresponding to each $p$CO$_2$ treatment (Table 1).
Table 3. Predicted photosynthetic parameters from non-linear regression model (Eq. 2) with irradiance and TCO$_2$ as independent variables. $\beta$ is the slope of linear relationship between photosynthetic rate and initial TCO$_2$ concentrations. Total sample size for *Z. marina* and *Z. japonica*: n = 98 and n = 93, respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>95% CI</th>
<th>tstat</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Z. marina</em></td>
<td>$P_{\text{max}}$</td>
<td>113</td>
<td>10.7</td>
<td>21.3</td>
<td>20</td>
<td>48.3</td>
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<tr>
<td></td>
<td>$\alpha$</td>
<td>0.840</td>
<td>0.211</td>
<td>0.420</td>
<td>18</td>
<td>34.2</td>
</tr>
<tr>
<td></td>
<td>$R_d$</td>
<td>24.7</td>
<td>69.6</td>
<td>138</td>
<td>20</td>
<td>32.1</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>-0.011</td>
<td>0.035</td>
<td>0.069</td>
<td>-0.327</td>
<td>0.745</td>
</tr>
<tr>
<td><em>Z. japonica</em></td>
<td>$P_{\text{max}}$</td>
<td>360</td>
<td>37.2</td>
<td>73.9</td>
<td>9.68</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$\alpha$</td>
<td>3.62</td>
<td>1.06</td>
<td>2.12</td>
<td>3.40</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>$R_d$</td>
<td>-1185</td>
<td>286</td>
<td>568</td>
<td>-4.15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>$\beta$</td>
<td>0.613</td>
<td>0.147</td>
<td>0.292</td>
<td>4.18</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Units: $P_{\text{max}}$ and $R_d = \mu$mol TCO$_2$ mg chl$^{-1}$ hr$^{-1}$; $\alpha = \mu$mol TCO$_2$ mg chl$^{-1}$ hr$^{-1}$ (\mu$mol$ photons m$^{-2}$ s$^{-1}$)$^{-1}$. 
Figure 4. P vs. E curves for *Z. marina* (open circles) and *Z. japonica* (closed squares) for all net photosynthetic rates collated from every $p\text{CO}_2$ treatment.
Table 4. ANOVA table from ANCOVA analysis examining $P_{\text{max}}$ values with main effects TCO$_2$ and species.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Sum Sq</th>
<th>F-stat.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>1</td>
<td>1.60E+06</td>
<td>109</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TCO$_2$</td>
<td>1</td>
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<td>8.67</td>
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<tr>
<td>Species*TCO$_2$</td>
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</tr>
<tr>
<td>Error</td>
<td>109</td>
<td>1.60E+06</td>
<td>•</td>
<td>•</td>
</tr>
</tbody>
</table>
Figure 5. Maximum photosynthetic rate ($P_{\text{max}}$) of *Z. marina* (open circles) and *Z. japonica* (closed squares) as a function of initial TCO$_2$ concentration. Initial TCO$_2$ concentrations correspond to each $p$CO$_2$ treatment (Table 1). $P_{\text{max}}$ values are photosynthetic rates above $I_k$, which was predicted from the model fit for *Z. marina* (131 µmol photons m$^{-2}$ s$^{-1}$) and *Z. japonica* (99 µmol photons m$^{-2}$ s$^{-1}$).
Model predictions and field irradiance. Irradiance data from the field, and average TCO₂ concentrations from both experiments (1972 μmol kg⁻¹ for Z. marina and 1956 μmol kg⁻¹ for Z. japonica) corresponding to the median 400 pCO₂ treatment were input into the two-parameter photosynthesis model to estimate the hourly change in photosynthetic rate for both species in Padilla Bay. The predicted net photosynthesis for Z. japonica reached a maximum of 379 μmol TCO₂ mg chl⁻¹ hr⁻¹ and 115 μmol TCO₂ mg chl⁻¹ hr⁻¹ for Z. marina for ~6 hours; this indicates that saturating irradiance for both species likely occurs from ~12:00 until ~18:00 during mid to late summer (Fig. 6). The upper and lower bounds are the predicted photosynthetic rate for Z. japonica if TCO₂ concentrations were 2051 μmol kg⁻¹ and 1771 μmol kg⁻¹, which correspond to the high (pCO₂ ~900) and low (pCO₂ ~140) treatments, respectively (Fig. 6).

The predicted photosynthetic rate (μmol TCO₂ mg chl⁻¹ hr⁻¹) over a diurnal period was used to estimate the change in TCO₂ from a starting point of 1964 μmol kg⁻¹. These values were then used to determine the associated change in pH and Ω_ar mg chl⁻¹ hr⁻¹ assuming a constant TA (Fig. 7). A combined average TA of the 400 pCO₂ treatment for both experiments was used for all calculations. Z. japonica induced a maximum change in pH of 0.65 units mg chl⁻¹ hr⁻¹ at a P_max of 379 μmol TCO₂ mg chl⁻¹ hr⁻¹. The induced change in pH by Z. japonica was ~0.4 units higher than the 0.25 pH unit change associated with Z. marina’s P_max of 115 μmol TCO₂ mg chl⁻¹ hr⁻¹ (Fig. 7). The Ω_ar change associated with the projected maximum photosynthetic rate for both species resulted in a change of 3.8 and 1.1 mg chl⁻¹ hr⁻¹ for Z. japonica and Z. marina, respectively. It should be noted that the change in pH was determined from the change in TCO₂ from a single seagrass segment in a 20 ml vial, and estimations beyond this scale are likely erroneous. We, therefore, present the change in pH and Ω_ar as the maximum potential possible
based on our findings, where extrapolation is constrained within direct proximity to a portion of a seagrass leaf.
Figure 6. Predicted mean net photosynthetic rate for *Z. japonica* (-) and *Z. marina* (--) under ambient irradiance at mean initial TCO$_2$ concentration of 1964 µmol kg$^{-1}$. Shaded region is the 95% CI. Upper (red) and lower (yellow) bounds are the predicted net photosynthetic rate of *Z. japonica* at initial TCO$_2$ concentrations of 2051 and 1770 µmol kg$^{-1}$, respectively.
Figure 7. Change in pH$_{\text{total}}$ (a) and Ω$_{ar}$ (b) at the predicted mean net photosynthetic rate for Z. japonica (-) and Z. marina (--) under ambient irradiance. Shaded regions are the 95% CI for photosynthetic rate.
Discussion

Species-specific photosynthetic rate and TCO$_2$ uptake are critical components that determine the capacity of seagrasses to remove dissolved CO$_2$ and, thus, the potential of seagrass beds as OA refugia. This study examined the photosynthetic potential of co-occurring *Z. marina* and *Z. japonica* seagrasses to a range of TCO$_2$ concentrations. Our results indicate that *Z. japonica* photosynthesis has a robust response to moderate increases in TCO$_2$, whereas *Z. marina* photosynthesis displays a minor or null response to the TCO$_2$ concentrations tested (~1770 – 2100 µmol kg$^{-1}$). These findings help elucidate how TCO$_2$ dependent instantaneous photosynthetic rates of *Z. marina* and *Z. japonica* will affect the carbonate system, and what implications exist for potential OA mitigation on short timescales. That is, if seagrasses are to mitigate the effects of acidification on vulnerable organisms, the timing of TCO$_2$ uptake and shift in favorable carbonate chemistry must be congruent with the organismal life-stages that are most sensitive to acidification. For example, recent studies have shown that the initial shell formation of *Crassostrea gigas* occurs in a matter of hours, and unfavorable carbon chemistry affects the development and growth of some bivalves and gastropods during the first 48 hours post-fertilization (Onitsuka et al. 2014, Waldbusser et al. 2015b). Instantaneous photosynthetic rates occurring on the hourly timescale, therefore, can correspond directly to the time period of shell formation for some bivalves, and have the potential to dampen the magnitude and exposure duration to acidification.

*Instantaneous photosynthesis.* The instantaneous photosynthetic rates measured indicate that non-native populations of *Z. japonica* in Padilla Bay have a maximum net photosynthetic rate and efficiency that is ~3 and 4.5-fold higher, respectively, than *Z. marina* occurring in the
same intertidal spatial zone when considered on a per chlorophyll basis. We did not observe inter-species differences when standardized to biomass (App. Table 2). Whereas the exact mechanism responsible for the discontinuity between standardization methods is not clear, standardizing to chlorophyll is more appropriate given the strong gradient in chlorophyll along a leaf and among shoots, which may correspond to changes in surface area and, therefore, pigment concentration (Zimmerman et al. 1997, Enríquez et al. 2002, Larkum et al. 2006 and references therein). Given that changes in thickness of the tissue can affect chlorophyll to biomass ratio (Enríquez et al. 1994), photosynthetic rate standardized to biomass may account for spatial variation in chlorophyll and how light is absorbed.

Similarly, a previous study comparing PNW populations of Z. marina and Z. japonica also found that Z. japonica has a maximum photosynthetic rate that is ~3-fold higher than Z. marina, when exposed to the same light conditions (Shafer and Kaldy 2014). The chlorophyll standardized rates reported in that study and in Shafer et al. (2011), however, were considerably lower than what we report (Fig. 2a-e). Differences in chlorophyll extraction methods, or inefficient extraction may have resulted in the higher photosynthetic rates found in our study. Additional factors such as intertidal location, canopy density, time of year, acclimatization to epiphytic growth, and age can also affect pigment concentration and photoacclimation amongst shoots and along leaves (Dennison and Alberte 1986, Major and Dunton 2002, Enríquez et al. 2002, Duarko and Kunzelman 2002, Drake et al. 2003, Cummings and Zimmerman 2003, Larkum 2006 and references therein), which may further explain the dissimilarity in rates. 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, Martin et al. 1995, Mateo et al. 2001); therefore, 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, based on 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. 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. Measurement of TCO$_2$ for our study, however, is appropriate given that carbon drawdown specifically, rather than photosynthetic rates, drives OA mitigation potential of seagrasses.

*Effects of TCO$_2$.* Increases in TCO$_2$ enhanced the maximum photosynthetic rate for Z. japonica, while Z. marina’s response was inconclusive (Fig. 5). Even though statistical analysis found a significant difference between mean $P_{\text{max}}$ values across initial TCO$_2$ concentrations for Z. marina, the level of significance was minor ($F_{4,53} = 3.01, p = 0.0258$). Across treatments, there was no observable increase in Z. marina $P_{\text{max}}$ and, therefore, no apparent trend of increasing $P_{\text{max}}$ with increasing TCO$_2$. An effect size of 19% was determined from the one-way ANOVA analysis (Appendix D), meaning that only 19% of $P_{\text{max}}$ variance was attributable to initial TCO$_2$ concentration. The lack of enhanced photosynthesis by Z. marina in response to increasing TCO$_2$ is clearly contradictory to what has been previously reported for instantaneous photosynthetic
rates (Beer and Koch 1996, Thom 1996, Zimmerman et al. 1997); however, the manipulated TCO₂ concentrations in those studies were, at minimum, 25% higher than our highest TCO₂ concentration. It is likely that the range of TCO₂ concentrations used for this study was not large enough to produce a response of increased $P_{\text{max}}$ for *Z. marina* that could be detected given the high variability within our treatments. Zimmerman et al. (1997) showed that *Z. marina* increased its $P_{\text{max}}$ 225% with an exposure to TCO₂ 77% higher than ambient (from 2074 to 3673 µmol kg⁻¹). Over this range of TCO₂, a linear relationship exists between $P_{\text{max}}$ and TCO₂ (Beer and Koch 1996). Using a linear equation derived from Zimmerman et al. (1997) and our mean $P_{\text{max}}$ values at the lowest and highest TCO₂ concentrations, an 18% increase in TCO₂ would induce a 55% increase in *Z. marina* $P_{\text{max}}$. Given that the coefficient of variation (CV) ranged from 30 to 60% over all initial TCO₂ concentrations, any response to TCO₂ by *Z. marina* was likely obscured by the variance. Interestingly, however, a robust response was observed for *Z. japonica*, which increased its mean $P_{\text{max}}$ 55% over the 18% increase in TCO₂, despite a CV identical to *Z. marina* (30 – 60%). Physiological and methodological factors contributing to the large variance within treatments may be a result of shoot to shoot variability of the photosynthetic apparatus, and the propagation of a diffusive boundary layer due to incremental stirring, which would increase the variability of the relative TCO₂ conditions experienced by the leaf segment compared to the entire vial.

The range of $p\text{CO}_2$ values and corresponding TCO₂ concentrations (Table 1) used for this study are representative of present day conditions in Padilla Bay, which exhibit large diurnal fluctuations in carbonate chemistry (Love et al. 2016). Based on our findings, *Z. japonica* will have a substantially greater response to TCO₂ fluctuation than will *Z. marina* in Padilla Bay. The differential response of $P_{\text{max}}$ to TCO₂ between species may be due to the mechanism by which
HCO$_3^-$ is utilized for photosynthesis. Evidence suggests that seagrass occurring in the intertidal, such as the Z. marina samples in our study, are adapted to more efficiently utilize HCO$_3^-$ than seagrass found at depth (Schwarz et al. 2000). Z. marina has been shown to take up HCO$_3^-$ as an inorganic carbon source via external carbonic anhydrase (CA) secretion, and is saturated with respect to HCO$_3^-$ at pH from 7.5 – 8.5 (Beer and Rehnberg 1996, Invers et al. 2001, Koch et al. 2013), which, corresponds to the range of pH values in our study (Table 1). Since HCO$_3^-$ appears saturating at present day and future TCO$_2$ levels for Z. marina, and any enhanced effect of CO$_2$ will be greater when pH is less than ~7.5, our results suggest that Z. japonica either has a lower pH threshold at which CO$_2$ concentrations are high enough to elicit enhanced photosynthetic rates, or that HCO$_3^-$ concentrations are not saturating over the range of TCO$_2$ concentrations used in this study.

Given that there are significant variations in the ability to utilize HCO$_3^-$, and that some species have a capacity to more efficiently utilize HCO$_3^-$ depending on the mechanisms exploited (Invers et al. 2002, Koch et al. 2013, Campbell and Fourqurean 2016) we, therefore, suggest that Z. japonica is less efficient than Z. marina in HCO$_3^-$ uptake. While we did not specifically measure the ability or mechanism of HCO$_3^-$ uptake in either species, there appears to be a higher threshold at which HCO$_3^-$ becomes saturating for Z. japonica, as evident by the shape of the response of increased $P_{max}$ to HCO$_3^-$ (Fig. 8a). The linear response of increased $P_{max}$ to HCO$_3^-$ is analogous to the response observed in figure 5 to TCO$_2$. This suggests that the dominant driver of enhanced photosynthetic rate over the range of TCO$_2$ tested is likely a response to HCO$_3^-$ and not CO$_2$, which produces a different $P_{max}$ response curve (Fig. 8b). Evidence supports that Z. marina has established mechanisms to efficiently use HCO$_3^-$ (Beer and Rehnberg 1997, Invers et al. 2001) when CO$_2$ concentrations are low; however, the mechanisms of HCO$_3^-$ utilization by Z.
*Z. japonica* remain unknown. If *Z. japonica* is indeed able to increase its maximum photosynthetic rate when HCO$_3^-$ concentrations are higher, than *Z. japonica* may be one of the few seagrass species to respond positively to moderate increases in seawater TCO$_2$, thus providing an increasingly more beneficial role in OA mitigation over *Z. marina*.

The high energetic cost behind HCO$_3^-$ utilization is due to the active mechanisms by which it occurs: H$^+$ pumping and CA secretion (Larkum 2006 and references therein, Koch et al. 2013); therefore, an energetic constraint may be a reason as to why HCO$_3^-$ uptake may not be as efficient for *Z. japonica*. Due to its distribution predominately in the high intertidal (Shafer et al. 2014), *Z. japonica* likely has to invest more energy into maintaining osmotic and thermal homeostasis due to the higher extremes of irradiance and salinity that occur throughout its distribution in the intertidal. Previous studies have found that *Z. japonica* invests in high concentrations of UV protective pigments and can tolerate chronic hypo and hyper saline conditions (Kaldy 2006, Shafer et al. 2011). Energy investment into these physiological characteristics which enable tolerance of unfavorable thermal and osmotic pressures likely result in a tradeoff, or lack of investment in other processes, such as active HCO$_3^-$ utilization. This does not, however, contradict the overall greater photosynthetic potential displayed by *Z. japonica* when TCO$_2$ concentrations are not elevated. Rather, mechanisms other than CO$_2$ substrate availability may be the reason for higher yields, such as the structure of the photosynthetic package, which determines the reflectance and absorbtance by the photosynthetic pigments (Cummings and Zimmerman 2003, Zimmerman et al. 2006).

The differential response of *Z. marina* and *Z. japonica* to increasing TCO$_2$ has implications for species’ success, performance, and expansion in a high CO$_2$ world. Based on our findings, it is likely that *Z. japonica* will benefit more from the increasing CO$_2$ baseline due to its
ability to increase HCO$_3^-$ utilization (Fig. 8a) as well as benefiting from increased CO$_2$ uptake. The greater response of *Z. japonica* to TCO$_2$ will lead to an increase in growth rate for above and below ground biomass. The increase in growth, however, does not necessarily translate to an increased competition effect with *Z. marina*. Evidence suggests that *Z. japonica* distribution offshore (i.e., an encroaching presence into *Z. marina*’s upper distribution) is limited by temperature, and that the morphological difference between the two species results in a competitive dominance of *Z. marina* over *Z. japonica* due to its broader leaves and canopy height, which shade *Z. japonica* (Harrison 1982b, Kaldy et al. 2015). While *Z. japonica* presence offshore is less likely due to thermal tolerance and competition with *Z. marina*, its continued colonization of unvegetated habitat higher in the intertidal may increase if growth is enhanced at higher TCO$_2$. The expansion into unvegetated habitat could provide additional ecological benefits for species (Shafer et al. 2014), as well as a spatial expanse of seagrass induced modification of carbonate chemistry throughout the intertidal zone.
Figure 8. Mean maximum photosynthetic rate ($P_{\text{max}}$) of *Z. marina* (open circles) and *Z. japonica* (closed squares) as a function of HCO$_3^-$ (a) and CO$_2$ (b). Error bars are SD. Discrete HCO$_3^-$ and CO$_2$ concentrations are the initial values for each treatment corresponding to $p$CO$_2$ and TCO$_2$ values (Table 1). Teal dashed lines are the linear fit applied to *Z. marina*’s response to HCO$_3^-$ and CO$_2$. Blue dashed lines are the linear (a) and non-linear ($2^{\text{nd}}$ order polynomial) (b) best fit of *Z. japonica*’s $P_{\text{max}}$ to HCO$_3^-$ and CO$_2$. 

\[ Z. marina \quad Z. japonica \]
Predicting OA mitigation potential. Measuring instantaneous photosynthetic rates of *Z. marina* and *Z. japonica* as a change in TCO$_2$, and assuming alkalinity constant, provides a constraint on the carbonate system; thereby providing a means to predict how the photosynthetic potential of each species will affect the carbonate chemistry of the surrounding water. Light sensor data from the field and initial TCO$_2$ concentrations were used to parameterize the two-parameter irradiance-TCO$_2$ model (Fig. 3), and predict how photosynthetic rate changes by the hour for *Z. marina* and *Z. japonica* in Padilla Bay (Fig. 6). Additionally, estimates of the corresponding change in pH and aragonite saturation state were calculated based on the photosynthetic rate of each species (Fig. 7). Importantly, the induced changes on the carbonate system are approximate values and correspond to seawater directly adjacent to a photosynthesizing leaf segment on a microscale level. Nevertheless, this gives better insight as to the potential change in the carbonate system made possible by *Z. marina* and *Z. japonica*. Our results indicate that *Z. japonica* displays a 3-fold higher photosynthetic potential and, therefore, has a stronger affect on the carbonate system on a per chlorophyll basis. Importantly, the estimated changes in pH and Ω$_{ar}$ are only representative at given temperature and salinity (Fig 7).

To integrate our findings into a more applicable context of OA-mitigation, the specific changes induced on the carbonate system by both species’ maximum photosynthetic potential were extrapolated to different volumes of water corresponding to various depths over a 1 m$^2$ patch of seagrass with a biomass of 100 gDW m$^{-2}$. Biomass estimates from Padilla Bay were approximated from Bulthuis (2013), where gDW m$^{-2}$ ranges from ca. 60 – 200 for both species. Assuming a well-mixed water column, average mg chl gDW$^{-1}$ ($C_b$) as measured for each species and projected $P_{max}$ values ($r$) were used to calculate a change in TCO$_2$ as:

$$\Delta TCO_2 = TCO_{2i} \left( C_b \cdot \left( \frac{b_{n}}{d} \right) \cdot \left( \frac{1}{d} \right) \cdot f \cdot r \right)$$
where $TCO_2$ is the initial TCO$_2$ concentration, $C_b$ is mg chl gDW$^{-1}$, $b_i$ is the gDW m$^{-2}$, $d$ is depth (m), and $f$ is the unit conversion m$^3$ 1000 L$^{-1}$ (summarized, Table 5). Changes in TCO$_2$ were then used to determine changes in carbonate chemistry parameters. It is important to note that the chlorophyll to biomass ratio for our experiment was considerably lower than what has been previously reported (Shafer and Kaldy 2014), and inefficiencies in our chlorophyll extraction could overestimate the predicted changes. Despite the differences in our chlorophyll to biomass measurements, the general trend and relative difference between Z. marina and Z. japonica photosynthesis on carbonate chemistry is clear. Specifically, the change in pH, aragonite saturation state, and the substrate-to-inhibitor ratio ([HCO$_3^-$]/[H$^+$], all of which have been shown to affect calcification and acid-base regulation of organisms sensitive to acidification (Pörtner 2008, Kroeker et al. 2013, Waldbusser et al. 2015a, b Thomsen et al. 2015, Fassbender et al. 2016) were approximated (Fig. 9). Theses changes, however, are specific to the timescale of TCO$_2$ uptake via photosynthesis, and will shift with changes in photosynthetic activity and respiration on an hourly scale. While our findings are based on Z. marina and Z. japonica populations in the lower intertidal of Padilla Bay, extrapolation of the induced changes on the carbonate system by species-specific photosynthesis may be applicable to other locales with similar physical conditions and ecological structure. Importantly, the distribution of both species needs to be taken into account when determining the total effect each species has on carbonate chemistry. For example, our findings suggest that Z. japonica has a stronger affect on carbonate chemistry than Z. marina; however, the 3:1 greater hectare coverage of Z. marina in Padilla Bay (Table 5) results in a larger-scale affect by Z. marina than Z. japonica. In addition, our findings could be applied and referenced when determining aquaculture techniques aimed at improving
carbonate chemistry conditions in more controlled environments, which is an initiative of the shellfish industry in Washington state (Blue ribbon panel on ocean acidification 2012).
Table 5. Analytical and predicted parameters mg chl gDW\(^{-1}\), ΔTCO\(_2\), and P\(_{\text{max}}\) for *Z. marina* and *Z. japonica*. Parameters were used in conjunction with approximated biomass m\(^2\) to calculate changes in the carbonate system (Fig. 9). *Reported from Padilla Bay (PB) in Bulthuis (2013).

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean initial TCO(_2)</th>
<th>Mean chl:biomass ratio</th>
<th>Predicted P(_{\text{max}})</th>
<th>Est. biomass* (gDW m(^{-2}))</th>
<th>Coverage species(^{-1}) in PB* (ha)</th>
</tr>
</thead>
<tbody>
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<td><em>Z. marina</em></td>
<td>1964</td>
<td>1.88 ± 0.89</td>
<td>115</td>
<td>100</td>
<td>3046</td>
</tr>
<tr>
<td><em>Z. japonica</em></td>
<td>1770</td>
<td>0.67 ± 0.33</td>
<td>193</td>
<td>100</td>
<td>836</td>
</tr>
<tr>
<td></td>
<td>1964</td>
<td>0.67 ± 0.33</td>
<td>379</td>
<td>100</td>
<td>•</td>
</tr>
<tr>
<td></td>
<td>2051</td>
<td>0.67 ± 0.33</td>
<td>490</td>
<td>100</td>
<td>•</td>
</tr>
</tbody>
</table>

Units: TCO\(_2\) = umol kg\(^{-1}\); P\(_{\text{max}}\) = µmol TCO\(_2\) mg chl\(^{-1}\) hr\(^{-1}\); chl:biomass = mg chl gDW\(^{-1}\).
Figure 9. Blue lines are the predicted change in pH, aragonite saturation state, and substrate-to-inhibitor (mol µmol$^{-1}$) ratio based on the predicted $P_{\text{max}}$ (µmol TCO$_2$ mg chl$^{-1}$ hr$^{-1}$) of *Z. marina* (open circles) and *Z. japonica* (closed squares) with an initial TCO$_2$ concentration of 1964 µmol kg$^{-1}$. Red and yellow lines are the upper and lower bound of the predicted change in pH, aragonite saturation state, and substrate–to-inhibitor ratio of the high and low $p$CO$_2$ treatment of *Z. japonica* at initial TCO$_2$ concentrations of 2051 and 1770 µmol kg$^{-1}$, respectively. Predicted change is over a 1 m$^2$ area and given depth assuming a biomass of 100 gDW m$^{-2}$ (Table 5, Eq. 4). Initial TCO$_2$ concentrations correspond to $p$CO$_2$ treatments at specific experimental conditions of TA (TA was averaged for 400 $p$CO$_2$ treatment), temperature, and salinity (Table 1).
Dynamics of seagrass beds. Recent studies have highlighted the extreme spatial and temporal variability that exists when attempting to determine potential mitigating effects macrophytes may have on unfavorable carbonate chemistry (Hendriks et al. 2014, 2015, Krause-Jensen et al. 2015, Challener et al. 2016). In addition to seagrass productivity and respiration, heterotrophic respiration, tidal exchange, and riverine input further modify carbonate chemistry on various spatial and temporal scales. Because organisms vulnerable to OA are more sensitive at particular life-stages, the temporal variability of carbonate chemistry becomes critical in determining organismal resilience to OA (Kurihara 2008, Hettinger et al. 2012, Barton et al. 2012, Waldbusser et al. 2015b). Importantly, the spatial heterogeneity of carbonate chemistry within seagrass beds is equally important. Variations in chlorophyll content along the leaf and amongst shoots will result in differential photosynthetic rates within the seagrass canopy, thereby creating disparate water parcels with respect to TCO₂ on various spatial scales. In addition, the density of seagrass will result in different mixing rates and flow regimes that will further modify carbonate chemistry in time and space (Peterson et al. 2005, Koch et al. 2006, Marbà et al. 2006, Hendriks et al. 2014). The flow of water, TCO₂ concentration, and light, interact in a way that, together, determine carbon assimilation and uptake (McPherson et al. 2015), which directly affects the scale of seagrass OA mitigation. It is evident, then that spatial microzones of differential carbonate chemistry exist within the canopy of a seagrass bed. Interestingly, vulnerable calcifying organisms may exploit these microzones of refuge, as carbonate chemistry chemical cues have been shown to drive substrate selectivity for bivalves transitioning to the benthos (Green et al. 2013, Clements et al. 2016). This suggests that the importance of spatial carbonate chemistry heterogeneity is critical when predicting seagrass OA mitigation potential.
A multitude of other biological factors also determine the variability of carbonate chemistry within seagrass beds. Epiphytes growing on seagrass leaves can account for over 50% of the primary production in some seagrass meadows, however, the dominance of epiphytic photosynthesis over seagrass varies depending on the season (Thom 1990, Wear et al. 1999, Borowitzka et al. 2006). Epiphytic growth can vary dramatically depending on the morphology of seagrass—that is, small changes in morphology can lead to large changes in epiphytic assemblages (Borowitzka et al. 2006). The large percent of primary production by epiphytes is likely a dual component of reduced photosynthesis by seagrass leaves from epiphytic shading, and surface area abundance of algae epiphytic growth. Since our experiment did not include epiphytic photosynthesis as they were removed from our samples, it is necessary to understand and consider the contribution to, and role of, epiphyte primary production on carbonate chemistry when examining seagrass OA mitigation potential.

The variability in carbonate chemistry driven by biological cycling of CO₂ in seagrass beds is not just a result of shoot and epiphytic autotrophy, but by the significant contributions of carbon fixation via the microphytobenthos (Kaldy et al. 2002) and elevated rates of heterotrophic respiration fueled by high concentrations of organic matter retained within the canopy (Barrón et al. 2006 and references therein). In addition, seagrasses excrete small quantities of DOM into the water column and sediments stimulating heterotrophy, which in turn reduces net community production (Benner et al. 1986). Roots and rhizomes diffuse oxygen into the sediments, creating rich oxic layers that are exploited by benthic heterotrophs; the increased benthic respiration not only produces a positive flux of CO₂ out of the sediment, but also generates corrosive waters that actively dissolve the carbonate rich sediments associated with seagrasses (Marbà et al. 2006, Mazarrasa et al. 2015). Dissolution of carbonate sediments has a direct affect on the carbonate
system—that is, higher carbonate availability from CaCO$_3$ dissolution increases the buffering capacity of the system. Together, the myriad biological processes add substantial heterogeneity to the carbonate system within seagrass beds and, although, recent studies have quantified the ecosystem effect on the carbonate system as a whole (Manzello et al. 2012, Hendriks et al. 2014), the complexity of the system can hinder extrapolation to other seagrass systems in time and space.

Conclusions. 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. In the higher intertidal zone where species overlap occurs, our results indicate that Z. japonica has a 3-fold greater photosynthetic potential than Z. marina when normalized to chlorophyll (Fig. 4). By measuring photosynthetic potential as a change in TCO$_2$, our findings could be extrapolated to estimate the predicted change in TCO$_2$ given measured mg chl gDW$^{-1}$ and reported biomass m$^{-2}$ in Padilla Bay (Bulthuis 2013) (Table 5, Fig. 9). Z. japonica appears to be better suited to mitigate OA on a per chlorophyll basis, given its higher photosynthetic rate and efficiency (Fig. 7 and 9). However, due to the 3:1 greater area covered by Z. marina over Z. japonica in Padilla Bay, Z. marina would have a 2.5x greater effect on carbonate chemistry when accounting for species total abundance. Additionally, our results indicate that Z. japonica stands to benefit more from moderate increases in TCO$_2$ by up regulating its photosynthetic potential (Fig. 5). The greater response of Z. japonica to TCO$_2$ may be a result of not only increased CO$_2$, but also increased HCO$_3^-$ concentrations, whereas Z. marina appears to be saturated with respect to HCO$_3^-$ (Fig 8a) (Invers et al. 2001). Due to the high variability within our treatments, any positive response to moderate increases in TCO$_2$ by Z. marina was likely obscured (Fig. 5, 8).
While a broader perspective of carbonate chemistry heterogeneity in time and space in situ will be needed when assessing potential seagrass OA mitigation, the photosynthetic potential of individual species with respect to not only irradiance and TCO$_2$, but temperature, salinity, and other environmental variables will need to be considered. This will become necessary when deciphering the favorable contributions each autotrophic group has on the carbonate system; particularly when two co-occurring seagrasses are in one specific region. Our results suggest that $Z$. japonica may increase its relative contribution to OA mitigation as TCO$_2$ rises in the coastal ocean. In addition, 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 a better understanding is needed (Blue ribbon panel on OA 2012). Further, our results could be integrated into larger scale biogeochemical models and observation networks aimed at deducing the individual contribution of seagrasses on reducing TCO$_2$, and the potential for creating areas of favorable carbonate chemistry (Khangaonkar et al. 2013, Alin et al. 2015).
References


Marbá, N., Arias-Ortiz, A., Masque, P., Kendrick, G.A., Mazarrasa, I., Bastyan, G.R., Garcia-


Appendix

A. Predicted photosynthetic parameters from hyperbolic tangent model (eq. 1) where $P_{\text{max}}$ is the maximum photosynthetic rate, $\alpha$ is the photosynthetic efficiency, and $R_d$ is the respiration rate normalized to gDW. Errors are 95% CI for individual parameters, and the root mean square error (RMSE) of the model fit. Trt. is the target PCO$_2$ treatment: 1, 2, 3, 4, 5 = 100, 250, 400, 800, and 1200 µatm, respectively.

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<th>$P_{\text{max}}$</th>
<th>$\alpha$</th>
<th>$R_d$</th>
<th>n</th>
<th>RMSE</th>
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<td>1200</td>
<td>319 ± 267</td>
<td>0.654 ± 1.0</td>
<td>38.9 ± 72</td>
<td>19</td>
<td>123</td>
</tr>
</tbody>
</table>

Units: $P_{\text{max}}$ and $R_d$ = µmol TCO$_2$ mg$^{-1}$ chl h$^{-1}$; $\alpha$ = µmol TCO$_2$ mg$^{-1}$ chl h$^{-1}$ (µmol photons m$^{-2}$ s$^{-1}$)$^{-1}$.
B. Summary of ANOVA results on respiration rates for *Z. marina* and *Z. japonica* across all *p*CO\(_2\) treatments.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum Sq.</th>
<th>d.f.</th>
<th>Mean sq.</th>
<th>F</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>3939</td>
<td>1</td>
<td>3939</td>
<td>1.2</td>
<td>0.2814</td>
</tr>
<tr>
<td><em>p</em>CO(_2)</td>
<td>25165</td>
<td>4</td>
<td>6291</td>
<td>1.92</td>
<td>0.1331</td>
</tr>
<tr>
<td>Spec* <em>p</em>CO(_2)</td>
<td>25958</td>
<td>4</td>
<td>6489</td>
<td>1.98</td>
<td>0.1233</td>
</tr>
<tr>
<td>Error</td>
<td>94833</td>
<td>29</td>
<td>3270</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Total</td>
<td>154168</td>
<td>38</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
</tbody>
</table>
C. Respiration rates for *Z. marina* (open circles) and *Z. japonica* (closed squares) across all $pCO_2$ treatments.
D. ANOVA table from one-way analysis examining $P_{\text{max}}$ values with main effect TCO$_2$.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F-stat.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCO$_2$</td>
<td>4</td>
<td>27094</td>
<td>6774</td>
<td>3.01</td>
<td>0.0258</td>
</tr>
<tr>
<td>Error</td>
<td>53</td>
<td>119078</td>
<td>2247</td>
<td>•</td>
<td>•</td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td>146173</td>
<td>•</td>
<td>•</td>
<td>•</td>
</tr>
</tbody>
</table>