

Critical Essay
Erin Morgan
July 30, 2009

Morgan

Predicted response of eelgrass (*Zostera marina* L.) photosynthesis, growth, and survival to ocean acidification

I. Introduction

Over the past 250 years, the composition of Earth's atmosphere has changed as a result of anthropogenic emissions of greenhouse gases. For example, activities such as deforestation, agriculture, and the burning of fossil fuels have produced a 31% increase in the concentration of atmospheric carbon dioxide ($[CO_2]$) since 1750 (IPCC 2001). Earth's atmosphere is linked to the oceans at the sea surface, such that alterations in atmospheric composition are reflected in the water. In particular, atmospheric CO_2 exists in equilibrium with dissolved CO_2 ($CO_{2(aq)}$) in the oceans. As the atmospheric $[CO_2]$ increases, gas exchange with the oceans causes a concomitant increase in $[CO_{2(aq)}]$, with concentrations rising toward a new equilibrium value (Goudriaan 1993, Guinotte and Fabry 2008).

The concentrations of carbon species in the ocean change according to the equilibrium equations in Figure 1. Dissolved CO_2 reacts with water to form carbonic acid, which dissociates into hydrogen, bicarbonate, and carbonate ions. As more CO_2 dissolves into the ocean, hydrogen ion (H^+) concentrations increase and ocean pH decreases (Goudriaan 1993, Orr et al. 2005). A decrease in pH inhibits the speciation of $CO_{2(aq)}$ into bicarbonate ions (HCO_3^-), such that increases in $CO_{2(aq)}$ are not followed by proportional increases in HCO_3^- (Goudriaan 1993). Essentially, this means that an increased atmospheric $[CO_2]$ results in both an actual increase in

oceanic $[\text{CO}_{2(\text{aq})}]$ as well as a proportional increase in the amount of $\text{CO}_{2(\text{aq})}$ relative to HCO_3^- in the oceans (Short and Neckles 1999).

Currently, surface ocean pH is 0.1 unit lower than preindustrial values (Orr et al. 2005) and is predicted to decrease 0.14-0.35 pH units by 2100 (Cao et al. 2007, IPCC 2007). The rapid rate of decreasing ocean pH, or "ocean acidification", is anticipated to negatively affect the survival of marine calcifying organisms that incorporate dissolved carbonate into their shells (Guinotte and Fabry 2008). However, it is predicted that ocean acidification will actually benefit some marine ecosystems. Specifically, seagrasses may experience enhanced photosynthesis and growth under more acidic conditions, translating into increased food and habitat resources for associated faunal communities (Wetzel and Grace 1983, Edwards 1995, Short and Neckles 1999, Hemminga and Duarte 2000, Palacios and Zimmerman 2007, Guinotte and Fabry 2008).

The purpose of this essay is to evaluate the claim that seagrass survival, growth, and reproduction will be enhanced in the more acidic marine environments predicted for coming centuries. Specifically, I will focus on research related to eelgrass (*Zostera marina* L.), as this widely studied species is the dominant seagrass in Pacific Northwest estuaries. This essay will: (1) review nine studies that quantify the affects of experimentally enhanced $[\text{CO}_{2(\text{aq})}]$ and decreased pH on eelgrass photosynthesis, growth, or productivity, (2) critically analyze their experimental methodologies and results, their relationship to global predictions of ocean pH, and their ecological relevance, and (3) discuss future directions for research.

II. Topic Development

This section provides background information on eelgrass identity and growth patterns. A detailed review of nine studies examining eelgrass response to increased $[\text{CO}_{2(\text{aq})}]$ and decreased pH follows.

Eelgrass Ecology

Zostera marina L. (eelgrass) is the most widespread of the 11 species within its genus and occurs throughout the temperate, northern regions of the Atlantic and Pacific oceans. It is found in muddy or sandy non-tidal, subtidal, and intertidal areas, and forms extensive meadows in many Pacific Northwest estuaries (Rasmussen 1977, Thom et al. 2003). Eelgrass has a modular structure with horizontal rhizomes divided into "nodes" where root and shoot growth occurs. Vertical shoots are composed of 2-6 strap-like leaves growing collectively from a single sheath, and a rhizome segment is produced with every new shoot (Rasmussen 1977, Short and Duarte 2001). Eelgrass can reproduce either vegetatively (clonally) or sexually, by the production of separate flowering shoots (Rasmussen 1977).

Eelgrass is a C_3 plant, meaning that one of the first stable products of its photosynthesis is a 3-carbon sugar (Larkum et al. 2006). CO_2 availability may limit the rate of photosynthesis in C_3 plants (Percy and Bjorkman 1983). Additional photosynthetic restrictions on submerged plants may include: (1) limited inorganic carbon uptake due to low external carbon concentrations, (2) slow diffusion rates through thick boundary layers over leaves, and (3) limited speed or activity of carbon transport and fixation within plant cells (Madsen and Sand-Jensen 1991, Noest et al. 1995). In seawater at pH 8.2, 90% of inorganic carbon exists as the

bicarbonate ion (HCO_3^-) and only 0.6% as $\text{CO}_{2(\text{aq})}$ (Millhouse and Strother 1986). As ocean pH drops, this balance will begin to shift toward increasing concentrations of $\text{CO}_{2(\text{aq})}$. The ability of eelgrass to utilize different types of dissolved inorganic carbon (DIC) and eelgrass response to CO_2 enrichment are reviewed in the following sections.

Rates of eelgrass photosynthesis

In C_3 plants, the enzyme RUBISCO catalyzes the initial step in carbon fixation, carboxylating CO_2 to form two, three-carbon sugars (3-phosphoglycerate). In aquatic environments, the primary source of carbon for RUBISCO is $\text{CO}_{2(\text{aq})}$ (Invers et al. 2001). However, the equilibrium concentration of $\text{CO}_{2(\text{aq})}$ in seawater (about 0.014mM at 15°C) is much lower than saturation levels of CO_2 use for either seagrasses or algae (Beer 1994). This means that photosynthesis in either group will be CO_2 limited under light-saturated conditions unless organisms are able to use abundant HCO_3^- as an inorganic carbon source (Sand-Jensen and Gordon 1984). A number of studies have quantified the ability of eelgrass to utilize bicarbonate in photosynthesis, and the changes in photosynthetic rate under more acidic conditions (i.e. when $\text{CO}_{2(\text{aq})}$ is present in higher than ambient concentrations). Such studies can provide some indication of how eelgrass will perform photosynthetically under increasingly acidic ocean conditions in the future.

In a laboratory study of the effect of pH on photosynthesis, eelgrass shoots in both dilute and natural seawater exhibited no change in photosynthetic rate when pH declined from 8.4 to 7.0, at constant DIC levels (Sand-Jensen and Gordon 1984). Rather, photosynthetic rates increased only as pH approached 6.0 and HCO_3^- was converted more fully to $\text{CO}_{2(\text{aq})}$. Other

studies have reported similar results. For example, in one experiment photosynthetic rates were reportedly three and a half times higher in acidic, $\text{CO}_{2(\text{aq})}$ -enriched water (pH 6.2, $[\text{CO}_{2(\text{aq})}] = 1340\mu\text{M}$) than under ambient seawater conditions (pH 8.2, $[\text{CO}_{2(\text{aq})}] = 14\mu\text{M}$) (Zimmerman et al. 1997). Specifically, leaf photosynthesis increased from 0.30 to 0.78 $\mu\text{mol O}_2 \text{ mg}^{-1}$ chlorophyll min^{-1} between ambient and enriched conditions, and dark respiration rates decreased (Figure 2). The concomitant increase in photosynthesis and decrease in respiration increased the overall ratio of gross photosynthesis to respiration 400% compared to that observed in ambient seawater (Zimmerman et al. 1997).

In a relative comparison of photosynthetic rates, marine angiosperms (including eelgrass) incubated under ambient conditions (15 $\mu\text{M CO}_2$, pH 8.3) only photosynthesized at approximately 80% of the rate observed under elevated conditions (350 $\mu\text{M CO}_2$, pH 6.9) (Madsen et al. 1993). Subsequent experiments show that eelgrass photosynthesizes at only about 40% of its maximum possible rate under ambient seawater conditions (Beer and Koch 1996). Note that the elevated rate examined in the former study (Madsen et al. 1993) was not reported as representative of eelgrass' maximal photosynthetic rate; this may account for the different relative net photosynthetic rates reported in these two experiments. Overall, data support the conclusion that eelgrass does not photosynthesize at its maximal rate under current seawater conditions, and that photosynthetic rates increase as pH decreases and $\text{CO}_{2(\text{aq})}$ becomes more abundant.

A plant's capacity to extract and utilize DIC is closely, positively correlated to its photosynthetic rate under varying CO_2 levels (Madsen et al. 1993). In other words, plants with a

high extraction capacity achieve a photosynthetic rate under ambient conditions closer to that observed under elevated CO_2 conditions. Madsen et al. (1993) considered eelgrass to have a high, relatively efficient DIC extraction capacity given its ability to photosynthesize under ambient conditions at approximately 80% of the rate it achieved under elevated $\text{CO}_{2(\text{aq})}$ conditions. This efficiency under ambient, $\text{CO}_{2(\text{aq})}$ -limited conditions indicates the presence of a "CO₂ concentrating system" – i.e. the ability of the plant to use HCO_3^- in photosynthesis (Madsen et al. 1993). This conclusion is supported by studies that observed a plateau in eelgrass photosynthetic rates across a range of pH values. For example, Sand-Jensen and Gordon (1984) state that photosynthetic rates did not change between pH 7.0 and 8.4, even though $[\text{CO}_{2(\text{aq})}]$ decreased from 0.27 to 0.01 mM; consequently, they inferred that eelgrass must use HCO_3^- as a carbon source in order to maintain the constant rates observed.

Not all authors consider seagrass bicarbonate assimilation efficient. Beer (1989) points out that although seawater bicarbonate concentrations are high enough to saturate photosynthesis, rates are still enhanced by the addition of $\text{CO}_{2(\text{aq})}$; therefore, he considers bicarbonate utilization systems to be "rather ineffective". Macroalgae and phytoplankton are also comparatively more efficient than seagrasses at assimilating bicarbonate (Figure 3). Macroalgal photosynthesis is often saturated with respect to inorganic carbon, while seagrass photosynthesis may be limited by carbon supply (Beer and Koch 1996). For example, the seagrasses *Zostera marina* and *Thalassia testudinum* photosynthesized at only about half their maximum rates under ambient seawater pH, while three species of macroalgae (*Ulva lactuca*, *Palmaria plamata*, and *Laminaria saccharina*) were at carbon saturation. Only as pH decreased

did eelgrass inorganic carbon affinity and photosynthetic rate increase, until at pH 6.0 both the seagrasses and algae studied were saturated with respect to DIC (Beer and Koch 1996).

The disparity in ambient photosynthetic rates between seagrasses and algae may be a result of their evolutionary age (Beer and Koch 1996). Macroalgae evolved approximately 1 billion to 750 million years ago, while seagrasses only evolved about 90 million years ago (Beer and Koch 1996). At the time seagrasses evolved, CO_2 concentrations were 3 to 12 times higher than current (Bernier 1991), and surface ocean pH may have been lower. For example, boron isotope data recovered from foraminifera tests in a deep sea sediment core indicate that surface ocean pH 20 million years ago may have been as low as 7.4 (Spivack et al. 1993). Under these conditions, the ratio of CO_2 : HCO_3^- would have been approximately 16 times its value today and eelgrass could have acquired and utilized DIC in photosynthesis at least as efficiently as some macroalgae (Beer and Koch 1996). Genetic limitations on bicarbonate assimilation present in seagrasses but not macroalgae may account for their differences in photosynthetic efficiency in today's oceans (Beer 1994, Beer and Koch 1996).

Storage of photosynthate

An important consequence of photosynthesis is the transformation of photosynthate into storable sugars that can be used for growth and anaerobic respiration. More than 90% of the carbohydrate pool in eelgrass leaves is present as sucrose and other hexoses (Zimmerman et al. 1995a). This represents an important energy source for tissues such as roots, immature fruits, and immature leaves that use more energy than they produce. These tissues transform sucrose reserves into energy via fermentation in light-limited, anaerobic conditions.

In a two hour experiment, eelgrass leaves incubated in acidified seawater (pH 6.0, $[\text{CO}_{2(\text{aq})}] = 1.8 \text{ mM}$) exhibited a light-saturated photosynthetic rate three times that of controls; this rate translated into a 43% increase in leaf sucrose content relative to control leaves incubated in ambient seawater (Zimmerman et al. 1995b). This was accomplished without an increase in the activity of the enzyme catalyzing sucrose formation (SPS). In fact, less than half of the available SPS enzyme was activated even under the $\text{CO}_{2(\text{aq})}$ -enriched conditions used (Zimmerman et al. 1995b). Since eelgrass leaves export about 60% of their fixed carbon, these results suggest that eelgrass photosynthesizing under enhanced $[\text{CO}_{2(\text{aq})}]$ will be able to export more sucrose to developing tissues and maintain larger sucrose pools within leaves (Zimmerman et al. 1995b). Sucrose reserves may aid in plant survival under light-limited conditions or sediment anoxia (Zimmerman et al. 1995b). This represents one way in which ocean acidification may enhance eelgrass growth and survival.

Eelgrass productivity, growth rates, and light requirements

Increased photosynthetic rates do not necessarily translate into enhanced aboveground growth for individual plants. Rather, plants may transfer energy into reproduction or storage. Therefore, measurements of plant growth and productivity are also important in determining whether the benefits of enhanced photosynthesis are visible on the level of individual shoots or of entire eelgrass populations.

Thom (1996) was the first to specifically examine changes in both eelgrass photosynthetic rates and growth rates in the context of predicted scenarios of human-induced $\text{CO}_{2(\text{aq})}$ enrichment. In his two-hour experiment, eelgrass leaves incubated at twice ambient

$\text{CO}_{2(\text{aq})}$ concentrations (~680 ppm) had net apparent photosynthetic (NAP) rates two and a half times those of leaves under ambient conditions (~340 ppm). However, in a second trial no difference was observed (Figure 4a). In a series of nine stepwise incubations with $[\text{CO}_{2(\text{aq})}]$ between one and five times the ambient concentration, eelgrass NAP was greatest at 2.5 – 3.0x ambient $[\text{CO}_{2(\text{aq})}]$, or approximately 850 – 1020 ppm (Figure 4b).

Eelgrass growth rates were untested at the high $[\text{CO}_{2(\text{aq})}]$ used in the stepwise experiments. However, under enrichment values between one and two times ambient $[\text{CO}_{2(\text{aq})}]$, growth rates increased from an average of 4.5 mg dry weight shoot⁻¹ day⁻¹ to 7.0 mg dry weight shoot⁻¹ day⁻¹. However, in a second trial, a slight decrease rather than an increase in growth rates occurred (Figure 5). Thom (1996) concluded that while eelgrass NAP may increase under the prediction of a doubling in ambient $[\text{CO}_2]$ by 2100 (Bazzaz and Fajer 1992), this may not actually translate into a change in the growth rate of individual eelgrass shoots. Discussion of the allocation of benefits between individual shoots and eelgrass populations under enhanced $[\text{CO}_{2(\text{aq})}]$ continues in the following section.

Effects of long-term $\text{CO}_{2(\text{aq})}$ enrichment

The experiments discussed above all examined the response of eelgrass growth or photosynthesis to $\text{CO}_{2(\text{aq})}$ enrichment over short time frames (hours). However, short-term responses to enrichment are not always indicative of responses to long-term exposure (Beer 1989, Zimmerman et al. 1997). In a 45 day experiment in which eelgrass was grown under ambient (pH 8.2) and enriched (pH 6.2) $[\text{CO}_{2(\text{aq})}]$ conditions, maximum net photosynthesis remained about 3 times higher under enriched conditions throughout the course of one study

(Zimmerman et al. 1997). These rates were maintained without any significant change in leaf respiration or consistent increase in leaf chlorophyll content. The increase in maximum net photosynthesis also translated into a decrease in the number of hours of light-saturated photosynthesis required for the plants to maintain a positive carbon balance – from about 7.0 hours to 2.7 hours. Plants grown under carbon-enriched but light-limited conditions maintained sizes, growth rates, tissue sugar contents, and sucrose enzymatic activity (SPS) similar to plants grown in unenriched but light replete treatments (Zimmerman et al. 1997). These results support the conclusion that eelgrass photosynthesis is carbon-limited under light-saturated conditions in normal seawater, that $[\text{CO}_{2(\text{aq})}]$ limitation drives high light requirements in eelgrass, and that eelgrass capacity for carbon fixation is three times greater than can be utilized under current $[\text{CO}_{2(\text{aq})}]$ in the ocean (Zimmerman et al. 1997). As global ocean pH decreases and $[\text{CO}_{2(\text{aq})}]$ rises, eelgrass could become an increasingly important sink for atmospheric carbon.

Year-long experiments on eelgrass response to $\text{CO}_{2(\text{aq})}$ enrichment yielded similar results. Under light-replete conditions, increases in shoot biomass were positively correlated with carbon enrichment (pH 7.75 and 7.5). This response was solely the result of more biomass allocated to the rhizome, as opposed to leaves and roots. In contrast, under light-limited conditions, $\text{CO}_{2(\text{aq})}$ enrichment did not affect biomass (Palacios and Zimmerman 2007).

In some cases, changes in seagrass morphology appeared to correlate with $\text{CO}_{2(\text{aq})}$ enrichment. Rhizome extension rates approximately doubled under light-replete, enriched conditions, and rhizome internode lengths were generally greater. In addition, flowering shoots

appeared earlier and matured more quickly, with the largest difference noted in the highest enrichment scenario. $\text{CO}_{2(\text{aq})}$ enrichment did not enhance flowering in light-limited treatments (Palacios and Zimmerman 2007).

While enrichment did not affect vegetative shoot abundance, shoots did survive for longer into the winter under light-replete conditions compared to non-enriched treatments. In these experiments, leaf area, growth rate, and sugar content did not respond to $\text{CO}_{2(\text{aq})}$ enrichment, but did change with seasonal light availability. Palacios and Zimmerman (2007) conclude that under light-replete conditions, "...eelgrass responds to CO_2 enrichment by increasing growth that benefits survival of the clone and/or population in ways that are not necessarily manifested at the level of individual shoots". Therefore, future monitoring studies should incorporate population-level measurements of eelgrass reproduction and survival in order to comprehensively characterize eelgrass response to ocean acidification.

Variations in seagrass response: eelgrass vs. other species

Eelgrass (*Zostera marina*) is only one of approximately 60 seagrass species that inhabit both temperate and tropical oceans worldwide (Short et al. 2001). Other than *Z. marina*, common temperate seagrass species include *Phyllospadix torreyi* (surfgrass), *Posidonia oceanica*, and *Cymodocea nodosa*. The two latter species inhabit the Mediterranean, while the two former are commonly found on the U.S. west coast. Invers et al. (2001) measured light-saturated leaf photosynthesis in these four species over the course of several hours under constant DIC concentrations and a pH range of 6.0 to 9.0, in order to compare their response to enhanced $[\text{CO}_{2(\text{aq})}]$.

The study found that all species exhibited the same pattern of photosynthetic response: high rates were observed under more acidic conditions (pH 6 to 7-7.5), a photosynthetic plateau occurred from pH 7.5 to 8.5, and finally rates declined above pH 8.5. However, the two west coast species exhibited a more dramatic range of photosynthetic rates than the Mediterranean species over the pH values tested. Eelgrass and surfgrass had higher rates under the most acidic conditions and lower rates under the most alkaline conditions, compared to the Mediterranean species (Figure 6). It is possible that the variation in photosynthetic range may result from a species' efficiency at using bicarbonate as a carbon source in photosynthesis; Mediterranean species may use bicarbonate more efficiently than west coast species and therefore not respond as dramatically to increases $[\text{CO}_{2(\text{aq})}]$ (Invers et al. 2001).

Like the temperate species listed above, the Caribbean seagrass *Thalassia testudinum* and the South Australian seagrass *Posidonia australis* also increased their net photosynthetic rates under acidified conditions. Interestingly, both species were reported to approximately double their net photosynthetic rates between ambient seawater pH and a pH of 7.5 (Durako 1993, James and Larkum 1996). These results contrast with those of Invers et al. (2001), who found that photosynthetic rates of all four temperate species studied remained relatively constant within this pH range.

It appears that certain seagrasses may begin to respond sooner than others to pH declines. Differences in the efficiency of bicarbonate use coupled with the sensitivity of photosynthetic rates to acidification will influence a species' response to changes in ocean pH. Variation in species' responses to increasingly acidic global ocean conditions may determine

shifts in species distributions, ecosystem composition, and a given plant's relative importance in global carbon fixation.

Summary of research

Under light saturated conditions, eelgrass photosynthesis is limited by current DIC concentrations in the ocean. Although eelgrass is able to utilize abundant bicarbonate as a source of inorganic carbon supplementing $\text{CO}_{2(\text{aq})}$ supply, photosynthetic rates remain undersaturated with respect to DIC (Beer 1989). Carbon-limited photosynthesis results in high light requirements for eelgrass, as longer periods of saturating irradiance are necessary to maintain a positive carbon balance (Zimmerman et al. 1997). Under acidified ($\text{CO}_{2(\text{aq})}$ -enriched) conditions, eelgrass photosynthetic rates increase (e.g. Sand-Jensen and Gordon 1984), more photosynthate is stored as sucrose (Zimmerman et al. 1995b), and growth, reproduction, and shoot survival are generally enhanced (Palacios and Zimmerman 2007). The pattern of eelgrass response to acidified conditions is similar to that of other seagrasses; however, species differ in the strength of their response (i.e. how drastically acidification affects photosynthetic rates) as well as the pH value at which changes in photosynthetic rate occur (Durako 1993, James and Larkum 1996, Invers et al. 2001). Overall, research to date supports the general statement that eelgrass photosynthesis, growth, and survival is enhanced under more acidic ocean conditions.

III. Discussion and Conclusions

This section provides a critical analysis of the studies reviewed in section II. Specifically, I evaluate inconsistencies in experimental methodologies and/or results, place data within the context of global changes in water quality, and suggest future directions for research.

Analysis of experimental methodologies

A majority of the studies reviewed provided information on changes in eelgrass photosynthetic rates under ambient and more acidic conditions. Photosynthetic rates are generally measured as rates of net oxygen exchange in the water around a plant over a short incubation period (Beer et al. 2001). All studies measured oxygen evolution under light-saturated conditions with constant water temperature and [DIC]. However, despite using similar methodologies, the experimental results reported were not always consistent. This may be due to (1) inappropriate incubation techniques, (2) timing of measurements, (3) length of acclimation periods prior to experimentation, and/or (4) low sample sizes and few replicate trials.

Oxygen evolution from epiphyte-free plant material is generally measured by oxygen electrodes inside sealed chambers (Beer et al. 2001). However, measurements of oxygen exchange are sensitive to stagnant media. Seagrass photosynthetic rates can be limited by boundary layers that develop around the leaves; as flow decreases, boundary layer thickness increases and gas diffusion from the surrounding water to the leaf surface occurs more slowly (Koch 1994). If seawater within closed measurement chambers is not mixed, then rates of oxygen exchange (and hence photosynthetic rates) may be underestimated. In addition, increasing oxygen concentrations within unstirred media may result in photorespiration, in which RUBISCO reacts with O₂ instead of CO₂ and the rate of photosynthesis slows (Beer 1989, Beer et al. 2001). Accurate representations of photosynthetic rates are important for predicting the magnitude of eelgrass response to increasing [CO_{2(aq)}].

Thom (1996) reported inconsistent eelgrass responses to $\text{CO}_{2(\text{aq})}$ enrichment. In his first experiment, he found that eelgrass net apparent photosynthesis more than doubled in one trial under twice ambient $[\text{CO}_{2(\text{aq})}]$, but showed no response in the second trial (Figure 4a). In his second experiment, photosynthetic rates appeared to decrease in seawater with $[\text{CO}_{2(\text{aq})}]$ 3.5 – 5 times ambient (Figure 4b). These results stand in contrast to those of Beer and Koch (1996), who found that eelgrass net photosynthetic rates increased and remained high under conditions up to 10x ambient DIC at pH values of 6 and 7 (Figure 7).

Stagnant incubation media may be partially responsible for Thom's inconsistent results. Thom (1996) is one of only two studies reviewed that did not detail the use of stirred incubation media in order to prevent oxygen accumulation and diffusion-limited photosynthesis (Zimmerman et al. 1995b). At high $[\text{CO}_{2(\text{aq})}]$, photosynthetic rates increase and oxygen accumulation occurs more quickly, which could account for the decline in photosynthetic rates above 3 times ambient concentrations. Additionally, the author did not quantify the amount of light reaching plants during the incubation period, but rather placed trials under "ambient mid-day sunlight". The first and second experiments were both conducted in July, but one year apart. It was assumed that conditions were light-saturated; however it is possible that differences in available light or angle of the sun (resulting in shaded blade surfaces) may have influenced photosynthetic rates observed in the two experiments.

Length of incubation can produce changes in media pH. In general, pH increases over time as plants use CO_2 , thereby altering the forms of inorganic carbon available and affecting photosynthetic rates (Beer et al. 2001). For example, a solution's alkalinity may influence

uptake of inorganic molecules by changing electrochemical gradients across leaf cells (Beer et al. 2001). For this reason, leaves should be incubated for short periods only, in unbuffered media (Beer et al. 2001). However, both Beer and Koch (1996) and Beer and Rehnberg (1997) used buffered seawater in their comparisons of net photosynthetic rates to maximal mean rates at given pH and [DIC]. It is possible that rates were inhibited in the buffered solution. Unfortunately, the authors only report measured photosynthetic rates in proportion to maximal rates (e.g. relative net photosynthesis), rather than as an absolute quantity. This makes it difficult to compare their results to studies in which net photosynthetic rates are reported per mg O₂ produced.

Seagrass photosynthetic activity may change throughout the day irrespective of light conditions; rates measured early in the morning may not be the same as those measured in the afternoon, even under similar irradiance levels (Beer et al. 2001). Few of the studies reviewed specified the time of day at which measurements of photosynthetic rates were made. Measurement timing may account for some of the variation in reported photosynthetic rates. For example, Zimmerman et al. (1997) record that eelgrass photosynthesized at a rate of 0.2 $\mu\text{mol O}_2 \text{ mg chl}^{-1} \text{ min}^{-1}$ at 15°C under ambient seawater conditions. Madsen et al. (1993) report a rate 2.4 times higher (0.48 $\mu\text{mol O}_2 \text{ mg chl}^{-1} \text{ min}^{-1}$, 18°C), while Sand-Jensen and Gordon (1984) report a rate 3 times higher (0.6 $\mu\text{mol O}_2 \text{ mg chl}^{-1} \text{ min}^{-1}$, 15°C). Each study obtained eelgrass from different populations in Denmark or Monterey Bay, CA, and it is possible that different eelgrass populations peak photosynthetically during different times of day. To depict the rate of eelgrass photosynthesis accurately, investigators should conduct measurements

throughout the day in order to place their results in the context of diurnal photosynthetic patterns (Beer et al. 2001)

In the studies reviewed, plants were acclimated to laboratory conditions for different lengths of time prior to experiments. Generally, plants were used within 24 hours of collection (Madsen et al. 1993, Invers et al. 2001), while only two studies specified an hour-long acclimation time to experimental set-ups (Beer and Koch 1996, Beer and Rehnberg 1997). In longer-term growth studies, Zimmerman et al. (1997) allowed plants to acclimate for 10 days in aquaria before beginning 45d experiments. Palacios and Zimmerman (2007) monitored plants over 5 months to assess any treatment affects from transplanting or containment. Length of acclimation time was related to methods of collection and storage. Investigators that carefully removed and repotted entire plants generally allowed for longer acclimation times than those that simply collected a number of leaves for immediate use.

I could find no studies that examined changes in eelgrass respiration related to collection wounds. However, Durako (1993) accounted for the possibility of wound respiration in his photosynthetic experiments with turtlegrass (*Thalassia testudinum*) by placing cut blades into aquaria and allowing for overnight equilibration. Similarly, Dawes and Tomasko (1988) waited 14 h before beginning photosynthesis-irradiance experiments on turtlegrass. Alteration of eelgrass photosynthetic rates due to collection wounds may partially account for reported variation. Further research is needed to determine whether eelgrass respiration changes in response to wounds, thereby affecting measured rates of net photosynthesis. In addition, a standard acclimation time across studies would make results more easily comparable.

Finally, it is important to note the variation in sample size and replication between experiments. Generally, researchers based photosynthetic rates on averages obtained from three to ten leaves (Thom 1996, Zimmerman et al. 1995b, Beer and Koch 1996, Invers et al. 2001). Madsen et al. (1993) apparently derived their data from measurements of single samples. Of these studies, only Thom (1996) reports conducting more than one trial of the same experiment using different groups of leaves. Larger sample sizes and a greater number of replicate trials would aid in resolving reported inconsistencies in eelgrass photosynthetic rates. A standardized way of reporting photosynthetic rates would also assist with data comparisons. Currently, photosynthetic rates are variously reported as O₂ evolved per mg chlorophyll, g fresh weight, and g dry weight.

Generally, longer-term growth experiments used larger sample sizes. For example, Zimmerman et al. (1997) included 30 plants in a given treatment and based growth measurements over 45 days on measurements of six plants per week. Palacios and Zimmerman (2007) distributed 512 plants between four treatments and measured effects on nine randomly-selected shoots per month over the course of a year. Each study only conducted one experimental trial; this is likely due to the difficulty of conducting multiple trials of long-term experiments that use large numbers of plants. More long-term studies of eelgrass photosynthesis are required to act as comparisons with the data from the two listed above.

Study implications in the context of predicted levels of ocean acidification

Average global surface ocean pH is predicted to decrease between 0.14 and 0.35 units by 2100 and 0.47 units by 2500, from a pre-industrial average pH of 8.17 (IPCC 2007, Cao et al.

2007). The deep oceans are expected to experience pH reductions less than those predicted for surface oceans (Cao et al. 2007). These anticipated scenarios equate to average surface ocean pH values between 7.82 and 8.03 for 2100 and 7.7 by 2500. These values are markedly different from the predictions of Palacios and Zimmerman (2007) who calculated ocean pH values of 7.75 for 2100 and 7.5 for 2200 based on atmospheric carbon concentrations they cite from Zeebe and Wolf-Gladrow (2001). However, Zeebe and Wolf-Gladrow (2001) reference Intergovernmental Panel on Climate Change (IPCC) estimates from 1995 that place ocean pH at approximately 7.87 in 2100 (p83). Therefore, in this section I evaluate the eelgrass research reviewed in the context of the most recent “worst case” scenarios of pH 7.82 (by 2100) and 7.7 (by 2500) given by IPCC (2007) and Cao et al. (2007).

Most of the studies reviewed that examined eelgrass photosynthetic rates were not undertaken to specifically evaluate eelgrass response to predicted scenarios of ocean acidification. Consequently, although experiments consistently demonstrate increases in eelgrass photosynthetic rates with decreasing pH, the range of pH values tested are outside those predicted under climate change scenarios. For example, Madsen et al. (1993), Zimmerman et al. (1995b), and Zimmerman et al. (1997) all compared eelgrass photosynthetic rates under two extremes: ambient $[\text{CO}_{2(\text{aq})}]$ with pH ~ 8.0 and elevated $[\text{CO}_{2(\text{aq})}]$ with pH from 6.0 to 6.9. Unfortunately, these studies do not provide enough resolution to make specific predictions about eelgrass response under likely pH levels associated with ocean acidification. Similarly, although Palacios and Zimmerman (2007) did seek to determine eelgrass response to chronic acidification under predicted scenarios, the pH values they chose do not correspond to IPCC predictions for 2100 (pH 7.75 vs. 7.82). The stepwise pH reductions utilized by the authors

(0.25 pH units) are somewhat coarse compared to predicted pH declines, and may only provide some indication of eelgrass response beyond the 21st century.

A few studies have examined eelgrass photosynthesis over a range of pH values which encompass those predicted. Eelgrass photosynthetic rates were reportedly unchanged between pH values of 7.0 and 8.5 under current DIC concentrations (Sand-Jensen and Gordon 1984, Invers et al. 2001). Rhizome biomass, internode length, rhizome extension rate, total shoot abundance, and length of shoot survival did increase under light-replete, enriched conditions at pH 7.75 (Palacios and Zimmerman 2007). These results suggest that eelgrass photosynthetic rates will not be significantly enhanced under predicted acidification scenarios for 2100 or 2500. However, positive changes in growth, biomass accumulation, and survival may occur under light-replete conditions subsequent to the 21st century.

Ecological relevancy under degraded environmental conditions

Unfortunately, human activities have degraded eelgrass habitats in a number of ways over the past 150 years. Any positive growth response that eelgrass may exhibit to declining ocean pH will occur in the context of habitats subject to anthropogenic nutrient loading, anoxia, pollution, disease, increases in turbidity and temperature, and changes in tidal range, velocity, and salinity regimes (Hemminga and Duarte 2000, Moore and Short 2006, Ralph et al. 2006). All of these factors tend to decrease seagrass survival, growth, reproduction, and expansion. For example, anthropogenic impacts over the last 30 years have resulted in a steady decline in eelgrass abundance and distribution along the U.S. east coast (Moore and Short 2006).

Conditions resulting in seagrass decline may be interrelated. Hemminga and Duarte (2000) and Ralph et al. (2006) provide concise summaries of such interrelated causes of habitat degradation. These two reviews provide the following example of linked problems stemming from eutrophication. As human populations along coastlines increase, anthropogenic nutrient loading to estuaries may lead to phytoplankton blooms. Blooms often decrease water clarity and light availability within the water column, thereby limiting seagrass photosynthesis. In addition, increased nutrient availability may enhance the growth of epiphytes and algae, further shading or smothering eelgrass leaves and potentially making the leaves a more desirable food source for grazers. As the plants die, their roots no longer act to bind sediments. Newly-mobilized sediments may produce a corresponding increase in turbidity, further reducing light availability in the water column. Such a cycle can severely limit seagrass distribution, or completely destroy a bed.

The studies reviewed that examined eelgrass response to enhanced $[\text{CO}_{2(\text{aq})}]$ were conducted under laboratory conditions of constant temperature, salinity, and light saturation. Seagrass leaves were usually cleaned of epiphytes, experienced no grazing pressure, and grew in well-flushed systems in which nutrients (other than carbon) were not limiting. Although maintenance of such controlled conditions was necessary in order to determine how carbon enhancement affected eelgrass photosynthesis and growth, such conditions are substantially different from those found in natural habitats.

Only two eelgrass studies altered multiple environmental variables: $[\text{CO}_{2(\text{aq})}]$ and light availability. Enhanced $[\text{CO}_{2(\text{aq})}]$ enabled eelgrass to survive under only four hours of light-

saturated photosynthesis – a period that would generally prohibit the maintenance of a positive carbon balance (Zimmerman et al. 1997). This result suggests that increased carbon availability could promote the survival of eelgrass meadows in turbid environments. For example, increased carbon fixation and sugar storage might help eelgrass roots survive multi-day episodes of light-limited and anoxic conditions (Zimmerman et al. 1995b).

However, cycles of natural illumination may not provide even four hours of light saturation per day (Palacios and Zimmerman 2007). Under such conditions, eelgrass growth becomes light limited rather than carbon limited. Outdoor experiments demonstrate that eelgrass grown in CO₂-enhanced but light-unsaturated conditions do not show any significant growth response to the increased availability of inorganic carbon (Palacios and Zimmerman 2007). It is also important to note that in natural systems, increases in [CO_{2(aq)}] may enhance the photosynthesis and growth of epiphytes and algae, and anthropogenic nutrient loading may relieve these organisms from nutrient-limited growth (Duarte 1995). Epiphytes and algae can shade seagrass leaves, once again limiting seagrass photosynthesis by light availability rather than carbon (Hemminga and Duarte 2000). As Palacios and Zimmerman (2007) succinctly state, “Whether rising atmospheric CO₂ can offset or keep pace with the effects of deteriorating water quality on eelgrass distributions remains an open question...”

Future directions for research

Evidence is inconclusive that eelgrass photosynthesis, growth, reproduction, or survival will be enhanced under predicted ocean acidification scenarios. In fact, I found relatively few studies that examined the response of *any* seagrass species to CO₂ enhancement in the context

of ocean acidification. With this in mind, there are a number of directions for future research that would supplement knowledge of eelgrass response to changing ocean conditions.

First, long-term, replicated laboratory experiments measuring eelgrass photosynthetic rates under actual scenarios of future ocean acidification would increase predictive ability. For example, investigators could quantify whether eelgrass ever experiences any photosynthetic down-regulation in response to enhanced conditions (Bazzaz and Fajer 1992). Research on endogenous rhythms of photosynthetic regulation and variation between global eelgrass populations would provide a context for more infrequent point measurements of growth or photosynthetic rates (Beer et al. 2001). In addition, laboratory studies similar to those of Palacios and Zimmerman (2007) are needed in which eelgrass growth, survival, and reproduction are measured under actual prediction scenarios.

Second, it would be helpful to conduct $\text{CO}_{2(\text{aq})}$ enhancement experiments that mimic degraded habitat conditions. For example, one might create a combination of treatments in which eelgrass is exposed to chronically elevated $[\text{CO}_{2(\text{aq})}]$ as well as elevated temperature. Similarly, one could create conditions of nutrient-loading and evaluate epiphyte growth, eelgrass palatability to grazers, and eelgrass survival. Such experiments would also allow researchers to begin to quantify whether any growth benefits experienced by eelgrass actually translate into food or habitat benefits for associated faunal communities.

Third, field comparisons would provide ecological realism lacking under laboratory conditions. Researchers should look for any areas where acidified waters and eelgrass may already coincide. Monitoring data available from the National Estuarine Research Reserve

system could assist in locating such areas in the United States. For example, in the Pacific Northwest, multi-year pH records exist for research reserves in Padilla Bay, WA and South Slough, OR. In South Slough, a monitoring site near Valino Island reported an average pH of 7.8 in four of the five years between 1999 and 2004 – a value comparable to that predicted for 2100 (CDMO 2009). In addition to examining plant photosynthesis and growth, experiments conducted at such “acidified” field sites could evaluate concurrent changes in species abundance, distribution, and faunal community composition.

Site-specific data from National Estuarine Research Reserves also provide an interesting contrast to recorded changes in global ocean pH. While average global ocean surface pH has declined 0.1 pH unit since the preindustrial period (Orr et al. 2005), average annual pH values in South Slough, OR and Padilla Bay, WA increased 0.1 – 0.2 units between 2001 and 2006 (CDMO 2009). Minimum and maximum pH also fluctuated over this period. At Valino Island in South Slough, a pH minimum of 6.1 was recorded in 2004 and a maximum of 8.7 in 2006. At Ploeg Channel in Padilla Bay, a pH minimum of 6.9 was recorded in 2004 and a maximum of 9.4 in 2001 (CDMO 2009, also see Table 1). Relatively acidic conditions (pH 6.2 – 7.3) were recorded as lasting from hours to days in 1999 at Valino Island (CDMO 2009). These data demonstrate that pH values may fluctuate widely over the course of a day or year. In addition, they highlight the need to examine seagrass response to changing ocean conditions within a regional context. Although ocean acidification may be an overall global trend, global averages may not reflect the actual pH changes plants experience on a yearly basis in a given bay or estuary. To achieve more accurate and realistic predictions, seagrass response to changing ocean conditions should be evaluated on a regional basis and should account for local environmental data.

Finally, terrestrial ecology may provide ideas for future areas of research. In this field, the idea that elevated atmospheric $[CO_2]$ will enhance the growth of terrestrial plants is known as the “ CO_2 fertilization effect”, and has been a research subject for several decades (Bazzaz and Fajer 1992). Bazzaz and Fajer (1992) synthesize the results of a number of studies that have reported both positive and negative effects on terrestrial plant growth. For example, while individual tree seedlings may thrive in enhanced CO_2 environments, competition between multiple species can constrain or negate any benefits to plant productivity. In addition, plants grown under enhanced $[CO_2]$ appear to reduce nitrogen concentrations in their leaves, such that leaf litter exhibits a reduced carbon:nitrogen (C:N) ratio. This changes the nutritional value of leaves for herbivores, as well as the amount of nutrients released into the soil during leaf decomposition. Finally, the authors note that observed changes in plant maturation and flowering times could disrupt pollination patterns.

All of these observations could be applied to seagrass studies. Although seagrass meadows are often monospecific, multi-species meadows do occur - particularly in the Indo-Pacific (Hemminga and Duarte 2000). Invasive species may also produce multi-species meadows. For example, Japanese eelgrass (*Zostera japonica*) has been introduced into Pacific Northwest estuaries such as Coos Bay, OR (pers. obs) which historically harbored *Zostera marina* monocultures. Laboratory and field studies in regions with multiple species could begin to quantify the effects of competition on seagrass growth and survival in enhanced $CO_{2(aq)}$ environments.

As in terrestrial plant studies, it would be useful to measure any changes in the C:N ratio in seagrass leaves grown under more acidic conditions. Grazers such as urchins, green turtles, dugongs, herbivorous fish, and waterfowl eat fresh leaves of various seagrass species; additionally, decomposing leaves that enter the detrital pathway are an important food source for seagrass-associated fauna (Hemminga and Duarte 2000). Even if seagrass growth and productivity increases under more acidic conditions, such growth may not benefit seagrass fauna if the nutritional content of the leaves declines.

Lastly, Palacios and Zimmerman (2007) did find that reproductive shoots appeared earlier and matured more quickly in eelgrass grown under enhanced levels of $\text{CO}_{2(\text{aq})}$. Although eelgrass does not rely on another organism for pollination or seed dispersal, it is possible that seasonal current patterns could alter eelgrass pollination and seed distribution. Determining the relationship between local currents and pollen or seed dispersal would allow for better prediction of how eelgrass distribution may change if reproduction occurs earlier in the year.

Conclusions

The prediction that seagrass beds will thrive under future scenarios of ocean acidification is premature. The eelgrass research reviewed does support the contention that photosynthetic rates and sucrose storage increase under certain values of low pH and high $[\text{CO}_{2(\text{aq})}]$ (Sand-Jensen and Gordon 1984, Beer 1989, Madsen et al. 1993, Beer and Koch 1996, Zimmerman et al. 1995b, Beer and Rehnberg 1997, Zimmerman et al. 1997, Invers et al. 2001). It also appears that certain aspects of eelgrass growth, reproduction, and survival are enhanced under more acidic conditions (Palacios and Zimmerman 2007). However, most of the studies

reviewed did not examine changes in eelgrass photosynthesis, growth, or productivity over a range of ocean pH values similar to those predicted for the remainder of the 21st century. Those that did examine photosynthetic rates over a pH range encompassing predictions found no change (Sand-Jensen and Gordon 1984, Invers et al. 2001). These results suggest that ocean acidification in the near future will not substantially enhance eelgrass growth, productivity, or overall population resistance to water quality decline.

Although data indicate that eelgrass may eventually reap some benefit from decreasing ocean pH, studies published to date ultimately lack ecological realism. None examine the effect of CO_{2(aq)} enhancement on eelgrass growth and survival under the degraded habitat conditions beds are likely to experience in the field. In addition, the generalization that acidification will benefit seagrasses is too broad; rather, seagrass species vary in the sensitivity of their response to increasing [CO_{2(aq)}] (Durako 1993, James and Larkum 1996, Invers et al. 2001). Populations of even a single species may respond differently in future decades, as global averages do not necessarily reflect the actual pH trends exhibited in different bays or estuaries. Thus, individual species and regional populations should be evaluated separately when determining whether acidification will enhance seagrass photosynthesis, growth, survival, or reproduction.

Seagrasses are well recognized for providing many ecosystem functions and services, such as water quality enhancement, sediment stabilization, and nutrient cycling, as well as habitat, food, and/or nursery resources for many fauna, epiphytes, and commercially important fish and shellfish (Hemminga and Duarte 2000). However, global seagrass populations are declining as a result of human-induced disturbances such as sediment runoff, commercial

fishing practices, and changes in hydrological regimes (Duarte 2002, Orth et al. 2006). Because of their ecological and economic roles, it is important to understand and accurately predict how seagrass distribution and abundance will change over time, and to determine whether ocean acidification will result in any compensatory benefits that buffer against water quality decline. Further investigation of the effect of ocean acidification on growth, reproduction, and survival is therefore warranted for both eelgrass and other seagrass species.

IV. Summary

Global average ocean pH is declining as increased amounts of atmospheric CO_2 dissolve into seawater. Seagrasses are one of the few organisms predicted to benefit from pH declines, since $[\text{CO}_{2(\text{aq})}]$, the primary type of inorganic carbon used in photosynthesis, should increase. The purpose of this essay was to evaluate the claim that eelgrass (*Zostera marina* L.) photosynthesis, growth, reproduction, and survival will be enhanced under future scenarios of ocean acidification.

Laboratory studies have reported that eelgrass photosynthetic rates increase when $[\text{CO}_{2(\text{aq})}]$ is elevated in experiments lasting from hours to weeks. Under high $[\text{CO}_{2(\text{aq})}]$, eelgrass has been shown to maintain a positive carbon balance with fewer hours of light-saturated photosynthesis and to store more photosynthate in leaves and rhizomes. Additionally, reproductive shoots mature and flower more rapidly, length of winter survival increases, and certain aspects of growth are enhanced.

However, the studies reviewed ultimately lacked ecological realism. The majority of experiments measured eelgrass response under conditions in which light and nutrients were

not limiting, water quality was optimal, and growth was not affected by the presence of epiphytes or grazers. Additionally, acidic laboratory conditions generally did not match those predicted in future acidification scenarios. This limits the applicability of such studies to predictions of change in eelgrass growth, distribution, and survival.

This field would benefit from laboratory and field studies examining long-term patterns in eelgrass photosynthesis, growth, and survival under explicit scenarios of acidification and degraded water quality. Measurements of competition between seagrass species for more abundant carbon resources, changes in leaf nutritional value, and alterations in pollination and seed dispersal would allow ecologists to better predict how eelgrass abundance and distribution will change in increasingly acidic oceans. Knowledge of regional pH trends will also provide an important context for predicting future population dynamics in specific seagrass meadows.

References

- Bazzaz, FA and ED Fajer. 1992. Plant life in a CO₂-rich world. *Scientific American* 266 (Jan): 68 – 74.
- Beer, S. 1989. Photosynthesis and photorespiration of marine angiosperms. *Aquatic Botany* 34: 153 – 166.
- Beer, S. 1994. Mechanisms of inorganic carbon acquisition in marine macroalgae (with special reference to the Chlorophyta). In FE Round and DJ Chapman (eds). *Progress in Phycological Research*, Volume 10. Biopress Ltd: Bristol, pp. 179 – 207.
- Beer, S and E Koch. 1996. Photosynthesis of marine macroalgae and seagrasses in globally changing CO₂ environments. *Marine Ecology Progress Series* 141: 199 – 204.
- Beer, S and J Rehnberg. 1997. The acquisition of inorganic carbon by the seagrass *Zostera marina*. *Aquatic Botany* 56: 277 – 283.
- Beer, S, M Björk, R Gademann, P Ralph. 2001. Measurements of photosynthetic rates in seagrasses. In FT Short and RG Coles (eds). *Global Seagrass Research Methods*. Elsevier Science B.V., Amsterdam, pp 183 – 198.
- Berner, RA. 1991. A model for atmospheric CO₂ over phanerozoic time. *American Journal of Science* 291: 339 – 376.
- Cao, L. K. Caldeira, and AK Jain. 2007. Effects of carbon dioxide and climate change on ocean acidification and carbonate mineral saturation. *Geophysical Research Letters* 34: L05607 (5pp).
- Centralized Data Management Office (CDMO) for the National Estuarine Research Reserve System. Accessed 2009. Downloads of yearly water quality data (1999-2006) for Valino Island at South Slough NERR and Ploeg Channel at Padilla Bay NERR. Available online at: <http://cdmo.baruch.sc.edu/QueryPages/data_metadata_select.cfm>.
- Dawes, CJ and DA Tomasko. 1988. Depth distribution of *Thalassia testudinum* in two meadows on the West Coast of Florida; a difference in effect of light availability. *Marine Ecology* 9(2): 123 – 130.
- Duarte, CM. 1995. Submerged aquatic vegetation in relation to different nutrient regimes. *Ophelia* 41: 87 – 112.
- Duarte, CM. 2002. The future of seagrass meadows. *Environmental Conservation* 29(2): 192 – 206.

- Durako, MJ. 1993. Photosynthetic utilization of $\text{CO}_{2(\text{aq})}$ and HCO_3^- in *Thalassia testudinum* (Hydrocharitaceae).
- Edwards, AJ. 1995. Impact of climatic change on coral reefs, mangroves, and tropical seagrass ecosystems. In D Eisma (ed). Climate change: Impact on coastal habitation. Lewis Publishers: London, pp 209 – 234.
- Goudriaan, J. 1993. Interaction of ocean and biosphere in their transient responses to increasing atmospheric CO_2 . *Vegetatio* 104/105: 329-337.
- Guinotte, JM and VJ Fabry. 2008. Ocean acidification and its potential effects on marine ecosystems. *Annals of New York Academy of Sciences* 1134: 320 – 342.
- Hemminga, MA and CM Duarte. 2000. *Seagrass Ecology*. Cambridge University Press: Cambridge.
- Intergovernmental Panel on Climate Change (IPCC). 2001. Climate Change 2001 Synthesis Report. Summary for Policymakers, Question 2. Available online: <<http://www.ipcc.ch/ipccreports/tar/vol4/008.htm>>. Accessed 7/24/09.
- Intergovernmental Panel on Climate Change (IPCC). 2007. Climate Change 2007 Synthesis Report: Summary for Policymakers. Available online: <http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr_spm.pdf>. Accessed 7/24/09.
- Invers, O, RC Zimmerman, RS Alberte, M Pérez, and J Romero. 2001. Inorganic carbon sources for seagrass photosynthesis: an experimental evaluation of bicarbonate use in species inhabiting temperate waters. *Journal of Experimental Marine Biology and Ecology* 265(2): 203 – 217.
- James, PL and AWD Larkum. 1996. Photosynthetic inorganic carbon acquisition of *Posidonia australis*. *Aquatic Botany* 55: 149 – 157.
- Koch, EW. 1994. Hydrodynamics, diffusion-boundary layers and photosynthesis of the seagrasses *Thalassia testudinum* and *Cymodocea nodosa*. *Marine Biology* 118: 767 – 776.
- Larkum, AWD, EA Drew, and PJ Ralph. 2006. Photosynthesis and metabolism in seagrasses at the cellular level. In AWD Larkum, RJ Orth, and CM Duarte (eds). *Seagrasses: Biology, Ecology, and Conservation*. Springer: The Netherlands, pp. 323 - 345.
- Madsen, TV and K Sand-Jensen. 1991. Photosynthetic carbon assimilation in aquatic macrophytes. *Aquatic Botany* 41: 5 – 40.

- Madsen, TV, K Sand-Jensen, and S Beer. 1993. Comparison of photosynthetic performance and carboxylation capacity in a range of aquatic macrophytes of different growth forms. *Aquatic Botany* 44: 373 – 384.
- Millhouse, J and S Strother. 1986. The effect of pH on the inorganic carbon source for photosynthesis in the seagrass *Zostera muelleri* Irmish ex Aschers. *Aquatic Botany* 24: 199 – 209.
- Moore, KA and FT Short. 2006. *Zostera*: Biology, Ecology, and Management. In AWD Larkum, RJ Orth, and CM Duarte (eds). *Seagrasses: Biology, Ecology, and Conservation*. Springer: The Netherlands, pp. 361 – 386.
- Noest, V, E van der Maarel, and F van der Meulen. 1995. Impact of climatic change on the ecology of temperate coastal wetlands, beaches, and dunes. In D Eisma (ed). *Climate change: Impact on coastal habitation*. Lewis Publishers: London, pp 189 – 208.
- Orr, JC, VC Fabry, O Aumont, L Bopp, SC Doney, RA Feely, A Gnanadesikan, N Gruber, A Ishida, F Joos, RM Key, K Lindsay, E Maier-Reimer, R Matear, P Monfray, A Mouchet, RG Najjar, G Plattner, KB Rodgers, CL Sabine, JL Sarmiento, R Schlitzer, RD Slater, IJ Totterdell, M Weirig, Y Yamanaka, and A Yool. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437: 681 – 686.
- Orth, RJ., TB Carruthers, WC Dennison, CM Duarte, JW Fourqurean, KL Heck Jr., AR Hughes, GA Kendrick, WJ Kenworthy, S Olyarnik, FT Short, M Waycott, and SL Williams. 2006. A global crisis for seagrass ecosystems. *BioScience* 56(12): 987 - 996.
- Palacios, SL and RC Zimmerman. 2007. Response of eelgrass *Zostera marina* to CO₂ enrichment: possible impacts of climate change and potential remediation of coastal habitats. *Marine Ecology Progress Series* 344: 1 – 14.
- Pearcy, RW and O Bjorkman. 1983. Physiological effects. In ER Lemon (ed). *CO₂ and Plants: The response of plants to rising levels of atmospheric carbon dioxide*. Westview Press: Boulder, CO, pp. 65 – 106.
- Prins, HBA and JTM Elzenga. 1989. Bicarbonate utilization: function and mechanism. *Aquatic Botany* 34: 59 – 83.
- Ralph, PJ, D Tomasko, K Moore, S Seddon, and CMO Macinnis-Ng. 2006. Human impacts on seagrasses: Eutrophication, sedimentation, and contamination. In AWD Larkum, RJ Orth, and CM Duarte (eds). *Seagrasses: Biology, Ecology, and Conservation*. Springer: The Netherlands, pp. 567 - 593.

- Rasmussen, E. 1977. The wasting disease of eelgrass (*Zostera marina*) and its effects on environmental factors and fauna. In CP McRoy and C Helfferich (eds). *Seagrass Ecosystems: A Scientific Perspective*. Marcel Dekker, Inc., New York, pp 1 – 51.
- Sand-Jensen, K and DM Gordon. 1984. Differential ability of marine and freshwater macrophytes to utilize HCO_3^- and CO_2 . *Marine Biology* 80: 247 – 253.
- Short, FT and CM Duarte. 2001. Methods for the measurement of seagrass growth and production. In FT Short and RG Coles (eds). *Global Seagrass Research Methods*. Elsevier Science B.V., Amsterdam, pp 155 – 182.
- Short, FT and HA Neckles. 1999. The effects of global climate change on seagrasses. *Aquatic Botany* 63: 169 – 196.
- Short, FT, RG Coles, and C Pergent-Martini. 2001. Global seagrass distribution. In FT Short and RG Coles (eds). *Global Seagrass Research Methods*. Elsevier Science B.V., Amsterdam, pp 5 – 30.
- Spivack, AJ, C You, and HJ Smith. 1993. Foraminiferal boron isotope ratios as a proxy for surface ocean pH over the past 21 Myr. *Nature* 363: 149 – 151.
- Thom, RM, AB Borde, S Rumrill, DL Woodruff, GD Williams, JA Southard, and SL Sargeant. 2003. Factors influencing spatial and annual variability in eelgrass (*Zostera marina* L.) meadows in Willapa Bay, Washington, and Coos Bay, Oregon estuaries. *Estuaries* 26(4B): 1117-1129.
- Thom, RM. 1996. CO_2 -enrichment effects on eelgrass (*Zostera marina* L.) and bull kelp (*Nereocystis luetkeana* (Mert.) P. & R.). *Water, Air, and Soil Pollution* 88: 383 – 391.
- Wetzel, RG and JB Grace. 1983. Aquatic plant communities. In ER Lemon (ed). *CO_2 and Plants: The response of plants to rising levels of atmospheric carbon dioxide*. Westview Press: Boulder, CO, pp. 223 – 280.
- Zeebe, RE and D Wolf-Gladrow. 2001. CO_2 in seawater: equilibrium, kinetics, isotopes. Elsevier Oceanography Series, 65. Elsevier: Amsterdam.
- Zimmerman, RC, JL Reguzzoni, and RS Alberte. 1995a. Eelgrass (*Zostera marina* L.) transplants in San Francisco Bay: Role of light availability on metabolism, growth and survival. *Aquatic Botany* 51: 67 – 86.
- Zimmerman, RC, DG Kohrs, DL Steller, and RS Alberte. 1995b. Carbon partitioning in eelgrass: regulation by photosynthesis and the response to daily light-dark cycles. *Plant Physiology* 108: 1665 – 1671.

Zimmerman, RC, DG Kohrs, DL Steller, and RS Alberte. 1997. Impacts of CO₂ enrichment on productivity and light requirements of eelgrass. *Plant Physiology* 115: 599 – 607.

Appendix

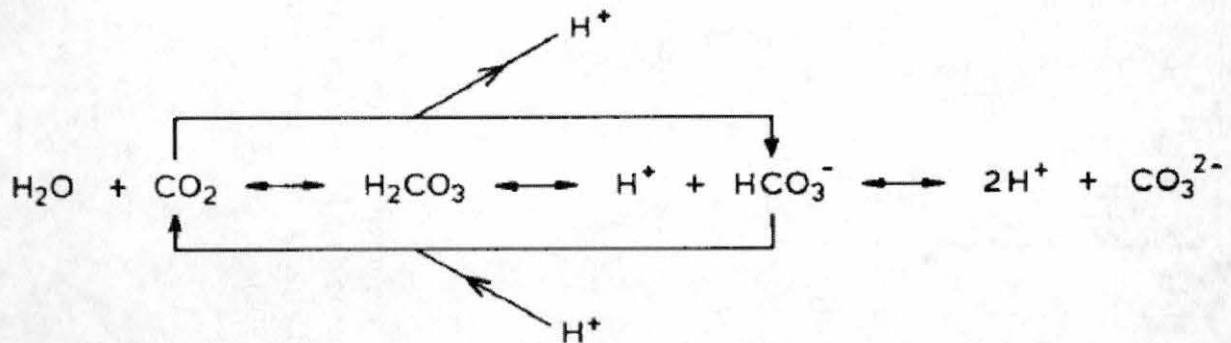


Figure 1: Carbon equilibrium in seawater, from Prins and Elzenga (1989). CO_2 = carbon dioxide, H_2CO_3 = carbonic acid, H^+ = hydrogen ion, HCO_3^- = bicarbonate ion, CO_3^{2-} = carbonate ion.

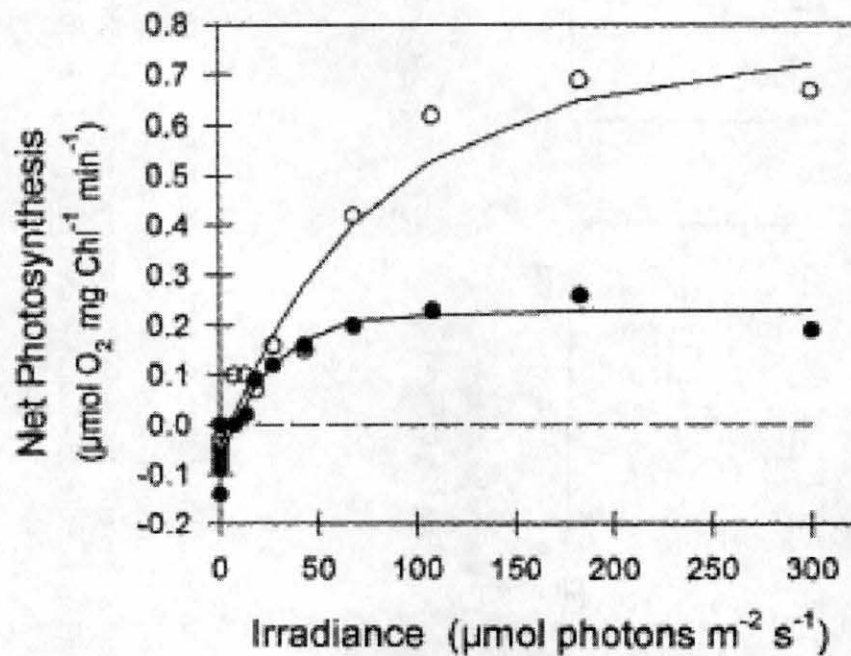


Figure 2: Net photosynthesis vs. irradiance under "normal" (pH 8.2) vs. CO_2 -enriched (pH 6.2) conditions. Normal conditions are designated by filled circles, while enriched conditions are open circles (Figure 1 and caption from Zimmerman et al. 1997).

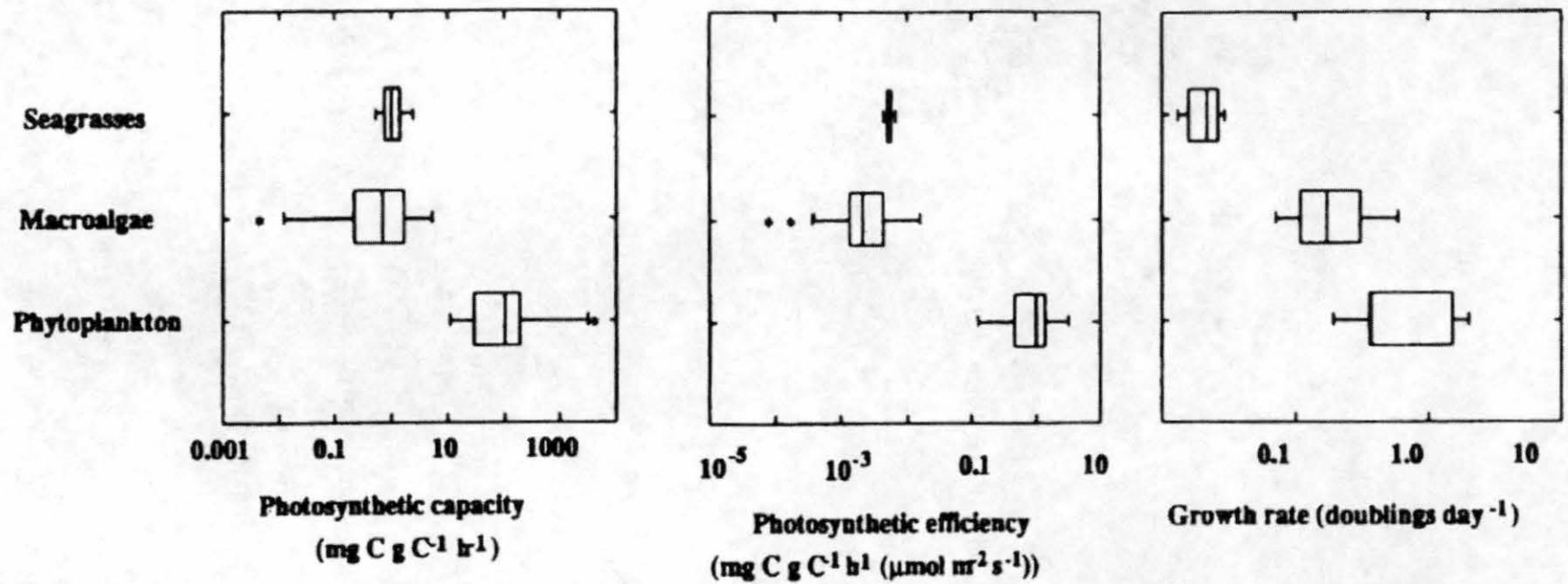


Figure 3: Box plots showing the distribution of photosynthetic capacity, efficiency, and specific growth rate for seagrasses macroalgae, and phytoplankton. Boxes encompass the 25 and 75% quartiles of all the data for each plant type, the central line represents the median, bars extend to the 95% confidence limits, and asterisks represent observations extending beyond the 95% confidence limits (Figure 2 and caption from Duarte 1995).

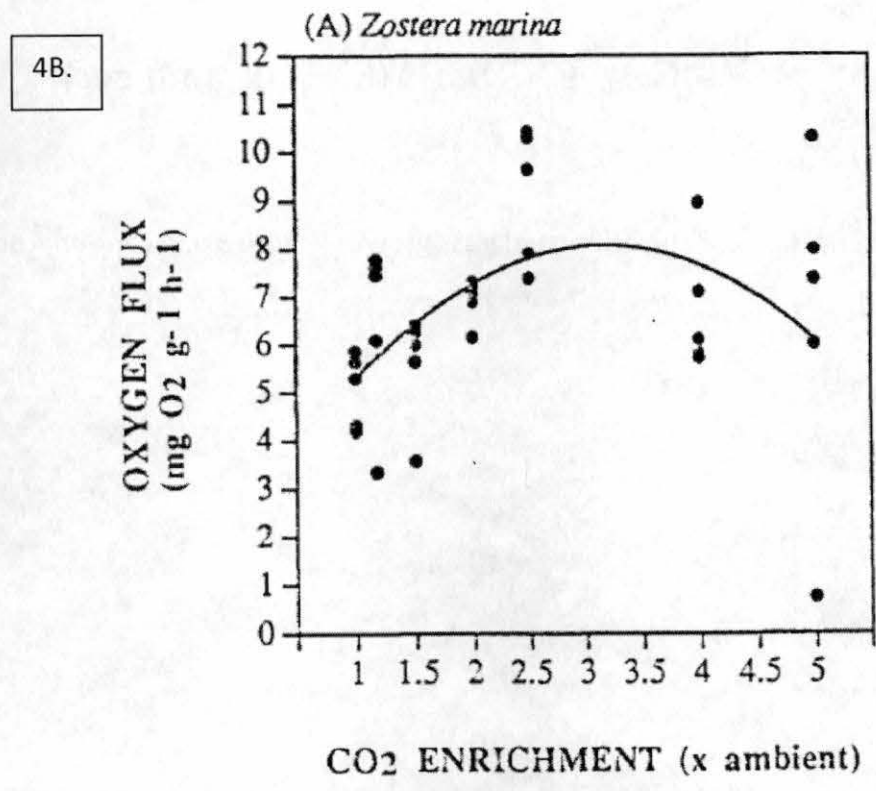
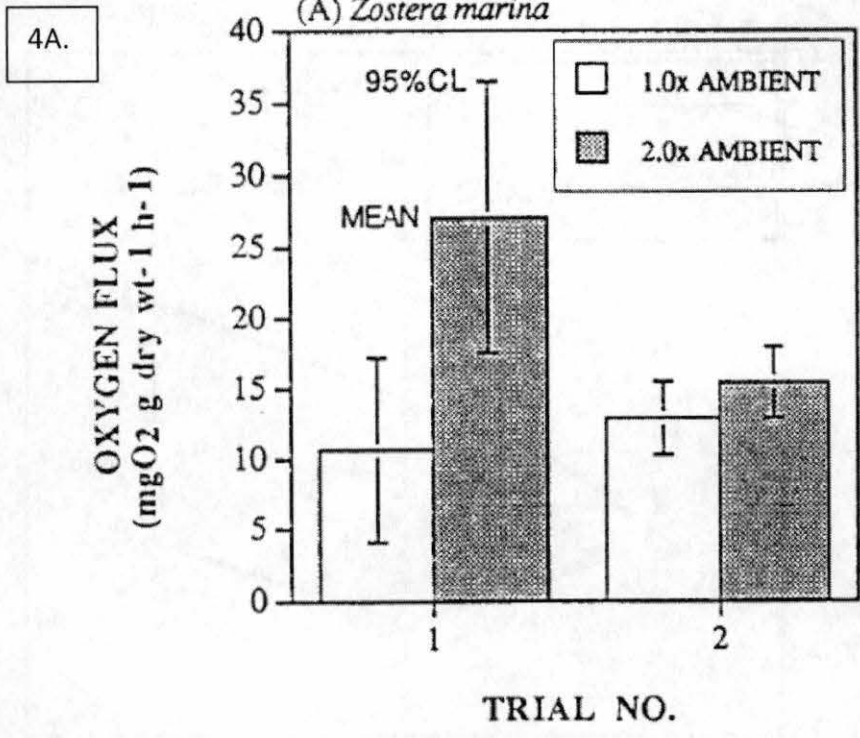


Figure 4: A.) Effect of doubling CO₂ enrichment on eelgrass net productivity (Figure 1a and caption from Thom 1996), B.) Effect of serial CO₂ enrichment on eelgrass net productivity (Figure 2a and caption from Thom 1996).

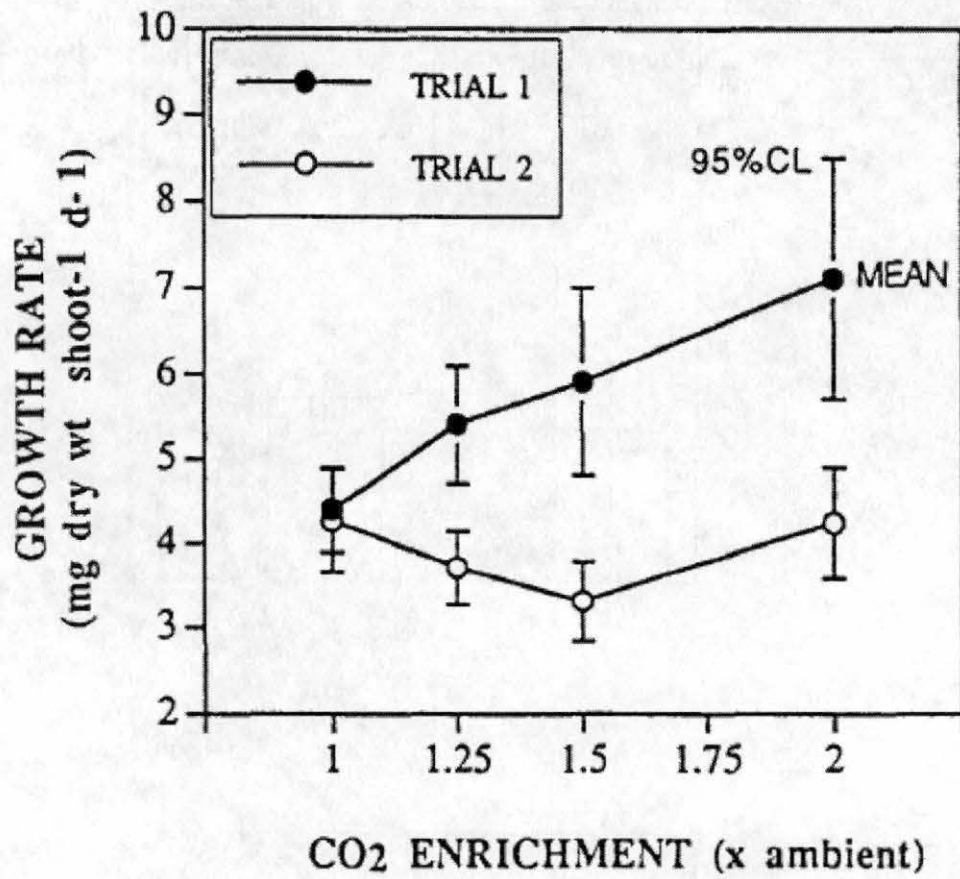


Figure 5: Effect of serial CO₂ enrichment on eelgrass growth (Figure 3 and caption from Thom 1996).

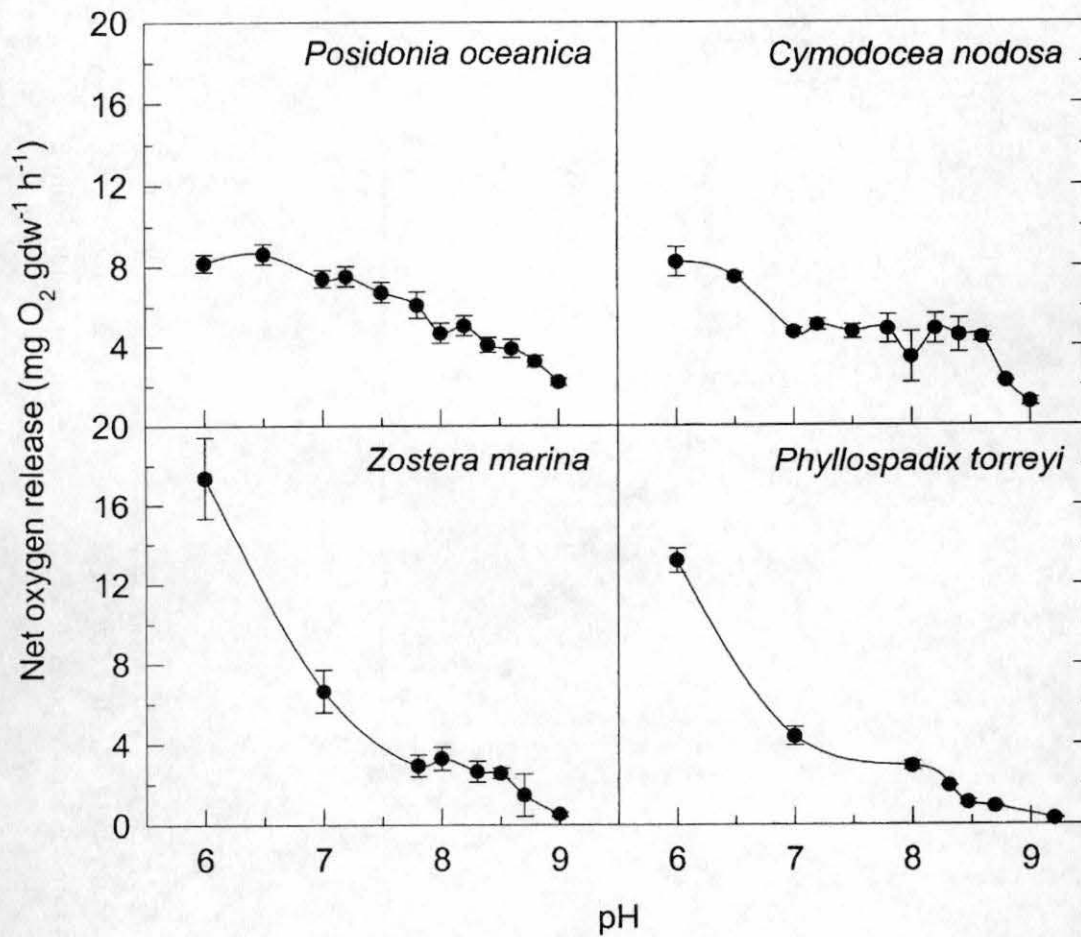


Figure 6: Changes in net oxygen release with pH in four seagrass species. Vertical bars represent the standard errors (n=3-4) (Figure 2 and caption from Invers et al. 2001).

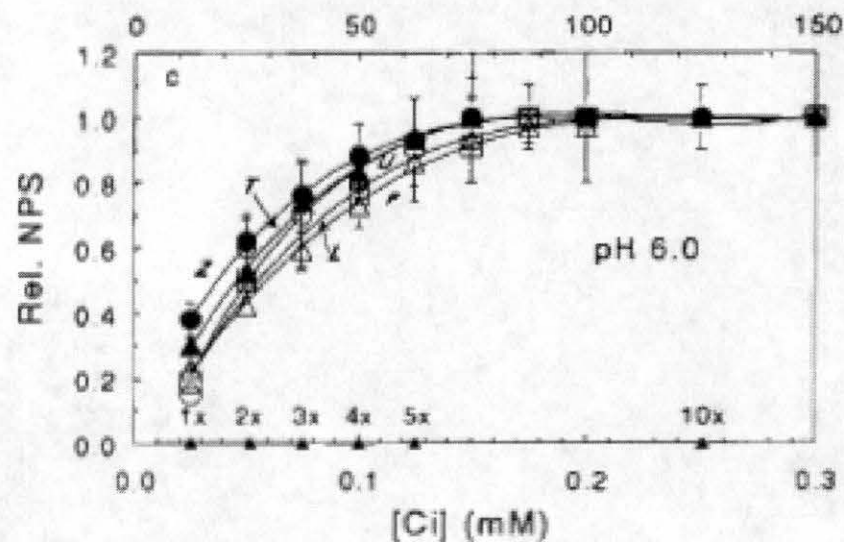
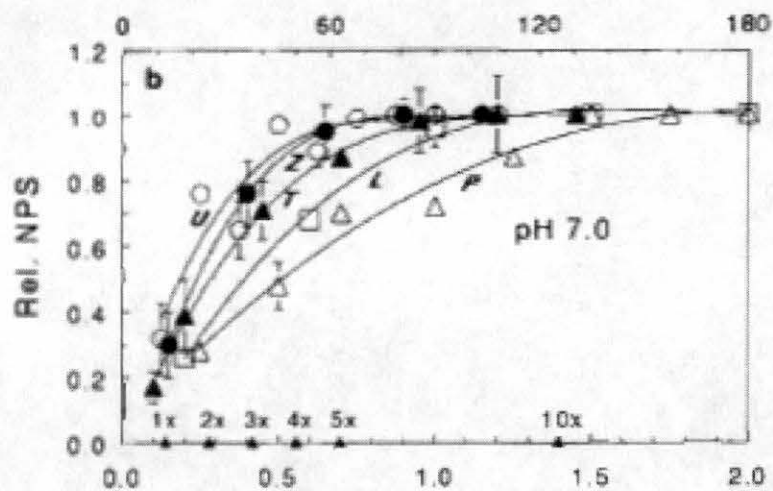


Figure 7: Net photosynthetic rates (NPS) of species of seagrass and marine macroalgae, in synthetic seawater to which different amounts of inorganic carbon were added. Eelgrass (Z) is represented as solid circles. Rates are given relative to the maximal mean rate for each species ($n = 9-10$). (Figure 2 and caption from Beer and Koch 1996)

Table 1: Synthesis of pH data collected from 1999-2006 for A.) the Valino Island monitoring site in the South Slough National Estuarine Research Reserve, OR and B.) the Ploeg Channel monitoring site in the Padilla Bay National Estuarine Research Reserve, WA. Raw data was obtained from CDMO (2009). A straight average pH for all values collected in one year is shown, as well as one standard deviation. The minimum and maximum pH values recorded in a given year are also shown.

A.) South Slough, OR – Valino Island

<i>Year</i>	<i>Average pH</i>	<i>Standard deviation</i>	<i>Minimum pH</i>	<i>Maximum pH</i>
1999	7.8	0.2	6.8	8.3
2000	7.9	0.2	6.7	8.5
2001	7.8	0.2	7.3	8.3
2002	7.8	0.2	7	8.3
2003	7.8	0.2	6.2	8.3
2004	7.9	0.3	6.1	8.4
2005	8.0	0.2	6.4	8.8
2006	8.0	0.2	6.9	8.7

B.) Padilla Bay, WA - Ploeg Channel

<i>Year</i>	<i>Average pH</i>	<i>Standard deviation</i>	<i>Minimum pH</i>	<i>Maximum pH</i>
2001	8.1	0.3	7.5	9.4
2002	8.2	0.4	7.1	9.3
2003	8.2	0.3	7.2	9.2
2004	8.0	0.3	6.9	9.2
2005	8.1	0.3	7.1	9
2006	8.2	0.3	7.3	9.3