ABSTRACT

Title of thesis: ACCLIMATION OF MARINE MACROPHYTES
                        (*SACCHARINA LATISSIMA* AND *ZOSTERA MARINA*) TO
                        WATER FLOW

                        Terry Jordan, Master of Science, 2008

Thesis directed by: Dr. Evamaria Koch

I examined the physiological response of two marine macrophytes, the brown alga

Saccharina latissima and the angiosperm *Zostera marina,* to water flow in nature and in

controlled experiments. Limitation of photosynthesis of both species by the availability

of dissolved inorganic carbon (DIC) was increased under low current velocities.

Physiological acclimation to low water flow occurred via upregulation of DIC uptake

mechanisms in both *S. latissima* and *Z. marina*. Both species increased their ability to

generate CO_2 in the boundary layer by increasing external carbonic anhydrase and in *Z.*

marina by also increasing proton extrusion and photosynthetic capacity. Changes in the

xanthophyll-cycle in low-flow grown *S. latissima* increased non-photochemical

quenching, thus reducing photodamage when photosynthesis was limited by DIC uptake.

Water flow also affected root length in Z. marina but root length and below ground

biomass were also significantly affected by sediment type, an indirect effect of water

flow.

ACCLIMATION OF TWO MARINE MACROPHYTES (*SACCHARINA LATISSIMA* AND *ZOSTERA MARINA*) TO WATER FLOW

by

Terry L. Jordan

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Master of Science
2008

Advisory Committee:

   Dr. Evamaria Koch
   Dr. Ian Davison
   Dr. Todd Kana

Acknowledgements

Table of Contents

List of Tables

Chapter 2

Table 1: Environmental conditions and morphological characteristics of *Saccharina latissima* at the two field sites in Maine with different water flows (SBC – low flow; Kresge – high flow).

Table 2: Physiological and biochemical characteristics of *Saccharina latissma* grown in the experimental flume at three current velocities.

Table 3: Physiological and biochemical characteristics of *Saccharina latissima* at the two field sites in Maine with different water flows (SBC – low flow; Kresge – high flow).

Chapter 3

Table 1: Morphology and pore water and water column nutrients of *Zostera marina* under three water velocity treatments (0, 1, and 20 cm $\cdot$ s^{-1}) and two sediment types (sand and mud) in outdoor tanks at Horn Point Lab

Table 2. Porewater nutrients (n=4), percent tissue nutrients (n=9) and physiology (n=4) of *Zostera marina* at low (Bullhead Bay) and high (Tyndal Point) sites including statistics.

Table 3. Physiology and biochemistry of *Zostera marina* under three water velocity treatments (0, 1, and 20 cm $\cdot$ s^{-1}) and two sediment types (sand and mud) in outdoor tanks at Horn Point Lab.

Table 4. Statistics of growth, morphology, pore water nutrients, and physiology of *Zostera marina* under three water velocity treatments (0, 1, and 20 cm $\cdot$ s^{-1}) and two sediment types (sand and mud) in outdoor tanks at Horn Point Lab.

List of Figures

Chapter 1

Figure 1: *Saccharina lattisima* morphology

Figure 2: *Zostera marina* morphology

Figure 3: Changes in species of inorganic carbon in response to pH

Chapter 2

Figure 1: Diagram of laboratory flume

Figure 2: DIC Kinetics. Oxygen production in field collected algae from low (SBC) and high (Kresge) flow sites in response to increasing concentrations of DIC

Figure 3: A. Percent Tissue Carbon. B. Growth. Carbon content and growth determined after 28 days of culture in the laboratory flume under three flow regimes: 0, 1 and 13 cm s^{-1}

Figure 4: The Effect of Flow on Carbonic Anhydrase. A. Percent inhibition of photosynthesis by acetazolamide in field collected algae from low (SBC) and high flow (Kresge) sites after injection of 50mM acetazolaminde B. Relative CAext activity determined after 28 days days of culture in the laboratory flume under three flow regimes: 0, 1 and 13 cm s^{-1} according to Mercado *et al.* (1997)

Figure 5: The Effect of Flow on Yield. A. Yield in field collected algae from low (SBC) and high (Kresge) flow (n = 3). B. Yield determined after 28 days days of culture in the laboratory flume under three flow regimes: 0, 1 and 13 cm s^{-1}

Figure 6: The Effect of Flow on Non-Chemical Quenching. A. qN in field collected algae from low (SBC) and high (Kresge) flow (SBC). B. qN determined after 28 days days of culture in the laboratory flume under three flow regimes: 0, 1 and 13 cm s^{-1}

Figure 7: The Effect of PAR on Non Chemical Quenching. Rapid light curve measured with a PAM fluorometer after 28 days days of culture in the laboratory flume under three flow regimes: 0, 1 and 13 cm s^{-1}

Figure 8: The Effect of Flow on Xanthophyll Pigments. A. Violaxanthin B. Antheraxanthin C. Zeaxanthin Samples were taken from treatment conditions in the flume at 50 μmol m^{-2} s^{-1} and after 45 minutes at 200 μmol. m^{-2} s^{-}

Chapter 3

Figure 1: Outdoor circulating flume in mesocosm tanks (3 x 0.6 m length x width, 0.5m water depth).

Figure 2: Depth profiles of NH_4 (A), PO_4 (B), SO_4 (C) and pH (D) in sediment pore water in *Zostera marina* beds at Bullhead Bay and Tyndal Point

Figure 3: Depth profile of DIC in sediment pore water in *Zostera marina* beds at Bullhead Bay and Tyndal Point

Figure 4: CA_{ext} (A) and inhibition of photosynthesis by erythrosin-B (B) of *Zostera marina* grown in different current velocities and sediment types.

Figure 5: DIC-uptake kinetics of photosynthesis of *Zostera marina* grown in different current velocities and sediment types: sand (A) and mud (B)

Figure 6: Electron turnover rate (ETR) of photosynthesis of *Zostera marina* grown in different current velocities and sediment types: sand (A) and mud (B)

Figure 7: Non-photochemical quenching of photosynthesis of *Zostera marina* grown in different current velocities and sediment types: sand (A) and mud (B)

Figure 8: CA_{ext} (A) and inhibition of photosynthesis (B) by erythrosin-B in *Zostera marina* beds at Bullhead Bay and Tyndal Point

Figure 9: Growth rate (A) number of shoots and (B) of leaves of *Zostera marina* exposed to three current velocities for 8 weeks

Figure10: Total (A), above (B) and below (C) ground *Zostera marina* biomass expressed per number of final shoots per tray at end of an experiment that exposed this seagrass to different current velocities for 8 weeks

Figure 11: Ratio of above to below ground biomass in *Zostera marina* at end of an experiment that exposed this seagrass to different current velocities for 8 weeks

Figure 12: Fresh weight expressed on the basis of *Zostera marina* leaf area at the end of the experiment that exposed this seagrass to different current velocities for 8 weeks

Figure 13: Root (A) and rhizome (B) biomass per final number of *Zostera Marina* shoots per tray. Root length (C) at end of an experiment that exposed this seagrass to different current velocities for 8 weeks

Figure 14: Chl-a expressed on the basis of *Zostera marina* leaf area at the end of the experiment.

Appendix 1

Figure 1: Changes in temperature (A) in *Zostera marina* measured and light levels (B) at Bullhead Bay and Tyndal Point

Figure 2: Depth profiles of NH_4 (A), PO_4 (B), SO_4 (C) and pH (D) in *Zostera marina* beds at Bullhead Bay and Tyndal Point.

Figure 3: Length (A) and width (B) of *Zostera marina* leaves 2 through 4 (oldest) at Bullhead Bay and Tyndal Point.

Figure 4: Number of *Zostera marina* roots per node (A) and average root length (B) at Bullhead Bay and Tyndal Point.

Figure 5: *Zostera marina* tissue nitrogen (A) and carbon (B) at Bullhead Bay and Tyndal Point.

Figure 6: Respiration, P_{max}, and Gross P_{max} (A) of *Zostera marina* and oxygen flux vs photosynthetically active radiation (B) at Bullhead Bay and Tyndal Point.

Figure 7: Electron turnover rate (ETR) (A) non-photochemical quenching of photosynthesis (B) in *Zostera marina* at Bullhead Bay and Tyndal Point.

Figure 8: CA_{ext} (A) and inhibition of photosynthesis (B) by erythrosin-B in *Zostera marina* at Bullhead Bay and Tyndal Point.

List of Abbreviations

CA_{ext}: external carbonic anhydrase

DBL: diffusive boundary layer

DIC: dissolved inorganic carbon

ETR: electron turnover rate of photosynthesis

P_{max}: maximum rate of light-saturated photosynthesis

R_d: dark respiration

Rubisco: ribulose-1,5-bisphosphate carboxylase oxygenase

Chapter 1: Introduction

Marine macrophytes (macroalgae and seagrasses) occur in shallow seas from the arctic to the tropics and are among the most widespread and productive marine ecosystems (Hemminga and Duarte 2000).

Both marine flowering plants (seagrasses) and macroalgae (seaweeds) are ecologically and economically important.   Macroalgae are commercially important, as seaweed is used by the textile, cosmetic, pharmaceutical and food industries (Gunstheimer and Jahreis 1998). The extracted polysaccharides of the algae (alginate from brown algae and agar and carrageen from red algae) are used in many food products (e.g., dairy products, fruit juices, dressings, pudding, jam and tinned meat) as texturing, thickening and gelling agents and are also important in the microbiology and biomedical research industries (e.g. agar plates for culturing bacteria and agarose gels for electrophoresis). Asia produces 80% of the global commercial algae (Luning and Pang 2003) and a considerable part is directly used for human nutrition. Algae have high vitamin content, that, in the case of A, B, and C vitamins surpass those of fresh fruits and vegetables. Additionally, they have high mineral and trace element (iron and iodine) content (Gunstheimer and Jahreis 1998). Macroalgae are also ecologically important as primary producers and providers of habitat and food for marine organisms including many commercially important fish and invertebrates (Kirkman and Kendrick 1997, Harris *et al.* 2004, Viera *et al.* 2005). This is most obvious in the case of the dense "forests" of the giant kelp *Macrosystis* that occur in the Pacific Ocean, but is also true of other species of seaweeds found in intertidal and shallow coastal waters. Because most seaweed grows attached to hard substrate, they are

generally most abundant on rocky bottoms, in contrast to seagrasses that grow rooted in sediments.

Seagrasses cover about 0.1-0.2 % of the global ocean and provide physical structure on sediment bottoms, enhancing community diversity, biomass and primary and secondary production (Duffy 2006). Historically seagrasses were utilized as commercial insulation, sound proofing material and roofing thatch (Urquhart 1824, Ostenfeld 1908, Wyllie-Echeverria and Cox 1999). Additionally, *Zostera marina* was used as a material for stuffing pillows, mattresses and upholstery. The crab industry in the Chesapeake Bay used seagrass extensively as a packing material for exporting crabs (Urquhart 1824, Ostenfeld 1908, Tubbs 1995). Currently, vital ecosystem functions are provided by nutrient recycling, detritus production and export, as well as sediment stabilization. The leaves provide a substratum for growth of epiphytic microalgae that fuel food webs and provide shelter and nursery areas for juvenile stages of commercially important finfish and shellfish and so contribute significantly to the economic importance of estuarine fisheries (Heck *et al.* 2003). Moreover because much seagrass production ends up in below ground tissues (i.e. roots and rhizomes) and ungrazed detritus, seagrass beds are an important global sink for carbon; estimated at 15% of net CO_2 uptake by marine organisms on a global scale despite contributing only 1% of marine primary productivity (Duffy 2006).

Ecology of *Saccharina*

Saccharina is a genus of 31 species of brown algae (Phaeophyceae) all sharing the common name 'kelp'. The life history of *Saccharina* consists of two stages. The large diploid sporophytes produce gametes with flagella after meiosis. The gametes settle on a hard substrate

and germinate into microscopic male and female gametophytes, which form branched filaments. The male gametophytes bear small unicellular antheridia, which produce sperm that are attracted to the egg cells within the oogonium, which remains attached to the female gametophyte. The zygote then develops on the female gametophyte, rapidly overgrowing it and grows to become the sporophyte. As the sporophyte and gametophyte generations look quite different, *Saccharina* is said to show heteromorphic alternation of generations.

 The species S*accharina latissima* is a circumpolar alga found at high latitudes in the northern and southern hemispheres and is commonly found in low littoral rock pools and from the sublittoral fringe to below 20 m.   *S. latissma* has a thallus that is up to 4 m long and attaches to rocks by a strong holdfast and stipe. It has a leathery blade, about 15 cm wide, which is unbranched and lacks a midrib. Typically blades are flat with wavy margins and bullations in hydrodynamically calm habitats (Figure 1). Alternately, under high water flow conditions, *S. lattisima* is narrow and thick, lacking wavy margins (Gerard and Mann 1979, Koehl and Alberte 1988, Dudgeon and Johnson 1992, Gutierrezand Fernandez 1992, Hurd *et al.* 1996 and references therein). This morphological difference can be used to identify algae from high and low water flow sites.

I chose to use *Saccharina latissma* in this experiment as it is readily available, grows well in culture, has been studied intensively from the perspective of photosynthesis and other aspects of physiology (e.g., Davison *et al*. 1991, Machalek *et al.* 1996, Kuehl *et al.* 2001, Klenell *et al.* 2002, Gevaert *et al*. 2003), and is known to change morphology in response to water flow (Gerard and Mann 1979, Gerard 1987).
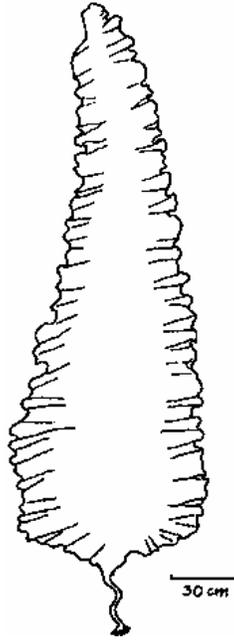
Figure 1. *Saccharina lattisima* morphology
Source: Chapman and Chapman (1980).

Ecology of *Zostera*

Zostera is a small genus of widely distributed flowering marine plants (angiosperms). It is found

in intertidal and shallow subtidal areas in sheltered, relatively shallow inlets and bays, estuaries

and saline lagoons in both hemispheres. *Zostera* can also be found in more wave-exposed areas

of intertidal mud and sand flats, as well as shallow subtidal sandbanks. *Zostera* plants have

inconspicuous flowers, which lack petals and are aggregated in inflorescences. Male and female

flowers are separate but occur on the same plants. In addition to producing seeds, *Zostera*

reproduces extensively by vegetative shoots. *Zostera* plants have an extensive network of

branched, creeping underground stems (rhizomes) that help bind the sand or mud substratum

(Figure 2). These horizontal rhizomes bear leafy shoots with abundant green, grass-like leaves.
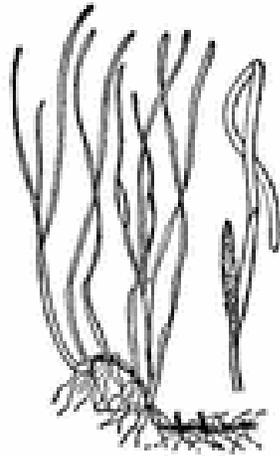
4

Figure 2. *Zostera marina* morphology .
Source: http://www.thefreedictionary.com/genus+Zostera

The leaves are flat and linear, with maximum length and width varying according to species. The leaves have large air spaces (lacunae), which transport oxygen to the sediment and keep the leaves upright in the water (Grice *et al*. 1996). *Zostera* exhibit considerable morphological plasticity in response to environmental conditions (Perlata *et al.* 2005).

The species *Zostera marina* is adapted to the cold waters of the North Atlantic and North Pacific and is found mostly in the sublittoral region, only rarely being exposed at low tide (Boese *et al.* 2004). In the United States, it extends southward to North Carolina in the Atlantic and Baja California in the Pacific. At the southern limits of its range, however, active growth is usually restricted to the cooler months of spring and autumn, with flowering occurring in the spring and plants dying back in the hotter summer months.

Zostera marina beds provide a vital contribution to sediment deposition and substrate stabilization. Slowing of water movement by leaves encourages accumulation of sediments and prevents resuspension (Ward *et al.* 1984, Almasi *et al.* 1987, Gacia *et al.* 1999, Terrados and Duarte 2000) while the dense rhizome and root system stabilizes the sediment preventing or

reducing sediment loss (Fonseca 1989). The consolidation of sediments in seagrass beds enables the development of richer infaunal communities with higher densities of individuals than those in adjacent bare sediments (Boström and Bonsdorff 1997). Additionally, seagrasses provide substrate for epiphytic algae and micro-invertebrates, and nursery grounds for many species of economically important fish and shellfish. Several species of waterfowl also use *Zostera marina* as an important food source.

I choose to work with *Zostera marina* because, like *Sacharina latissima,* it is readily available, grows well under experimental conditions, has been studied intensively from the perspective of photosynthesis and other aspects of physiology and shows considerable morphological and physiological plasticity in response to environmental factors (Evans *et al.* 1986, Zimmerman *et al.* 1989, Lee *et al.* 2007).

Definitions

Several terms and concepts used in my thesis are commonly used in the literature, but are loosely defined. To avoid confusion and ambiguity I have provided the following definitions, which are consistent with most, but not all of the previous literature. These definitions follow those of Davison (1991).

Physiological responses are immediate or short-term reversible adjustments of metabolism to changes in environmental conditions.

An example would be the instantaneous response of photosynthesis to water-flow induced by changes dissolved inorganic carbon (DIC) transport. Although physiological responses are rapid

and reversible, they may act as the trigger or sensing mechanism to induce physiological acclimation.

2) Physiological acclimations are phenotypic modifications in metabolism in response to environmental change that involve the up or down-regulation of enzymes or other cellular constituents.

An example would be an up-regulation of external carbonic anhydrase (CA_{ext}) to compensate for the reduction in CO_2 transport in low-flow grown plants or algae. Acclimation responses are frequently reversible, but over a longer timescale than physiological responses because of the time required to synthesize or break down enzymes and other key components of metabolic pathways.

3) Physiological Adaptations are genetic differences between species or ecotypes, by which a species or individual improves its fitness in relationship to its environment.

A classic example of physiological adaptations is the photosynthetic differences between "sun" and "shade" plants.

Acclimation and **Adaptation** are connected in that the ability to acclimate is under genetic control, whereas the distinction between **physiological response** and **acclimation** is dependent

on the degree of to which the cellular apparatus changes following a disturbance in environmental conditions.

4) Metabolic compensation is the degree to which an acclimation response overcomes changes in environmental conditions. Compensation may be partial or perfect.

An example of compensation is provided by the response of metabolism to temperature. The physiological response of photosynthesis to temperature is for rates to instantaneously increase with elevated temperature. Physiological acclimation of Calvin cycle enzymes either eliminates (perfect compensation) or reduces (partial compensation) this effect so that, for example, the rates of photosynthesis achieved at 5 and 15°C are either the same, or less different that would be predicted from the instantaneous metabolic responses (Davison 1991).

5) Rate limitation is the restriction of a metabolic process by the supply of a resource.

6) A Limiting factor is the environmental factor that restricts the overall rate of growth, and is based upon Liebig's law.

A **rate limitation** is not necessarily a **limiting factor** because **metabolic compensation** may be perfect and compensate for **rate limitation** or a **limiting factor** (such as N) may limit growth so that the **rate limitation** has no overall impact on growth.

Effect of Water Motion on Seaweeds and Seagrasses

Both seaweeds and seagrasses occupy dynamic habitats characterized by constant changes in environmental conditions such as light, salinity, temperature, and nutrients over both seasonal and tidal timescales. Additionally, marine macrophytes inhabit a fluid environment in constant motion due to the action of currents and waves. These hydrodynamic parameters have received less attention than other factors such as light (Koch 2001). Seaweeds and seagrasses inhabit a wide range of surroundings that vary greatly in water motion, ranging from rocky shores exposed to extremely high and variable current and drag forces from breaking oceanic waves to constricted areas that increase the velocity of tidal currents (Marba *et al.* 1994, Boller and Carrington 2006, Hepburn *et al.* 2007, Mach *et al.* 2007) and sheltered bays protected from wave action and also characterized by very low current velocities.

One well documented morphological response to water motion in some macrophytes is the development of high and low flow phenotypes (Gerard, 1987, Blanchette 1997, Boller and Carrington, 2006). Seaweeds from sites with high water motion are generally longer, narrower, thicker, stronger, and more streamlined than members of the same species growing in calm sites, which tend to be thin, broad and often undulate or ruffled (Gerard and Mann 1979, Koehl and Alberte 1988, Dudgeon and Johnson 1992, Gutierrez and Fernandez 1992, Hurd *et al.* 1996). Morphological response to water flow in seagrasses is not as well documented and must be differentiated from the morphological response to sediment type, an indirect effect of water flow.

Additionally, water motion has an important effect on most of the major biotic and abiotic factors that affect macroalgae and seagrasses (Hurd 2000, Koch *et al.* 2005). Water flow affects

marine primary productivity, as it alters resource acquisition. Water flow can affect rates of photosynthesis and nutrient uptake by changing the thickness of the diffusive boundary layer (DBL) (Koch 1994). The boundary layer is the area next to the leaf where water flow is restricted by friction. This restriction in flow sets up a gradient in velocity that is lowest right next to the surface of the leaf. This area closest to the leaf is the diffusive boundary layer. Water flow within this layer is laminar and movement of molecules across it is dominated by molecular diffusion. Diffusion of CO_2 in water is a very slow process (diffusion coefficient for CO_2 in water at 25°C is 1.55 x 10^{-9} m^2 s^{-1}). This creates a resistance to the transfer of nutrients and dissolved inorganic carbon (DIC) to the leaf or blade surface. Further, the DBL thickness is inversely proportional to water flow leading to an even lower flux of carbon and other nutrients from the water column to leaf or blade surfaces under low water flow conditions. Since both carbon and nutrients in macroalgae are taken up solely from the water column, photosynthesis could be restricted by the slow rate of diffusion of either DIC or nutrients across the DBL with nitrogen the most likely nutrient to be limiting (Ryther and Dunstan 1971, Fisher *et al.* 1992, Conley 1999).

In the case of seagrasses, although DIC and nutrients are available both in the water column and sediment, DIC uptake occurs primarily from the water column via the leaves, whereas nutrient uptake occurs mainly from the pore water by the roots. The concentration of nutrients available in the pore water of seagrass beds is generally higher than in the water column. Globally, water column ammonium and phosphate levels in seagrass beds have a median of 1.7 and 0.35 μM respectively, while pore water median concentrations are 60 and 6.5μM (Hemminga 1998). In carbonate sediments, however, phosphate is bound to the particles and not readily available for

uptake by seagrass roots (Jensen *et al.* 1998). Therefore, leaf nutrient uptake may only be relatively important when nutrient levels in the water column are high (Stapel *et al.* 1996) or when the substrate is carbonate in origin (Jensen *et al.* 1998). Therefore although rate limitation of photosynthesis by nutrients could be induced by increased DBL thickness, carbon limitation is more likely. Sand-Jensen (1977) evaluated the reduction of photosynthesis in *Zostera marina* due to epiphyte load, which limits uptake from the water column, and concluded that reduction of light saturated photosynthesis was due to carbon deficiency not nutrient limitation. Carbon uptake through the pore water has been shown in some species of freshwater vascular plants (Wium-Anderson 1971) and one study of *Zostera marina* showed limited inorganic carbon uptake from the pore water (McRoy and Goering 1974). However, they concluded that carbon levels in the water column were too high to make pore water carbon uptake a significant contribution to total carbon uptake.   Therefore, while the main source of nutrients for seagrasses is the sediment, the main source of carbon is the water column. As a result increased DBL thickness is more likely to affect DIC dynamics than nutrient dynamics in seagrasses.

Rate Limitation of Photosynthesis by Carbon

When carbon dioxide from the atmosphere dissolves in water, it forms a balance of several ionic and non-ionic species (DIC) (Raven *et al.* 1999). These species are: aqueous carbon dioxide (CO_2), carbonic acid (H_2CO_3), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) (Miller 1985). The equilibrium equation for carbon in seawater is as follows:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2\ H^+$$

The ratio of these species depends on alkalinity. In strongly basic conditions, the carbonate ion, CO_3^{2-}, predominates. In weakly basic conditions, the bicarbonate ion, HCO_3^- is prevalent. In acid conditions, aqueous carbon dioxide, CO_2 is the main form. At the pH of seawater (~ 8), HCO_3^- is the predominant form with much smaller amounts of CO_2 available (Figure 3). The total DIC concentration of seawater is around 2 mM of which 1.9 mM is bicarbonate and 0.1mM is carbon dioxide. CO_2 is the species used in photosynthesis where it is fixed by the enzyme ribulose-1, 5-bisphosphate carboxylase oxygenase (rubisco) and eventually converted to glucose. CO_2 for photosynthesis must be taken up directly, or, if another species of carbon (i.e. HCO_3^-) is actively transported into the cell it must be converted to CO_2 before use. CO_2 is not a charged ion and therefore moves into the cell by passive transport down the concentration gradient. Therefore, CO_2 is the preferred ionic species of carbon for macrophytes (Brouns 1994, Beer and Koch 1996) including *Saccharina latissima* and *Zostera marina.* The mechanism by which
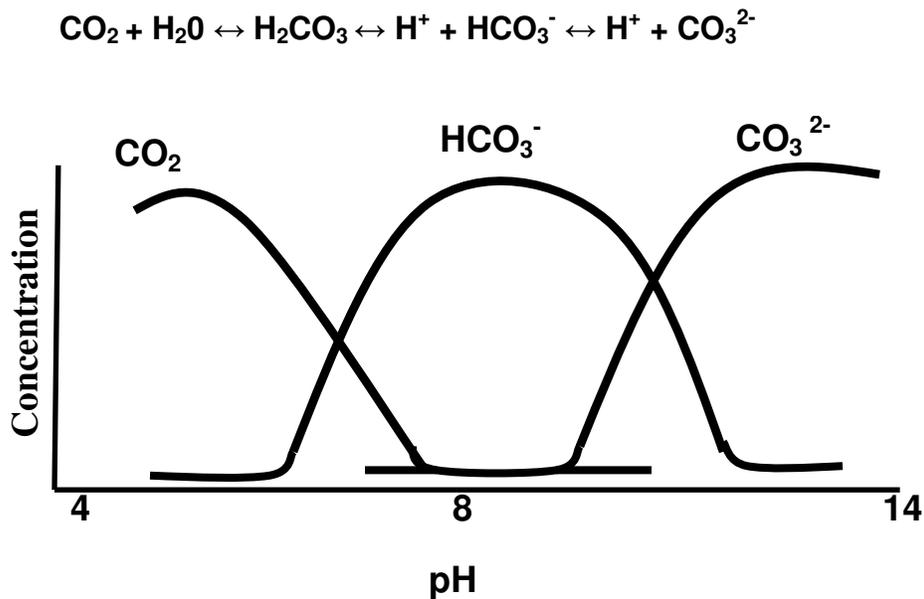
$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow H^+ + CO_3^{2-}$$

Figure 3. Changes in species of inorganic carbon in response to pH

carbon limits photosynthesis is a function of CO_2 concentration in the water column, water flow, and carbon uptake rate.

Rate limitation of photosynthesis by DIC at ambient concentrations has been established in macroalgae (Beer and Shragge 1987, Holbrook *et al.* 1988) and seagrasses (Beer & Rehnberg 1997, Björk *et al.* 1997, Schwarz *et al.* 2000) specifically in *Zostera marina* (Thom 1995, Beer and Koch 1996, Invers *et al.* 2001). In fact, one study of *Zostera marina* found that CO_2 enrichment increased photosynthesis three fold (Zimmerman *et al.* 1997). The global increase in atmospheric CO_2 is leading to the acidification of the oceans (Sabine *et al.* 2004, Lippsett 2005), which may be beneficial for macrophytes, especially seagrasses (Beer and Koch 1996) due to the relatively higher availability of DIC in the form of CO_2. Even under elevated CO_2 concentrations (unless saturated) water flow can reduce DIC availability due to the existence of the DBL (see above).

In the DBL of leaves and fronds of marine macrophytes, the available CO_2 is quickly taken up for photosynthesis because it diffuses rapidly across the cell wall and cell membrane and into the chloroplast down the diffusion gradient created by the fixation of CO_2 by rubisco. To replenish the carbon dioxide in the DBL, CO_2 must diffuse across the DBL from the higher concentration in the bulk medium (seawater). The thicker the DBL, the further CO_2 must diffuse to reach the leaf. Average DBL thickness in seawater at 25°C has been estimated at 50μm in high water flow and 1000μm in medium to low water flow (Larkum *et al.* 1989). Flux of CO_2 across the DBL would then be 3.1×10^{-7} mM m^{-2} s^{-1} and 1.6×10^{-8} mM m^{-2} s^{-1} in high and low flow respectively. Pmax then would then be 1.12×10^{-2} μmol O_2 cm^{-2} h^{-1} in high flow and 5.76×10^{-4}

in low flow.   These calculated rates of photosynthesis supported by diffusion are far lower than measured rates.

As the available CO_2 is drawn through the DBL, it is replenished by HCO_3^- (see equilibrium equation above), readily obtainable in the DBL due to greater concentration, and acts as an external reservoir for CO_2. However, the conversion of HCO_3^- to CO_2 is not instantaneous and depends upon salinity, temperature and pH. The reestablishment of equilibrium after the addition of HCO_3^- requires tens of seconds (Schulz *et al.* 2006) which is extremely slow for a chemical inter-conversion as many reactions take place in milliseconds. This conversion can become the rate limiting step in photosynthesis.

Rate limitation of photosynthesis due to reduced carbon dioxide availability brought on by increased boundary layer thickness has been shown to exist under stagnant conditions. Crossley *et al.* (2002) compared the effects of flowing and stagnant water on growth of *Aponogeton elongatus*, a freshwater rooted macrophyte. He found the limiting factor in still water was DIC. This plant depended on its roots for mineral uptake so nutrient levels were not greatly affected by DBL thickness. The freshwater macrophytes *Elodea canadensis* and *Ranunculus peltatus* acclimated to increased DBL thickness by upregulating photosynthetic performance in response to CO_2 limitation (1µM of CO_2) as seen in an increase in P_{max}, an increasing affinity for CO_2 (Madsen *et al.* 1996), and increasing rubisco activity (Olesen and Madsen 2000).

Acclimation to Carbon Limitation

One possible response to DBL carbon limitation would be to physiologically acclimate by mechanisms responsible for increasing carbon uptake. Most macroalgae, including *Saccharina latissima*, are able to utilize HCO_3^- by means of extracellular carbonic anhydrase (CA_{ext}) (Beer and Israel 1990, Johnson 1992, Beer 1998, Axelsson *et al.* 2000, Klenell *et al.* 2004), which catalyzes the conversion of HCO_3^- to CO_2 (Flores-Moya and Fernández 1998). Marine algae are known to increase CA_{ext} and rubisco levels in response to reduced carbon availability (Bozzo and Colman 2000, Andria *et al.* 2001), and similar responses might compensate reductions due to low water flow. Seagrasses also utilize facilitated diffusion of CO_2 in conjunction with CA_{ext}, (Invers *et al.* 2001, Hellblom *et al.* 2001, Beer *et al.* 2002). In one study, acetazolamide (AZ), an inhibitor of CA_{ext} activity reduced photosynthetic rates in *Zostera marina* by 20% (Hellblom *et al.* (2001) whereas another study showed a 65% reduction in photosynthesis in response to AZ (Hellblom and Bjork 1999).

Moreover, recent research has described a buffer-sensitive inorganic carbon utilization system in *Zostera marina* as well as other seagrasses (Uka *et al.* 2005). Hellblom *et al.* (2001) found that the addition of 50mM TRIS (pH 8.1) to natural seawater reduced net photosynthetic rates of *Z. marina* by some 70% as compared with rates in non-buffered seawater of the same pH. Additionally in *Zostera noltii* a large inhibitory effect was produced by TRIS buffer addition (Mercado *et al.* 2003). This suggests that proton extrusion, and the maintenance of acidic zones in the DBL, is also of importance in increasing availability of CO_2. Acidification of the DBL increases the concentration of CO_2 as the HCO_3^- - CO_2 equilibrium in seawater is pH dependent.

As the higher levels of CO_2 are taken up in the DBL, the rate limiting step becomes the conversion of HCO_3^- to CO_2, which is catalyzed by CA_{ext}. Therefore, these two mechanisms working in concert should be more effective than either alone.

These carbon uptake mechanisms have been linked to DIC limitation but not to water flow. It has been suggested that macrophytes acclimate physiologically to water flow (Koch 1994, Hurd 2000, Koch *et al.* 2005) but this has not yet been investigated. Acclimation to low water flow may include increases in one or both of these carbon uptake mechanisms.

At what current velocity, however, does DBL thickness increase rate limitation of DIC uptake? In algae, Wheeler (1980, 1982) established that photosynthesis and nitrate uptake by *Macrocystis* were limited at current velocities of less than 6.0 cm s^{-1}. Hurd and Stevens (1997) found that the transition from a laminar to a turbulent velocity boundary layer (i.e., DBL rate limitation) occurred at mainstream velocities of 1.5 cm s^{-1} in single blade specimens of algae whereas the transition in multiple bladed specimens was higher at 2.5-3.0 cm s^{-1}. Another study reports rate limitation of photosynthetic rates due to decreased carbon availability at velocities below 3-5 cm s^{-1} in *Thalassia testudinum* and *Cymodocea nodosa* (Koch, 1994). In this paper, due to the fluctuation of friction velocity, which measured mass transfer within the boundary layer on seagrass leaves observed *in situ*, it was concluded that rate limiting conditions do not persist in nature for prolonged periods. As the above-mentioned studies indicate, the velocity at which uptake is affected by increased boundary layer thickness is species specific and it is not clear that such low flows persist over long periods of time *in situ* (e.g., see Gerard 1982, Koehl and Alberte 1988, Koch 1994, and discussion in Hurd 2000). Unlike typical algae beds, low flow rates do

occur *in situ* in seagrass beds because dense seagrass beds attenuate water flow substantially. Current velocity decreases with distance downstream in a seagrass canopy and with the distance below the canopy surface (Fonseca and Koehl, 2006).

Rate limitation of DIC uptake in the DBL also reduces the amount of light used in photosynthesis. Therefore, another possible physiological response to a reduction in carbon availability would be an increase in photoprotective mechanisms that dissipate excess light energy. The xanthophyll cycle protects against high light stress by dissipating excess light via heat, referred to as non-photochemical quenching (see review Demmig-Adams and Adams 1992). The xanthophylls (violaxanthin, antheraxanthin, zeaxanthin) are carotenoids which undergo rapid and reversible changes in concentration due to high-light exposure. During the day, as irradiance increases, most of the violaxanthin is transformed into antheraxanthin and then zeaxanthin. Higher levels of zeaxanthin correlate with periods of reduced photosynthetic activity. Antheraxanthin and zeaxanthin are responsible for dissipation of excess energy from light-harvesting complexes. As irradiance decreases most of the xanthophyll pool converts back to violaxanthin. The ability of macrophytes to cope with high irradiance is related to the size of their pool of xanthophyll-cycle compounds and within a species the xanthophyll pool has been found to change as an acclimation to environmental parameters (Franklin *et al.* 1996). When stressed with high light, S*accharina latissima* has been shown to have a very effective xanthophyll cycle (Harker *et al*. 1999, Gevaert *et al*. 2003, Rodrigues and Pereira dos Santos 2002). Additionally, non-photochemical quenching increases much more rapidly in shallow water algae during high light treatment and maintains a higher level than deep water algae

(Grobe unpublished) indicating that *S. latissima* adjusts to light level by regulating the xanthophyll pool. This cycle also functions in *Zostera marina* in response to high light stress (Ralph *et al.* 2002). An investigation of seven seagrass species showed that shallow water seagrasses receiving high light had a high capacity for non-photochemical quenching compared to seagrasses in deep water (Ralph *et al.* 1998). Acclimation to low water flow may involve an increase in non-photochemical quenching.

Effect of Sediment on Seagrasses

Seagrasses experience direct effects of water flow on their leaves (e.g., drag and DBL limitation) but they also experience indirect effects of water flow as water flow modifies the sediment and consequently, the nutrients available to the roots. Sediments in high current flows are coarser and less organic than the fine, nutrient-rich sediments typical of more quiescent waters (Davis 1985) due to the removal of fine organic particles from the sediment by high current velocities. However, Lee and Dunton (1999) found that although organic and non-organic sediment had different amounts of nitrogen in the pore water, the same amount was taken up through the roots of *Thalassia testudinum* because it acclimated and increased below ground biomass to increase nutrient uptake. Similarly, sediment nutrients have been shown to affect biomass allocation in *Zostera marina* (Short 1987, Wicks 2005) and *Syringodium filiforme* (Short *et al.* 1985); low nutrient sediments produced less above ground biomass and more root biomass.

It is difficult to distinguish between indirect effects of water flow mediated by the sediment (a response to pore water nutrients) and direct effects of water flow (a response to leaf DBL thickness and drag) on seagrass morphology. Field observations indicate that leaves of *Posidonia australis* are longer in wave-exposed areas than in sheltered areas (Larkum 1976), those of *Thalassodendron ciliatum* are narrower in a rocky exposed area in contrast to a sheltered sandy area (Bandeira 2002), and leaves of *Thalassia testudinum* and *T. hemprichii* are shorter in wave exposed areas (Van Tussenbroek 1996, Tomascik *et al.* 1997). In a controlled lab experiment, which eliminated sediment composition and genetic differences as variables, Peralta *et al.* (2005) showed that *Zostera noltii* had shorter and narrower leaves and greater below ground biomass at high (30 cm s^{-1}) than low current velocities (2 cm s^{-1}).

Hypotheses

My thesis focuses on the ecophysiological response of marine macrophytes to water flow. Although water motion due to waves and hydrodynamic interactions between individuals in algal forests and seagrass canopies are important (Fonseca and Kenworthy 1987, Hurd 2000), for simplicity, I have decided to focus my experimental work on responses of individual plants to currents, in order to provide a fundamental understanding of the physiological basis of the response to water motion.

I tested the general hypothesis that marine macrophytes physiologically acclimate to water flow. One alga (*Saccharina latissma* L.) and one seagrass (*Zostera marina* L.) were used in this research. The rationale for this approach is that algae have a simple system that draws all the nutrients and DIC needed from the water column through the blades. They are attached to rock

with a holdfast, which is used exclusively for anchorage. Seagrasses, however, are rooted angiosperms that take up nutrients from both the water column and the sediment, whereas DIC uptake occurs mainly through the leaves.

I will address these specific hypotheses 1) Seagrasses and macroalgae acclimate physiologically to low water flow due to diffusive boundary layer (DBL) limitation and 2: Seagrass physiology and morphology are affected by water flow directly via DBL and indirectly via sediment composition. Particularly, I am looking at the following questions: Does increased DBL thickness due to low flow increase rate limitation of photosynthesis? What is the initial physiological response? Does acclimation take place and how? Does sediment type, an indirect effect of water flow, impact leaf DBL acclimation?

Chapter 2.   Physiological responses to water flow in the brown alga

(*Saccharina latissima* Linnaeus) Lane, Mayes, Druehl and Saunders

Abstract

I examined the response of the brown alga *Saccharina latissima* to water flow in order to test the hypothesis that this alga acclimates its physiology in response to a flow-dependent decrease in carbon availability. I compared physiological characteristics of *S. latissima* from high and low flow sites on the coast of Maine (Kresge Point and Sand Beach Cove, respectively) and of alga grown under controlled conditions in a laboratory flume at three flow velocities (0, 1, and 13 $cm \cdot s^{-1}$). Dissolved inorganic carbon (DIC) uptake kinetics indicate that ambient seawater concentrations of DIC are insufficient to saturate photosynthesis in *S. latissima.* Measurement of light-saturated photosynthetic electron transport rate (ETR) using a PAM fluorometer show that photosynthesis declines immediately following a transfer from high to zero flow conditions, which I attribute to rate limitation of photosynthesis due to decreased concentrations of DIC in the diffusive boundary layer (DBL). Growth rates and tissue carbon contents were significantly lower under stagnant conditions than at 1 or 13 $cm \ s^{-1}$ suggesting that the reduction in DIC in the DBL can become a limiting factor at flows between 0 and 1 $cm \ s^{-1}$ despite physiological acclimation of DIC uptake. Photosynthetic transport of DIC in *S. latissima* occurs via diffusion of CO_2 produced in the DBL by external carbonic anhydrase (CA_{ext}) and localized acidification from a proton pump in the cell membrane. CA_{ext} activity was highest in algae from the low flow site and in the stagnant treatment in the flume experiment, suggesting that CA_{ext} is upregulated to increase availability of CO_2 in the boundary layer. However, there were no differences in the

dependence of photosynthesis on the proton pump that could be associated with water flow, suggesting that the limiting step in DIC uptake under DBL-limited conditions is the rate of interconversion of HCO_3^- to CO_2 catalyzed by CA_{ext} rather than the equilibrium concentration of CO_2, which is determined by pH. Photosynthetic rates (ETR) were highest in algae collected or grown at high flow. I attribute this to a down-regulation of other aspects of photosynthetic metabolism such as Rubisco and other Calvin-cycle enzymes to balance carbon uptake with carbon assimilation. In contrast, the capacity for non-photochemical quenching (qN) was highest in algae from low water flows, which also exhibited larger increases in the xanthophyll cycle pigments, antheraxanthin and zeathanthin, when exposed to high light under low flow conditions. Tissue nitrogen contents suggest that laboratory grown algae were N-replete (3% N), whereas contents of field algae are consistent with summer N-limitation of growth (1.2% N), indicating that physiological acclimation of DIC uptake mechanisms in response to water flow occurs regardless of the N-status of the algae. Overall my data support the hypothesis that *S. latissima* responds to low current velocities by increasing its ability to generate free CO_2 in the boundary layer via increased activities of CA_{ext} and also increases the dissipation of excess light energy via qN to reduce photodamage when photosynthesis is rate limited by decreased DIC concentrations in the DBL under stagnant or low water flow conditions.

Introduction

Interest in the response of marine algae to inorganic carbon uptake has been heightened by the anthropogenic increases in atmospheric CO_2 (Short and Neckles 1999, Beer and Koch 1996). These changes have increased oceanic DIC concentrations (Houghton et al. 2001) and reduced

pH (Caldeira and Wickett 2003), increasing the relative and absolute concentrations of free dissolved CO_2, the species most easily absorbed by marine phototrophs (Madsen and Sand Jensen 1991, Beer and Koch 1996, Haugan and Drange 1996, Beer and Rehnberg 1997). Current ambient concentrations of oceanic DIC (~ 2 mM) are insufficient to saturate photosynthesis in both seagrasses and macroalgae, both of which generally exhibit an immediate increase in photosynthesis when supplied with additional DIC (Beer and Shragge 1987, Holbrook *et al*. 1988, Lavavasseur *et al.* 1991, Raven and Osmond 1992, Beer and Koch 1996, Schwarz *et al* 2000, Invers *et al*. 2001). The availability of DIC to marine phototrophs is not only a function of atmospheric concentrations and pH but also water flow, which affects the thickness of the DBL on the plant surface (Raven *et al.* 1985, Koch 1994). DBL thickness is inversely proportional to water flow leading to a reduction in the diffusion of DIC and nutrients to the surface of the plant or alga under low flow conditions (Koch 1994, Gonen *et al.* 1995).

It is well-established that some macroalgae exhibit morphological differences in responses to water flow (Cotton 1912, Mathieson *et al.* 1981, Norton 1982, Gutierrez and Fernandez 1992). Seaweeds from sites with high water motion are generally longer, narrower, thicker, stronger, and more streamlined than members of the same species growing in calm sites, which tend to be thin, broad and often undulate or ruffled (Gerard and Mann 1979, Koehl and Alberte 1988, Dudgeon and Johnson 1992, Gutierrez and Fernandez 1992, Hurd *et al.* 1996). The low flow morphology increases the capacity for carbon and nutrient uptake by increasing blade area relative to blade biomass (thickness) or by increasing turbulence and reducing the DBL either with an uneven blade surface, (Hurd *et al.* 1996), or by inducing flapping of the blade under low flow conditions (Denny and Roberson, 2002). Although in some macrophytes these

morphological differences have a genetic basis, i.e., ecotypic adaptation (Roberson and Coyer 2004), in most seaweeds there is a phenotypic (i.e., acclimation) response to water flow (Sundene 1964). In the case of *S. latissima* morphological differences are phenotypic and responses to mechanical drag (Gerard, 1987). In contrast to morphology, the physiological response of macroalgal photosynthesis to water flow has received relatively little attention (Hurd 2000).

The response of photosynthesis to water flow is intricately linked to the mechanisms for DIC uptake. Most macroalgae are able to utilize HCO_3^- (Beer and Israel 1990, Johnson 1992, Beer 1998). Although some algae are capable of uptake of HCO_3^- by active transport, most depend upon diffusion of CO_2 produced in the DBL by means of CA_{ext}, which catalyzes the interconversion of HCO_3^- and CO_2, as well as the active extrusion of protons into the DBL which lowers the pH to increase the equilibrium concentration of CO_2 (Drechsler and Beer 1991, Larsson *et al.* 1997, Flores-Moya and Fernández 1998, Axelsson *et al.* 1999). *S. latissima* appears to depend on proton extrusion and CA_{ext} for DIC uptake (Axelsson *et al.* 2000, Klenell *et al.* 2004).

The response of macroalgae to changes in ambient DIC suggests algae may also respond to flow-induced reductions in DIC in the DBL. Marine algae are known to increase CA_{ext} and Rubisco in response to reduced DIC availability (Bozzo and Colman 2000, Andria *et al.* 2001). The coral *Pocillopora damicornis* increases photosynthesis and Rubisco in response to high current velocities, whereas CA_{ext} activity increases in low flows, possibly to compensate for a reduction in DIC availability in the DBL (Lesser *et al.* 1994). Because the Calvin cycle affects the light-

harvesting reactions of photosynthesis by controlling the rate of ATP and NADPH utilization, DBL-effects on DIC uptake may also affect the protective mechanisms that dissipate excess light energy. The xanthophyll cycle protects against high light stress by dissipating excess light energy via heat, in non-photochemical quenching (Demmig-Adams and Adams 1992), and is known to be important in *Saccharina latissima* (Harker *et al.* 1999, Gevaert *et al.* 2002, Rodrigues and Pereira dos Santos 2002).

My hypothesis is that the alga *Saccharina latissima* will exhibit phenotypic changes (i.e., acclimation responses) of the DIC uptake apparatus and other aspects of photosynthetic metabolism in response to growth at different water-flow regimes. In order to test this hypothesis, I compared the physiology of *S. latissima* from high and low water motion sites in the field in Maine and grown under controlled conditions in a laboratory flume. I anticipated that *S. latissima* would up-regulate mechanisms for DIC-uptake (CA_{ext}, H^+ extrusion) and non-photochemical quenching, when grown under low-flow conditions.

Materials and Methods

Field Sites: Field studies and collection of algae were done by SCUBA at sites close to the Darling Marine Center in Walpole, Maine using the University of Maine's RV Silversides. Two sites were selected: Kresge Point (43°50.037'N, 69°30.926'W) and Sand Beach Cove (SBC; 43°50.411'N, 69°33.354'W), Maine. Kresge Point is a wave-exposed site that experiences considerable surge even under low wind conditions, whereas SBC is a very sheltered site inside the mouth of the Damariscotta River. Both sites were of similar depth at about 3 m below MLW.

The sites were marked with a buoy and three concrete blocks were randomly deployed within sight of the anchor attached to the buoy line (inside a 3-4 m radius) to facilitate re-location. Each block was drilled to anchor a threaded plastic pipe designed to accept custom-built strain gauges (Bell and Denny 1994). The strain gauges were deployed for several 48 h periods (in one case 72 h) between August 31 and September 11, 2005. Plaster of Paris clod cards were deployed over the same period to provide a relative measure of water motion with the inclusion of latex paint to slow dissolution rate (Gerard and Mann 1979). The plastic bases of the clod cards were anchored to the concrete blocks with small bungee straps

Morphological characterization. I measured blade length, width and thickness with a measuring tape and digital calipers. Blade area was determined by accurately tracing the algae on paper, cutting out and weighing the outline of the blade and calculating surface area using a calibration factor of paper weight per unit area.

Experimental material used in Maine: Entire sporophytes of *Saccharina latissima* (Linnaeus) Lane, Mayes, Druehl and Saunders approximately 1 m in total length were collected from the two field sites each time they were visited (~ every two days from August 31 to September 11, 2005). Algae were placed in a dive collection bag and immediately on reaching the surface, placed in the dark in an insulated container filled with ambient seawater for the trip back to the laboratory. The kelp were held in an indoor tank in flowing ambient seawater at $18^{o}C$ and low light (<10 μmole m^{-2} s^{-1}) and used for experiments within 36 h of collection from the field.

Sample discs (2.97 cm^2) were taken from the blade margin between 10 and 20 cm above the stipe.

Algal material for flume study: We collected 5 to 15 cm sporophytes of *Saccharina latissima* at the field site at SBC from a depth of 8 to 10 m in January, 2006 by SCUBA diving. The algae were transported to the laboratory in Maryland in the dark in an insulated container containing seawater cooled to 0-5°C with plastic bottles filled with frozen seawater at -20°C. They were subsequently held for 2-3 days in aerated ambient seawater at 80 μmole m^{-2} s^{-1} light levels and 10°C prior to being used in the flume experiment. Blades were cut back to 7cm and measurements of blade length were taken every week.

Flume experiments: The 50 cm wide flume channels were divided into two sections. A bent partition (Figure 1) deflected the flow in order to create two flow regimes within the same channel (~ 1 cm s^{-1} and 13 cm s^{-1}). Flow straighteners and collimators were used to obtain homogenous flows across the channels as observed in dye experiments and flow measurements using a Marsh McBirney Flo-Mate 2000 current meter. Additionally, two aquaria were set up between the two flume channels as controls (0 flow). Two algae were placed side-by-side without overlapping on each side (flow regime) of the partitions in the two flume channels, and two in each of the control aquaria (total 12 algae). The seawater was changed each week and enriched with Provasoli enriched seawater (Provasoli 1968). Fluorescent lights provided 50 μmole m^{-2} s^{-1} (saturating light level) in a 16:8 L:D cycle and the environmental chamber temperature was maintained at 10°C.
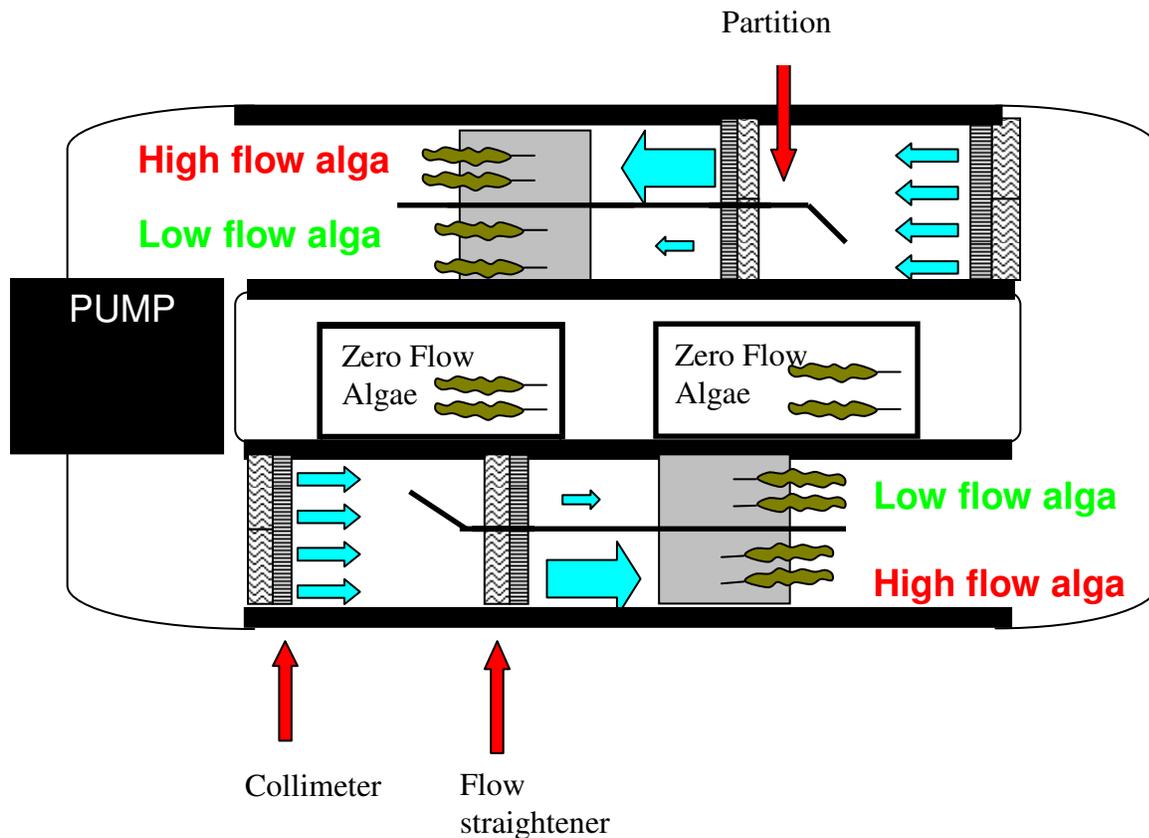
Figure 1. Diagram of laboratory flume used in experiment. See details in Methods.

Photosynthetic Measurements: I used a Clark-type oxygen electrode chamber (DW3, Hansatech, Norfolk, UK) to measure DIC uptake kinetics of photosynthesis at pH 8 at 15°C using 2.97 cm^2 disks excised from the blades of field-collected algae (Collén and Davison 1999). Light was provided by a 50 W tungsten-halogen lamp in an LS-2 light source (Hansatech) attenuated to a saturating light level of 200 μmoles m^{-2} s^{-1} with neutral density filters. The chamber contained 10 mL of DIC-free seawater prepared as described by Forster and Dring (1992) that was filtered through a 0.45 μm nitrocellulose membrane filter and buffered to pH 8.0

with 5 mM Tris-HCl. Oxygen production was measured following sequential additions of

NaHCO$_3$ (0.05mM to 10mM) injected into the chamber. Each measurement was continued until

a stable oxygen flux was achieved (usually 5-10 mins).

Photosynthetic responses were studied with the rapid light curve program of a diving Pulse

Amplitude Modulated (PAM) Fluorometer (Walz, Effeltrich, Germany) using a 1-second

saturating flash intensity of 6000 µmoles m^{-2} s^{-1}. Algae were dark-adapted for 10 minutes after

which light levels were increased every 10 s and relative electron transport rate (ETR),

measurements of yield (variable light-adapted fluorescence), and incident light were used to

calculate non-photochemical (qN) and photochemical quenching (qP).

The PAM fluorometer was also used to measure yield, ETR, qN and qP under stagnant (stirrer

off in oxygen electrode chamber) vs high water flow (stirrer on) conditions. After 10 minutes in

the dark the algae were exposed to a single 1 second saturating flash intensity of 6000 µmoles m^{-2} s^{-1} to measure the initial dark-adapted F_v/F_m used to calculate the subsequent fluorescence

parameters. Immediately afterwards (t = 0) the LS-2 light source (200 µmol. m^{-2} s^{-1}) was

switched on under rapidly stirred conditions. The stirrer was switched off at t = 15 min and back

on at t = 30 min for a 15 min recovery period. Fluorescence measurements were taken every

minute for 45 minutes using a saturating flash intensity of 6000 µmoles m^{-2} s^{-1}.

Dry weight:fresh weight and N analysis: Tissue samples (~ 0.5 g) were blotted dry and

weighed to determine the fresh weight prior to drying in an oven at 60°C for 48 h. Tissue was

allowed to cool in a desiccator prior to determining dry weight for calculations of dry:fresh weight ratios. Dried samples were ground to a fine powder in a mortar and pestle, redried at 60°C, and stored in tightly capped glass vials in a desiccator prior to measuring total nitrogen and carbon content using a CE Flash Elemental 1112 Analyzer.

Photosynthetic pigments: Chlorophyll-a, chl-c and fucoxanthin concentrations were quantified spectrophotometrically following extraction with dimethyl sulfoxide and methanol, as described by Seely *et al.* (1972) and Duncan and Harrison (1982).

Samples for Xanthophyll analysis: Samples for xanthophyll pigments were excised from the blade of a single *S. latissima* individual taken directly from the growth experiment using a number 13 cork-borer (2.97 cm^2). The samples were blotted dry and weighed (0.1 – 0.15 g), wrapped in aluminum foil and immediately frozen and stored in liquid nitrogen until used to measure xanthophylls pigments. Xanthophylls were also measured in the disks used in the flow on-off experiment described above, using disks frozen in liquid nitrogen immediately after the 45 min incubation period. Xanthophyll pigments were measured using an Agilent 1100 HPLC, Agilent Technologies, Santa Clara, CA as described by Van Heukelem and Thomas (2001).

Carbonic anhydrase and proton pump: Measurements of external carbonic anhydrase (CA$_{ext}$) were made using two approaches. In the field study, CA$_{ext}$ was measured indirectly by examining the inhibition of photosynthesis by injecting 10μl of 50mM acetazolamide (AZ), an inhibitor of external carbonic anhydrase, into the 10ml Hansetech chamber and measuring oxygen

production before and after the injection. The greater the % inhibition the higher the dependence of photosynthesis on CA_{ext}. In the flume experiment, direct measurements of relative CA_{ext} activity were made as described by Mercado *et al.* (1997). The dependence of photosynthesis on the external proton-pump was measured by the response of photosynthesis to the injection of 10μl of 4mM erythrosin B, a proton-pump inhibitor into the 10 ml chamber.

Statistical Analyses: Data were analyzed using 1 and 2 way ANOVA with Duncans test for post-hoc multiple comparisons using Statmost3[2] (DataMost Corp, South Sandy UT). Only one flume was available for the laboratory experiments. We used each side of the flume as a replicate with two sub-replicates which were averaged for analysis. Therefore n = 2. The only other possibility was to run the experiment three times with each run being a replicate. After consideration we decided against this because the well-documented seasonal changes in *Saccharina* physiology (e.g., Davison *et al.* 1984) would have resulted in different starting material for each run.

Results

Saccharina latissima from the two field sites exhibited distinctly different morphologies Blades at Kresge were significantly narrower than at SBC at 5, 10, 15, 20 and 40 cm from the stipe, whereas blade thickness was significantly greater (Table 1). Clod cards lost weight almost three times faster at Kresge than SBC, consistent with the greater force exerted on the fronds at Kresge (9.2 N m^{-2}) than at SBC (3.2 N m^{-2}) (Table 1).

Table 1. Environmental conditions and morphological characteristics of *Saccharina latissima* at the two field sites in Maine. Data are means ± SE. Also shown are probability and F values from one way ANOVA. df = 1 in each instance, n = 5 unless noted.

Measurement (units)	SBC	Kresge Point	Statistics
Light at 4m (% surface)	25	20	n/a
Depth (m)	3 m	3 m	n/a
Drag on *Laminaria* ($N \cdot m^{-2}$)	3.2	9.2	$p < 0.0001$, F = 86.6784
Clod card erosion (% d^{-1})	5.8	16.3	
Stipe length (cm)	6.1	4.9	$p = 0.2152$, F = 1.8113
Stipe diameter (mm)	6.0	6.4	$p = 0.5764$, F = 0.3391
Blade length (cm)	100.2	98.2	$p = 0.9010$, F = 0.0165
Blade area (m^2)	0.28	0.12	$p = 0.0196$, F = 7.4444
Blade width measured from stipe – blade transition			
5 cm	21.4	9.50	$p = 0.0017$, F = 21.5869
10 cm	25.1	12.8	$p = 0.0035$, F = 16.7634
15 cm	26.4	14.4	$p = 0.0038$, F = 16.1888
20 cm	24.8	13.8	$p < 0.0001$, F = 42.7899, (n = 14)
40 cm	29.6	18.1	P = 0.0049, F = 10.4370, (n = 8)
Thickness (mm)	0.29	0.65	$p < 0.0001$, F = 64.1192, (n = 18)

DIC kinetics of photosynthetic O_2 production in field-collected algae measured at pH 8.0, indicated that photosynthesis was not saturated at ambient DIC concentrations (~ 2 mM) at the maximum stirrer speed of the electrode chamber (Figure 2). There were no differences however in the response of algae from the two sites except at 10mM where oxygen production was 1.67 and 1.99 µmol cm^2 s^{-1} at Kresge and SBC respectively. There were no significant differences in percent tissue carbon between the field sites (34 and 36 at Kresge and SBC (Table 2). However, in the flume, the control plants (exposed to stagnant conditions) had significantly lower tissue

2.5

2.0

1.5

PS (μmol cm^{-2} s^{-1})

1.0

0.5

0.0

-0.5

■ **Kresge**
■ **SBC**

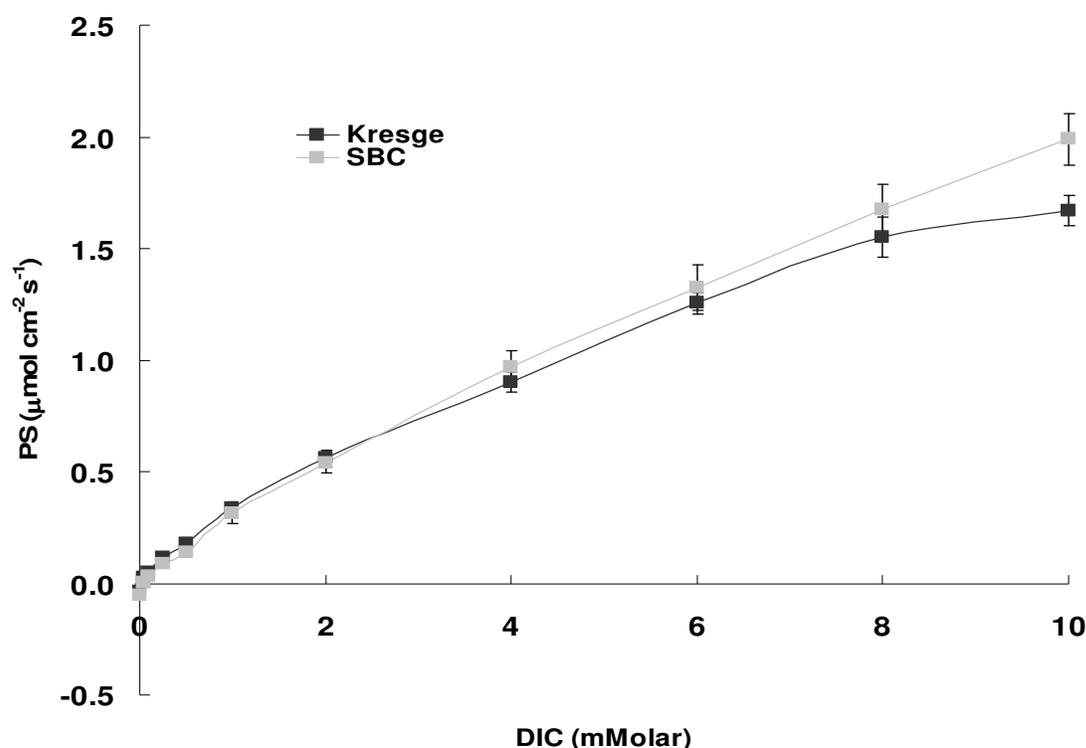**0   2   4   6   8   10**

DIC (mMolar)

Figure 2. DIC Kinetics. Oxygen production in field collected algae from low (SBC) and high (Kresge) flow sites in response to increasing concentrations of DIC. Vertical bars denote standard error of means (n = 4).

Table 2. Physiological and biochemical characteristics of *Saccharina latissma* grown in the experimental flume at three current velocities. Data are means ± SE Also shown are probability and F values from one way ANOVA. (df = 2, n = 2)

Measurement (units)	0 cm s^{-1}	1 cm^{-1}	13 cm s^{-1}	Statistics
Growth rate (cm · cm^{-1} · d^{-1})	0.24	0.48	0.44	p = 0.0181, F =20.2984
Carbon content (% dw)	22.9	26.2	26.0	p = 0.0321, F =13.3624
Nitrogen content (% dw)	3.2	3.4	3.3	p = 0.7902, F = 0.2550
Chl-*a* (nmol · cm^{-2})	21.2	20.4	22.5	p = 0.5962, F = 0.6174
Chl-*c* (nmol · cm^{-2})	4.2	3.8	4.2	p = 0.6808, F = 0.4382
Fuxoxanthin (nmol · cm^{-2})	9.8	8.5	10.1	p = 0.9839, F = 0.0163
Ratio Chl-*c*:Chl-*a*	0.21	0.19	0.19	p = 0.0099, F = 8.0390
Ratio Fucoxanthin: Chl-*a*	0.46	0.44	0.43	p = 0.0521, F = 4.1777
Dark-adapted F$_v$/F$_m$	0.74	0.76	0.77	p = 0.0644, F = 3.7778
Carbonic Anhydrase (% inhibition by Acetazolamide)	68	57	17	p = 0.0553, F = 8.8338
Proton pump (% inhibition to erythrosin B)	15	21	22	p = 0.373, F = 1.40

carbon levels than both high flow and low flow (Figure 3A, Table 2). Percent tissue nitrogen was similar between treatments in the flume and between sites in the field (Table 2 and Table 3, respectively).

The rate of growth in the flume was significantly lower in the stagnant controls than under high and low flow conditions (Figure 3B and Table 2). There were significant differences in pigment densities per unit area measured between the two field populations (Table 3). Chlorophyll *a* and fucoxanthin were significantly higher at Kresge when compared to SBC although the ratios of chl *c*:chl *a* and fucoxanthin:chl *a* were similar in both treatments (Table 3). There were no significant differences between flow treatments in chlorophyll *a* or fucoxanthin density per unit area in the flume, however chl *c*:chl *a* and fucoxanthin:chl *a* ratios were higher in the 0 flow treatment than in either the 1 cm s^{-1} or 13 cm s^{-1} (Table 2).

Inhibition of photosynthesis by AZ produced significantly higher inhibition at SBC (86%) than at Kresge (67%) indicating that algae from the low-flow site were more dependent on CA_{ext} (p = 0.016, F = 7.28, df = 1) (Figure 4A). Similar results were obtained in the flume experiment using the Mercado *et al.* (1997) technique, with relative activities being 68, 57 and 17% at 0, 1 and 13 cm s^{-1}, respectively (Figure 4B), although these results were on the border of significance (p = 0.055, F = 8.84, df = 2). The percent inhibition in response to the proton pump inhibitor was 28 at Kresge and 24 at SBC and 15, 21 and 22 in the flume at 0, 1 and 13 cm s^{-1} which was not significantly different either in the field or in the flume (p = 0.496, F = 0.510, df = 4 and p = 0.373, F = 1.40, df = 1).

Table 3. Physiological and biochemical characteristics of *Saccharina latissima* at the two field sites in Maine with different water flows (SBC – low flow; Kresge – high flow). Data are means ± SE. Also shown are probability and F values from one way ANOVA. df = 1 in all instances, n = 5 except where noted).

| Measurement (units)	SBC	Kresge Point	Statistics
Carbon content (% dw)	35.0	33.3	$p = 0.1229$, $F = 0.9733$
Nitrogen content (% dw)	1.3	1.2	$p = 0.3226$, $F = 1.1111$
Chl-a (nmol · cm^{-2})	25.3	36.4	$p = 0.0049$ $F=14.8346$
Chl-c (nmol · cm^{-2})	7.5	10.2	$p = 0.0812$ $F=3.9767$
Fuxoxanthin (nmol · cm^{-2})	17.7	24.4	$p = 0.0204$, $F= 8.0320$
Ratio Chl-c: Chl-a	0.29	0.28	$p = 0.4484$, $F= 0.6353$
Ratio Fucoxanthin: Chl-a:	0.70	0.67	$p = 0.3697$, $F= 0.9032$
Dark-adapted F_v/F_m	0.76	0.76	$p = 0.8180$, $F=0.0602$, (n = 3)
Carbonic Anhydrase (% inhibition by Acetazolamide)	86	67	$p = 0.0158$, $F = 7.2831$
Carbonic Anhydrase (relative)	81	68	$p = 0.367$, $F = 0.887$
Proton pump (% inhibition to erythrosin B)	28	23	$p = 0.496$, $F = 0.510$
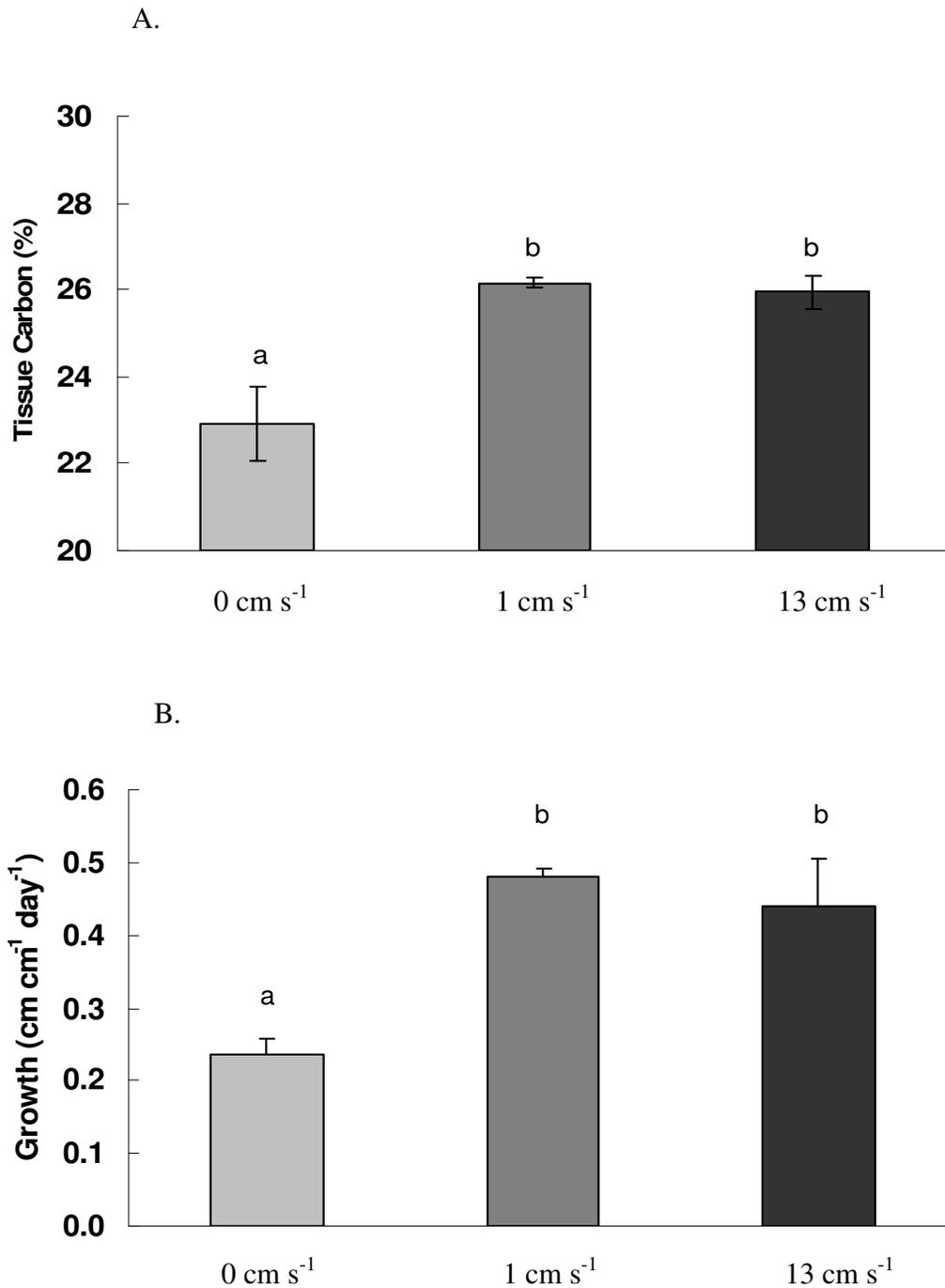
A.

B.

Figure 3. A. Percent Tissue Carbon B. Growth. Carbon content and growth determined after 28 days of culture in the laboratory flume under three flow regimes: 0, 1 and 13 cm s^{-1}. Vertical bars denote standard error of means (n = 2). Different letters denote treatments that differ by $\leq$ 0.05 as determined by Duncan's post-hoc multiple comparisons.
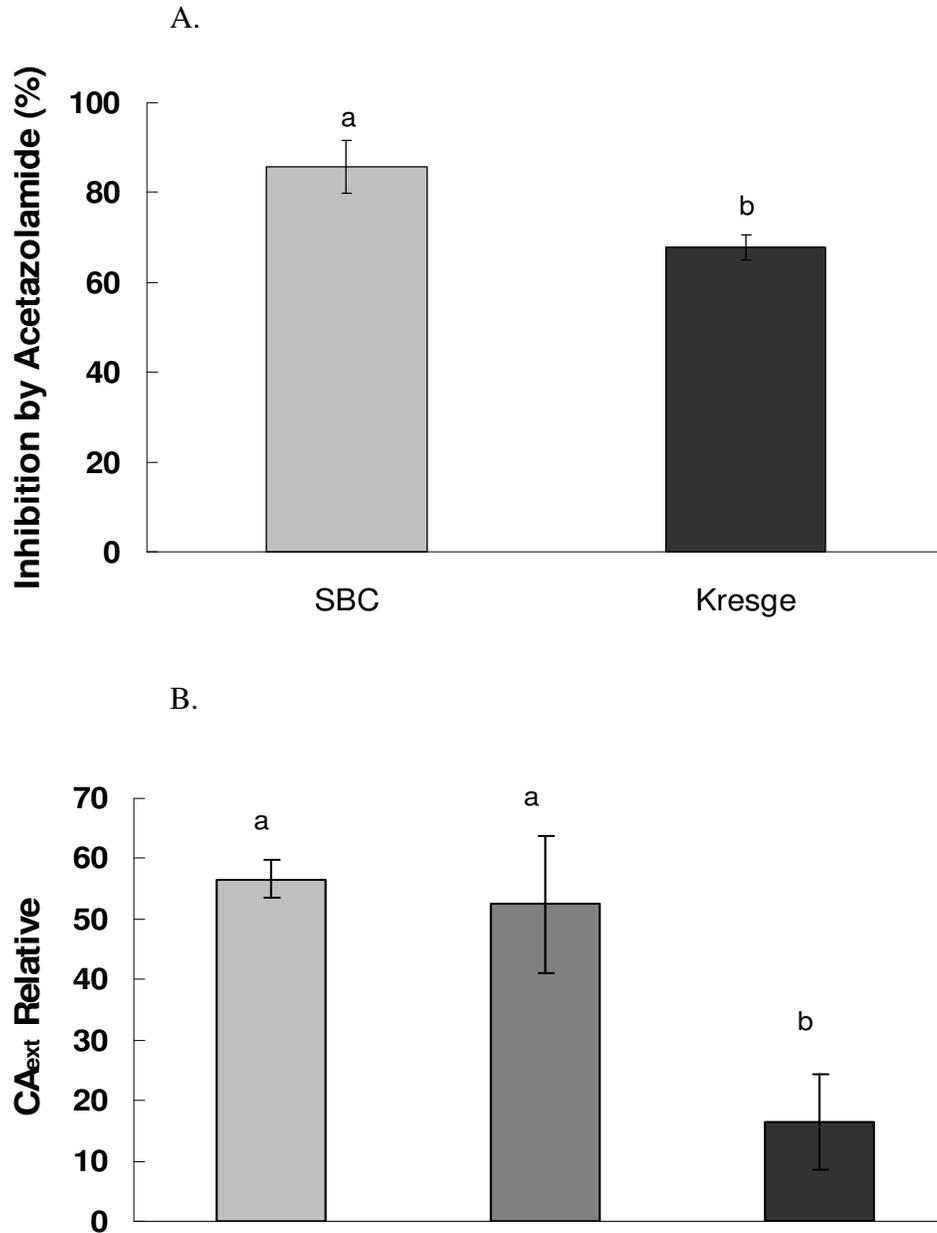
Figure 4. The Effect of Flow on Carbonic Anhydrase. A. Percent inhibition of photosynthesis by acetazolamide in field collected algae from low (SBC) and high flow (Kresge) sites after injection of 50mM acetazolaminde (n = 9). B. Relative CA_{ext} activity determined after 28 days days of culture in the laboratory flume under three flow regimes: 0, 1 and 13 cm s^{-1} according to Mercado *et al.* (1997) (n = 2). Vertical bars denote standard error of means. Different letters denote treatments that differ by ≤ 0.05 as determined by Duncan's post-hoc multiple comparisons.

Algae collected from the high flow site or grown at 13 cm s^{-1} exhibited higher rates of light-saturated photosynthesis, measured as ETR, than those from lower water flows (Fig 5, 7A). When transferred from high to no flow in the cuvette experiments ETR declined immediately in both field and flume algae, recovery was rapid when flow was switched on in the field grown algae, but less immediate in those from the flume (Figure 5A,B). Non-photochemical quenching increased in all groups of algae when they were transferred from high flow to no flow conditions. The increase in qN was greatest in field-collected algae from SBC and low and stagnant flow-acclimated algae in the flume both in response to low flows, and in the case of the flume, when exposed to high light (Figure 6A, 6B and 7B). There was a gradient in qN in the flume with the highest levels of qN in the controls, followed by low flow algae with the least in high flow algae. The differences in qN in the flow on-off experiment were consistent with the xanthophyll data. Although there were no differences in the levels of the three pigments when the algae were sampled from the growing conditions, violaxanthin decreased and the two pigments involved in heat dissipation (zeaxanthin and antheraxanthin) increased in the order 13<1<0 cm s^{-1} during the flow on-off experiment in high light (Figure 8).

Discussion

Several lines of evidence indicate that there are hydrodynamic differences between the Kresge Point and SBC study sites, with algae at Kresge being exposed to greater water motion from wave or tidal-induced currents. First, although unreliable as an absolute indicator of current velocity (Porter *et al.* 2000), the erosion of the Plaster-of-Paris clod
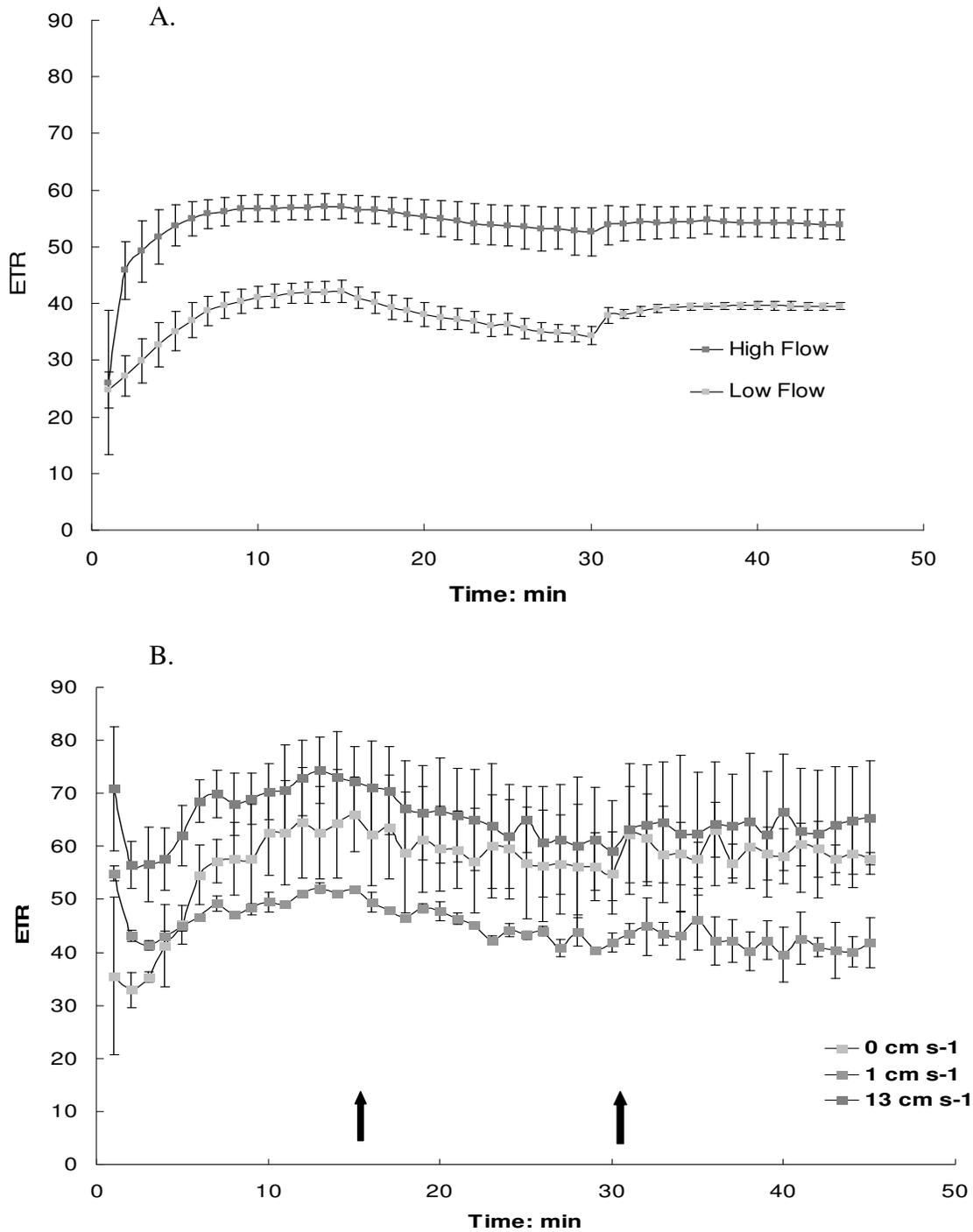
Figure 5. The Effect of Flow on Yield. A. Yield in field collected algae from low (SBC) and high (Kresge) flow (n = 3). B. Yield determined after 28 days days of culture in the laboratory flume under three flow regimes: 0, 1 and 13 cm s^{-1} (n = 2). Yield was measured with a PAM fluorometer. Algae were put in a chamber with the stirrer turned on for 15 minutes then the stirrer was turned off for 15 minutes and on again for 15 minutes. A measurement was taken once every minute during the 45 minute experiment. Vertical bars denote standard error of means.
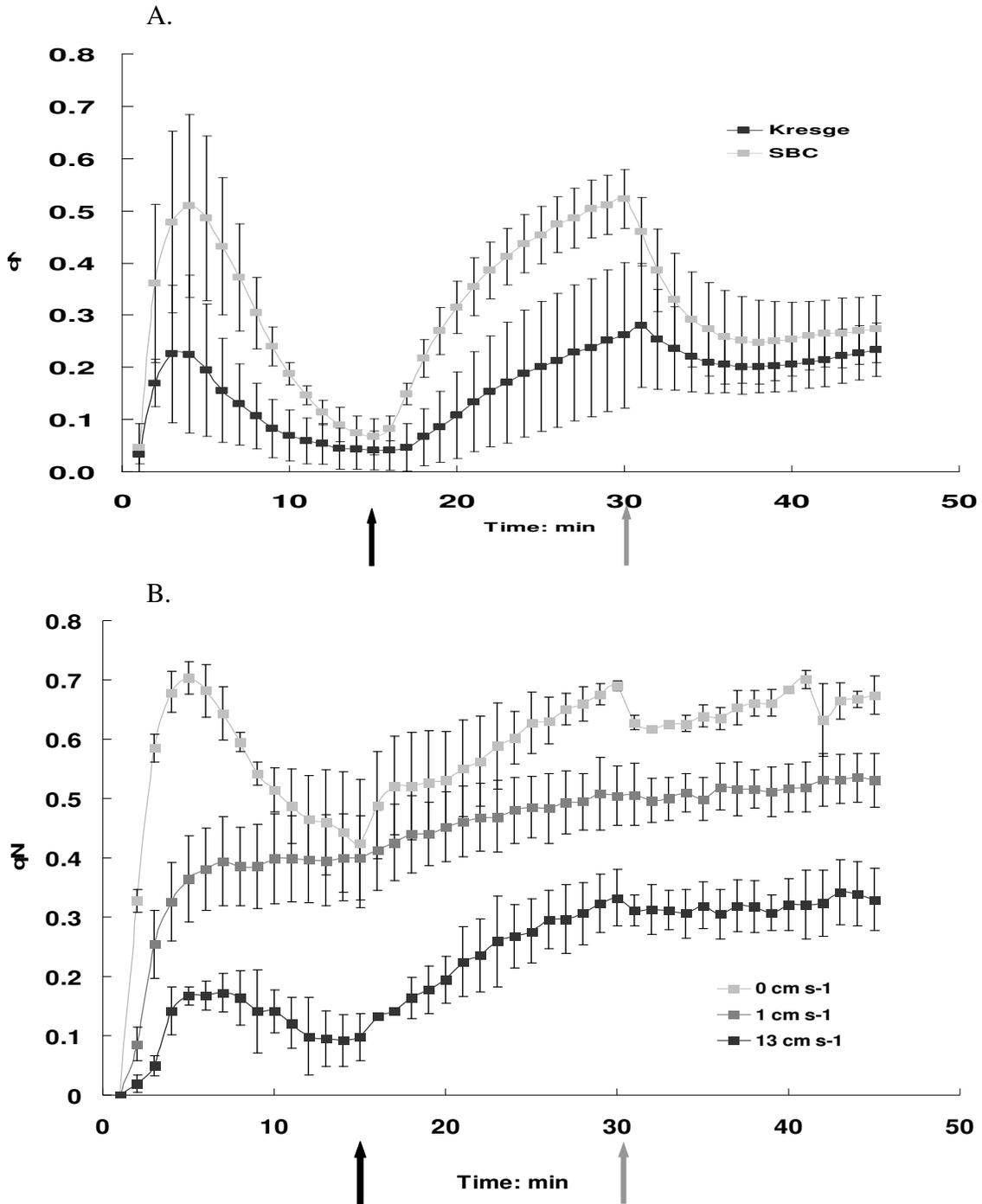
Figure 6. The Effect of Flow on Non-Chemical Quenching. A. qN in field collected algae from low (SBC) and high (Kresge) flow (SBC) (n = 3). B. qN determined after 28 days days of culture in the laboratory flume under three flow regimes: 0, 1 and 13 cm s^{-1} (n = 2). Quenching was measured with a PAM fluorometer. Algae were put in a chamber with the stirrer turned on for 15 minutes then the stirrer was turned off for 15 minutes and on again for 15 minutes. A measurement was taken once every minute during the 45 minute experiment. Vertical bars denote standard error of means.
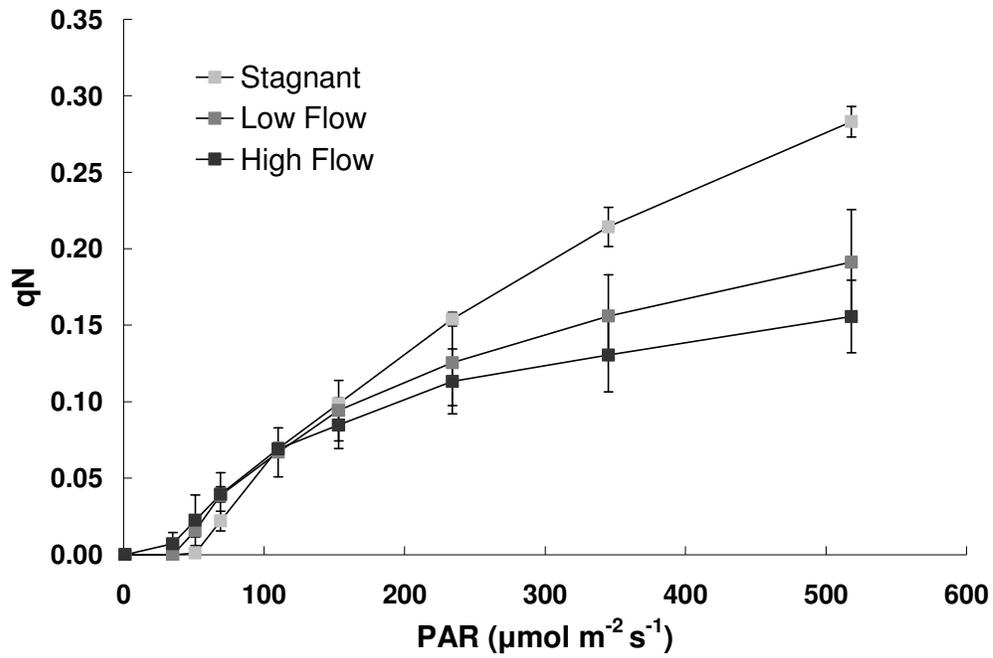
Figure 7. The Effect of PAR on Non Chemical Quenching. Rapid light curve measured with a PAM fluorometer after 28 days days of culture in the laboratory flume under three flow regimes: 0, 1 and 13 cm s^{-1} (n = 2). Vertical bars denote standard error of means.
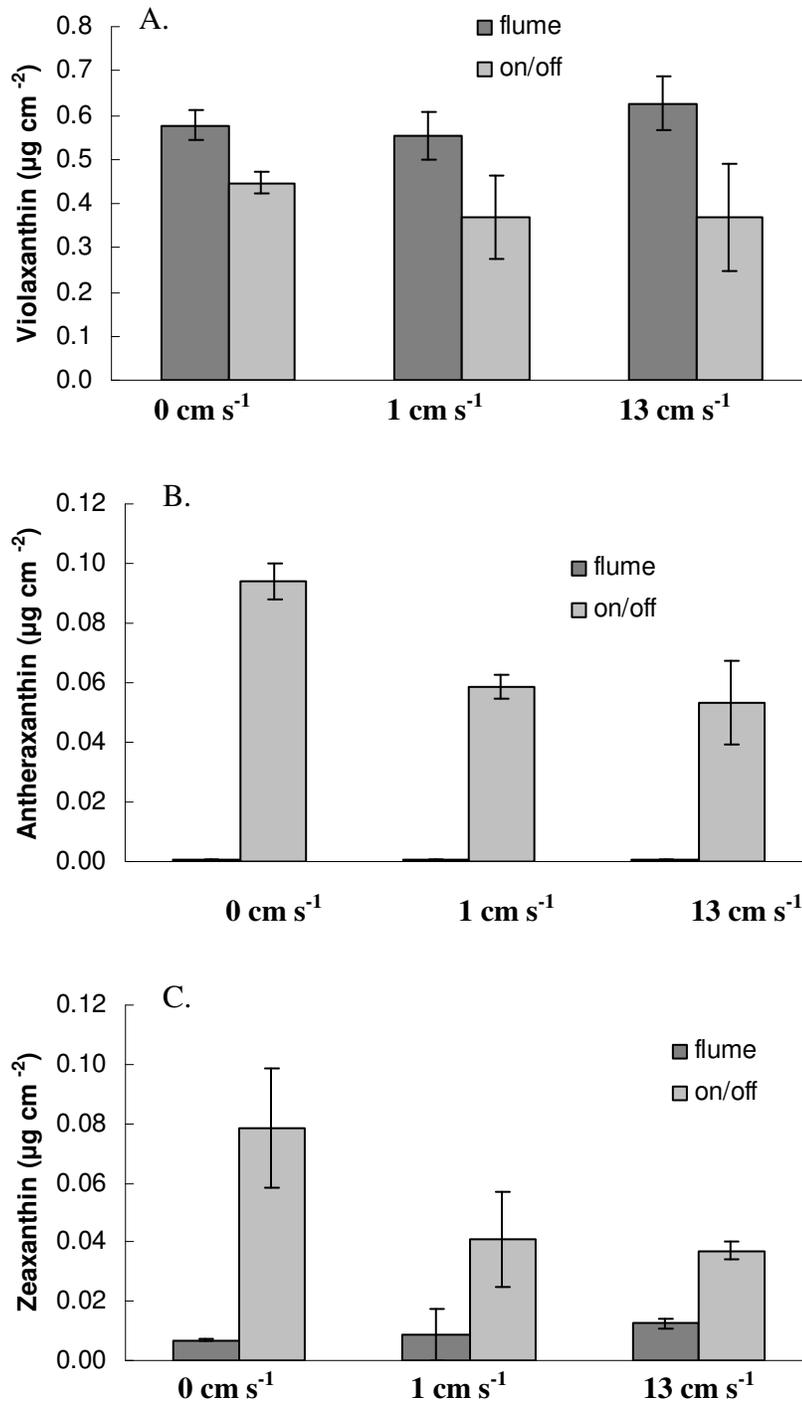
Figure 8. The Effect of Flow on Xanthophyll Pigments. A. Violaxanthin B. Antheraxanthin C. Zeaxanthin Samples were taken from treatment conditions in the flume at 50 μmol m^{-2} s^{-1} and after 45 minutes at 200 μmol. m^{-2} s^{-1}. Vertical bars denote standard error of means (n = 5). Different letters denote treatments that differ by ≤ 0.05 as determined by Duncan's post-hoc multiple comparisons.

cards occurred more rapidly at Kresge than SBC. Second, direct measurements of drag forces experienced at the two sites indicate that *S. latissima* growing at Kresge are exposed to three times the drag force than algae at SBC, although the alga's surface area at SBC is twice as large as at Kresge. Third, the morphological differences between the two sites, with thicker, narrower, and smaller blades at Kresge are consistent with the well-documented phenotypic plasticity of *S. latissima* (Gerard and Mann 1979, Gerard, 1987) and suggest that algae at Kresge experience greater hydrodynamic forces than those at SBC.

The small sporophytes used for the flume experiment were collected from the low flow site at SBC and there was no change in their morphology during the course of the experiment, suggesting that the forces from the highest experimental current speed ($13 \text{ cm} \cdot \text{s}^{-1}$) are insufficient to induce the morphological changes observed at hydrodynamically energetic sites *in situ*. The evidence for physiological acclimation of DIC uptake mechanisms in the flume experiment in the absence of morphological changes suggests that the phenotypic responses of morphology to drag (e.g., Gerard 1987) and those of DIC-uptake are not linked, but are controlled by different mechanisms.

DIC-acquisition mechanisms exhibited an acclimation to water flow consistent with the hypothesis that *Saccharina latissima* compensates for rate limitation of photosynthesis by up-regulating carbon uptake. Activities of CA_{ext} were greater under low flow in both the field and flume experiments. These data are consistent with those of Haglund and Pedersén (1992) who found that the relative activity of CA_{ext} increased when the red alga *Gracilaria tenuistipitata* was

aerated with CO_2-free air, and decreased under supra-ambient concentrations of DIC. The

increase in CA_{ext} occurred at both 0 and 1 cm · s^{-1} with the threshold for this response being

somewhere between 1 and 13 cm · s^{-1} and therefore higher than that for growth which saturated

at 1 cm s^{-1}. The acclimation of CA_{ext} was not able to overcome carbon limitation under stagnant

conditions although it presumably contributed to the ability of the 1 cm · s^{-1} algae to grow at

maximum rates. Although photosynthesis was susceptible to the proton pump inhibitor

erythrosin B, consistent with previous studies on *S. latissima* (Axelsson *et al.* 2000, Klenell *et al.*

2004), this parameter did not appear to respond to changes in water flow either in the field or the

flume experiment. These data suggest that the limiting step in DIC uptake under DBL-limited

conditions is the rate of conversion of HCO_3^- to CO_2 rather than the equilibrium concentration of

CO_2.

My data strongly suggest that photosynthesis of *Saccharina latissima* is not saturated by the

availability of DIC for photosynthesis in ambient seawater (~2 mM) with a maximum

photosynthetic capacity in excess of 2-3 times that realized in nature. This is in agreement with

Forster and Dring (1992) and Mercado *et al.* (1998) who found evidence of rate limitation of

photosynthesis by ambient DIC concentrations in eight out of eleven red and brown macroalga,

but not in green seaweeds. However, Gordillo *et al.* (2001) found that elevated DIC increased

growth rates in the green alga *Ulva rigida*, but attributed this to the stimulation of nitrogen

assimilation. Despite the up-regulation of CA_{ext} in *S. latissima* grown at low flows, maximum

rates of photosynthesis, measured as ETR, were higher in high-flow grown algae. This suggests

that the increases in CA_{ext} ameliorate but do not overcome the reduction in DIC availability in

the DBL at low flows, with the capacity for P_{max} being down-regulated to maintain metabolic balance. However, the reduction in P_{max} does not affect growth except at very low flows, indicating that carbon fixation is not the growth limiting factor. This lack of tight coupling between photosynthesis and growth is consistent with previous literature on macroalge (e.g., Kübler and Davison 1993).

In contrast to freshwater macrophytes grown under different concentrations of DIC, (Madsen *et al.* 1996, Olesen and Madsen 2000) there were no differences in the affinity of photosynthesis for DIC between algae from high and low flows. It is possible that this is because the measurements of DIC kinetics were made with an oxygen electrode at flows well above those required to saturate photosynthesis, and that the acclimation of DIC-uptake in low-flow grown algae is only exhibited when transport is limited by diffusion across the DBL.

Recognizing that photosynthesis is already rate-limited by DIC availability even at high flows, the critical question is, does DBL limitation produce more severe limitation of DIC-uptake in nature? Wheeler (1980, 1982) established that photosynthesis and nitrate uptake by *Macrocystis* were limited by DBL transport at current velocities of less than 6.0 cm s^{-1}. Hurd and Stevens (1997) found that the transition from a laminar to a turbulent velocity boundary layer (i.e., DBL limitation) occurred at mainstream velocities of 1.5 cm $\cdot$ s^{-1} in species with a single blade but at 2.5-3.0 cm $\cdot$ s^{-1} in those with multiple blades. It is not clear how pervasive low flows are *in situ* (e.g., see Gerard 1982, Koehl and Alberte 1988, Hurd 2000), although they appear more likely to occur in very dense seaweed or seagrass beds that attenuate flow. Rate limitation of

photosynthesis due to reduced carbon concentrations in the DBL occurs at velocities below 3-5 cm s^{-1} in the seagrasses *Thalassia testudinum* and *Cymodocea nodosa* (Koch, 1994), although these authors concluded that carbon-limitation did not persist *in situ* for prolonged periods due to the observed natural fluctuations of friction velocity on the seagrass leaves. Kyung-sil Choo *et al.* (2002) suggested photosynthesis could be limited by water flow within dense *Cladophora glomerata* bed where currents were reduced by the algae and where high photosynthetic rates produce increased pH and lowered carbon concentrations. My data on the up-regualtion of CA$_{ext}$ in *S. latissima* from the low-flow field site provides evidence that algae experience flows sufficiently low to create rate limitation of photosynthesis in nature. However, the flume data suggests that the ability to acclimate DIC uptake by increasing CA$_{ext}$ compensates for the DBL transport limitation so that growth is not reduced except at very low flows between 0 and 1 cm s^{-1} that are unlikely to occur in nature except in sheltered habitats such as very dense seaweed beds or tide pools.

Another indicator of the importance of up-regulation of DIC uptake to allow *Saccharina latissima* to optimize photosynthesis across a range of water flows is provided by the fact that it occurred in algae that were N-replete (flume) and N-limited for growth (field). The algae in the field measurements were nitrogen-limited based on the low N in the tissue (e.g., Chapman and Craigie 1978, Davison *et al*. 1984). The differences in pigment content between the two sites suggests that N-limitation may have been less severe at Kresge than SBC, although these differences might also be attributable to other environmental factors known to affect pigmentation such as light and temperature (Machalek et al. 1996, Cayabyab and Enriquez

2007), or to the difference in thickness of the blade between the sites. The relationship between blade thickness and pigmentation is evident from the comparison of the pigment content of the thin blades of the small sporophytes used in the flume experiment that were not nitrogen-limited (Table 3) with that of the larger and thicker algae collected from the field (Table 2). .

Another aspect of the physiological acclimation to water flow by both field-collected and laboratory-acclimated *Saccharina latissima* was an increase in non-photochemical quenching in low flow algae. This may reduce the susceptibility of *S. latissima* to photo-damage when DBL-limitation reduces the ability to use light in photosynthesis. The immediate reduction in ETR following transfer to unstirred conditions is presumably a consequence of carbon limitation because *S. latissima* lacks the internal inorganic carbon stores to maintain photosynthesis in DIC-free seawater. It may also reflect the effect of DBL transport on N-uptake as photosynthesis in *S. latissima* has been shown to respond immediately to changes in inorganic nitrogen availability (Williams and Herbert 1989). However, we consider this unlikely given that the same response occurred in both N-limited (field) and N-replete (flume) algae. Dark-adapted F_v/F_m and initial yield were higher in the algae grown at the highest flows possibly indicative of some chronic photo-damage in the low flow algae. However, those from low flows in both the field and the flume exhibited a greater ability to dissipate excess light energy via non-photochemical quenching, which in the case of the flume experiments was correlated with changes in the xanthophyll-cycle pigments. These data suggest that the ability to cope with light in excess of that required for photosynthesis is a function of the ability to adjust to DBL-induced carbon limitation.

In summary, this is one of the first studies to examine the physiological acclimation of algal

photosynthesis to water flow. The response to low water flow involves: 1) an increase in the

activity of CA_{ext}, a key enzyme involved in DIC uptake and 2) the ability to dissipate excess light

energy via non-photochemical quenching. Although growth and

tissue carbon were only affected by very low flows ($< 1 \text{ cm} \cdot \text{s}^{-1}$) the physiological responses

occur at higher flows strongly suggesting that they play an important role in nature

Chapter 3. Physiological and morphological acclimation to water flow in the marine

angiosperm *Zostera marina* L.

Abstract

I examined the physiological and morphological responses of the marine angiosperm *Zostera marina* L. (eelgrass) to water flow under three current velocities (0, 1, and 20 cm s^{-1}) and in two sediment types (fine, sand with higher organic content and coarse sand with lower organic content), under controlled conditions in outdoor mesocosms, as well as at two sites in the field in Peconic Bay, Long Island, N.Y. My goals were to test the hypotheses that: 1) low water flow increases rate limitation of photosynthesis due to decreased carbon availability on the plant surface, a result of a thicker diffusive boundary layer (DBL), 2) *Z. marina* physiologically acclimates to low water flow by increasing the efficiency of DIC-uptake, and 3) the effects of water flow are mediated by sediment characteristics, an indirect effect of water flow. Measurements of photosynthetic electron turnover rates (ETR) in the mesocosm indicated that zero and low-flow (1 cm s^{-1}) grown plants in higher organic content sediment and zero flow treatment plants in the sand were able to achieve greater rates of photosynthesis under stagnant conditions than those grown at higher current velocities. This ability to up-regulate photosynthesis under low-flow conditions was attributable to physiological acclimation of external carbonic anhydrase (CA$_{ext}$) and proton extrusion, both of which were inversely proportional to water flow, and may also have involved Rubisco, explaining the dependence of the reponse on sediment nutrients. Equal growth rates under all water flows tested (0 to 20 cm s-1) suggest that there was perfect metabolic compensation of carbon uptake mechanisms in response to reduced DIC availability under low water flow conditions. In contrast, there were no

differences in either CA_{ext} or dependence of photosynthesis on H^+ extrusion between field sites indicating that water flow at these sites were both above the threshold to induce acclimation of DIC uptake or that other factors such as high sediment DIC were more important than water flow. The field sites were, Tyndal Point, characterized by high currents, coarse sandy substrate and low sediment organic content (0.8%) and Bullhead Bay, a sheltered, low current site, with soft muddy sediment and high sediment organic content (12%) and very high (>10 mM) pore water DIC. While physiological acclimation of eelgrass leaves in mesocosms seems to be a direct response to water flow, morphological acclimation of roots and rhizomes seems to be a stronger function of sediment characteristics than water flow. Only root length responded to current velocity, increasing under high flow conditions, presumably to enhance anchorage. Root length as well as root and rhizome biomass were also higher in sand-grown plants to increase nutrient uptake. Even so, nutrients in the sandy substrate may have been limiting to eelgrass as shoot growth was lower in sand than mud. In summary water flow has a complex effect on the growth, morphology, and physiology of eelgrass, reflecting direct and indirect effects, which are mediated through mass transport across the DBL on the leaves, and mainly by sediment characteristics on the roots.

Introduction

Despite the well studied occurrence of physiological acclimation of seagrasses to environmental factors such as light, temperature, nutrients and CO_2 availability (e.g., Tanaka and Nakaoka 2007, Collier *et al.* 2008, Nejrup and Pedersen 2008) very little is known about the physiological acclimation of marine algae and angiosperms to water flow. As water flow affects every aspect

of the existence of aquatic plants, from the substrate they colonize, to the flux of nutrients to their surface (Hurd 2000 Koch *et al.* 2005), this knowledge gap needs to be addressed.

Water flow affects the flux of carbon to the plant surface, exerts drag on the leaves and changes the composition of the sediment in which seagrasses are rooted. As a result water flow can have a direct effect on the leaves and an indirect effect on the whole plant, via the sediments. Unlike nutrients, which are mainly taken up from the sediment, seagrasses depend upon the water column for carbon uptake (Sand-Jensen 1977). Based on Fick's First Law, the availability of DIC at the leaf surface for photosynthesis is a function of ambient concentration and the thickness of the diffusive boundary layer (DBL) on the plant surface (Raven et al. 1984, Koch 1994). Ambient DIC concentrations (~2 mM) are well known to be limiting to seagrass photosynthesis in seawater (Thom 1995, Beer and Koch 1996, Beer and Rehnberg 1997, Björk *et al*. 1997, Schwarz *et al* 2000, Invers *et al* 2001). DBL thickness is inversely related to water flow leading to a longer diffusional path for DIC from the water column to the plant surface under low flow conditions (Koch 1994, Gonen *et al.* 1995). This may impose rate limitations to seagrass photosynthesis. There is some evidence that flow-induced rate limitation occurs in nature. In the freshwater macrophyte *Aponogeton elongatus,* the limiting factor to growth in still water was inorganic carbon (Crossley *et al.* 2002). Similarly, growth rate and primary production of the marine angiosperm *Posidonia oceanica* was reduced in areas with limited water exchange (La Loggia *et al.* 2004). In the seagrasses *Thallasia testudinum* and *Cymodocea nodosa*, decreased photosynthetic rates due to reduced carbon availability occurred at velocities below 2.5 cm $\cdot$ s^{-1} (Koch 1994, Enríquez and Rodríguez-Román 2007).

Seagrasses have been suggested to physiologically acclimate to water flow-induced rate

limitation (Koch et al. 2005) but there is currently little evidence in the literature. Enríquez and

Rodríguez- Román (2006) observed changes in photosynthesis in *Thallasia testudinum* that are

consistent with acclimation to low water flow. One mechanism possibly involved in acclimation

to water flow may be an increase in protective mechanisms, such as the xanthopyll cycle that

prevents high light stress by dissipating excess light via heat, in non-photochemical quenching

(Demmig-Adams and Adams 1992). Because reduced availability of carbon under low water

flow creates a rate limitation to photosynthesis, less light energy is used in photosynthesis

possibly leading to an increase in non-photochemical quenching. In addition to regulation of

photosynthesis, acclimation to water flow may also occur via regulation of mechanisms that

increase the availability of carbon to the plant surface. Two such mechanisms that increase the

concentration and the flux of CO_2 to the site of photosynthesis have been identified in

seagrasses: 1) the production of external carbonic anhydrase (CA_{ext}) which catalyzes the

conversion of HCO_3^- to CO_2 in the DBL and/or 2) the acidification of the DBL via the release of

protons increasing the equilibrium concentration of CO_2 (Hellblom *et al.* 2001, Uka *et al.* 2005).

In addition to the direct effects of water flow on resource acquisition, there are indirect effects of

water flow, mediated via the influence of waves and currents on sediment, and consequently,

nutrient availability to the roots. Sediments in high water flow environments are coarser and less

organic than the nutrient-rich fine sediments typical of more quiescent waters (Davis 1985).

Sediment nutrients have been shown to affect the morphology of *Zostera marina* and

Syringodium filiforme (Short *et al* 1985, Short, 1987), with low nutrient sediments producing less

above ground biomass and more roots. Therefore, the indirect effect of high water flow on

seagrasses (via sediment composition) is expected to be seen in the ratio of above:below ground biomass.

Here I address several questions related to the physiological and morphological acclimation of *Zostera marina* to water flow. First I address the questions, "what effect does water flow have on physiology in *Z. marina*" and "does metabolism acclimate to changes in flow to ameliorate the effect of DBL-limitation of DIC uptake"? Secondly, "what effect does water flow have on growth and morphology"? Thirdly, "are the physiological and morphological effects of water flow mediated by the sediment"? We examined these questions by comparing the physiology and morphology of *Z. marina* grown in outdoor mesocosms under three different flow regimes in low and high nutrient sediments as well as measuring carbon uptake mechanisms in the field.

Materials and Methods

Mesocosms: Outdoor mesocosms (3 x 0.6 m) at Horn Point Lab, Cambridge, Maryland ($38^o35.0$'N, $76^o8.1$'W) were filled to a depth of 0.5 m for use as circulating flumes. A constant current velocity was maintained by trolling motors and homogenized across the channel by flow straighteners and banks of collimators (Figure 1). Dye experiments and flow measurements using a Marsh McBurnie Flo-Mate 2000 were made to assure that the flow was homogeneous across and in both channels of the tank. Three flow treatments were established in six mesocosms (two mesocosms for each flow regime) 0, 1 and 20 cm s^{-1}. The seawater (24 salinity) was obtained by mixing filtered water from the Choptank River and Instant Ocean

(Aquarium Systems Inc., Mentor Ohio) and was changed weekly. Each of the six mesocosms

contained one tray (0.33 x 0.26 x 0.11 m) with sediment type placed downstream of a tray

containing sand to minimize turbulence at the edge of the trays (Figure 1). Six seedlings were

planted in each tray. The duration of the experiment was eight weeks from April 20, to June 14,
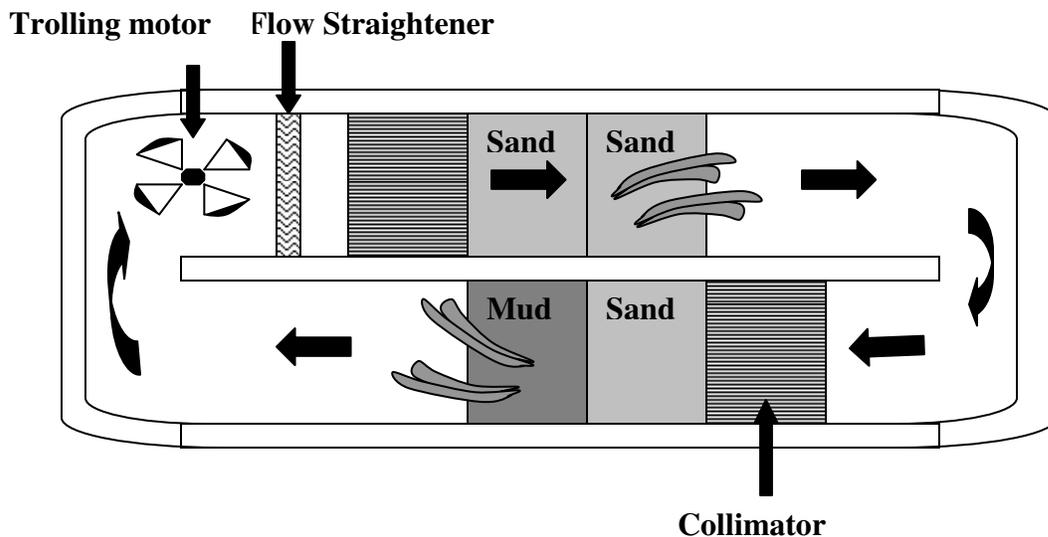
2006.

Figure 1. Outdoor circulating flume in mesocosm tanks (3 x 0.6 m length x width, 0.5m water depth). Flow was provided by an electric trolling motor and homogeneized by collimators and banks of flow straighteners upstream of the plants. Trays filled with sand were place between the flow straighteners and trays containing plants to reduce edge turbulence.

Field Sites: Two sites were selected in Peconic Bay on Long Island, N.Y. , Tyndal Point and

Bullhead Bay (43^o50.037'N, 69^o30.926'W and 43^o50.411'N, 69^o33.354'W, respectively). The

sites were chosen due to obvious differences in water flow. Tyndal Point is at a narrow

constriction in the bay system that experiences high current velocities at both outgoing and

incoming tides. Bullhead Bay is in a sheltered cove with negligible currents at all stages of the

tide. Water flow was measured with a Marsh McBurnie Flo-Mate 2000 current meter.

Velocities at Tyndal Bay exceeded 40 cm s^{-1} whereas those at Bullhead Bay were below the level

of detection of the instrument. Plants standing straight up also suggest that water flow was

negligible. Both sites were of similar depth at about 1.0 m at high tide. The seagrass bed at

Bullhead Bay was very dense, with leaves of almost 50 cm in length that form a dense canopy at

the water surface at low tide. The seagrass bed at Tyndal Point was sparse and the plants were

much smaller with leaves of less than 20 cm in length.

Experimental materials (mesocosm): Plants of *Zostera marina* L. were collected by hand at

low tide from a seagrass bed at Chincoteague, Maryland (37°56.0'N, 73°21.8'W), transported

from the site to the lab in ambient seawater and planted in the outdoor mesocosms within six

hours. Sediment was collected at the Chincoteague site and from a sand bar at Horn Point Lab,

Cambridge, Maryland (38°35.0'N, 76°8.1'W). For simplicity, the sediment treatments are

referred to as "mud" (Chinoteague) and "sand" (Horn Point). Although there is an order of

magnitude difference in organic content between the two sediments, the mud contains less

organic material and nutrients than sediments from many seagrass beds (Koch, 2001).

Experimental material (field): Entire plants of *Zostera marina* were collected from each site

and immediately placed in a cooler filled with ambient seawater for the trip back to the

laboratory at Cornell University Marine Environmental Center in Southold N.Y. The plants

were held in a greenhouse in an indoor tank in flowing ambient seawater, ambient light, and temperature. Plants were used for experiments within 24 h of collection from the field.

Light and Temperature Levels (mesocosm): Plants in the flume were exposed to full natural sunlight, attenuated by 25 cm of water in the tanks. The tanks were not temperature controlled and water temperatures increased from 10 to 28°C over the course of the eight-week experiment. Temperatures also varied diurnally by approximately 6.5°C between pre-dawn and late afternoon.

Temperature Levels (field): Temperature (Stowaway Tidbit, Onset Computer Corporation, Bourne, MA) and light sensors (Odyessey, Data Flow Systems PTY Limited, Christ Church, New Zealand) were deployed on June 23 and collected on June 27, 2007. Temperature sensors were deployed at the top and mid canopy recording every five minutes. Water temperatures varied diurnally at both sites and exhibited a progressive increase from June 22 to June 27, 2007 (Figure 1). Compared to Tyndal Point, temperatures at Bullhead Bay had greater diurnal amplitude (4^oC cf.1.5^oC), were 3-5°C higher at the hottest part of the day, and increased at a faster rate reaching a maximum of 28^oC. The temperature was the same at the top and bottom of the canopy at both sites.

Organic content and grain size: Samples of the initially collect sediment for the mesocosms and samples from the field were oven dried at 60°C for seven days and weighed; after being ashed in a furnace at 450°C for four hours the samples were reweighed and organic content was

calculated from the loss of weight. Grain size was determined by putting a dried sediment sample through a series of geological sieves (Erftemeijer and Koch 2001).

Porewater Measurements (mesocosms): Porewater nutrients in the sediments were measured by allowing porewater constituents to equilibrate with a membrane protected water sample for 12 days (10mL deoxygenated - N_2 bubbled), deionized water contained in plastic cells covered with semi-permeable polysulfonate membrane protected by nylon screen (Hesslein 1976). For a discussion of equilibrium dynamics of porewater samples see Webster *et al*. (1998). The pore-water samplers deployed in the sediment trays were positioned 1.5 cm below the sediment surface on July 24, 2006, collected on August 6, 2006, and processed immediately. Samples were filtered through a 0.45µm membrane filter attached to a polystyrene syringe and placed into plastic vials. Samples for analysis of NH_4, NO_3 and PO_4 were frozen at $-20^{o}C$, 80µl chilled diamine (prepared by adding 8g N-N-dimethyl-p-phenylenediamine and 12g $FeCl_3$ to 500 mls 6M HCl for the samples from the sand treatments and 20g and 30g respectively for the samples from the mud treatments) was added to 1 ml samples used for H_2S and 1.5 µl mercuric chloride was added to 1 ml DIC samples; both of these samples were stored at room temperature until processed. NH_4, NO_3 and PO_4 samples were processed by a Bran + Luebbe autoanalyzer, (Bran + Luebbe Inc., Delavan, WI). H2S and DIC samples were measured using a Shimazu TOC 5000A (Shimazu Company Ltd., Kyoto, Japan) autoanalyzer.

Porewater Measurements (field): Porewater nutrients in the sediments were measured by allowing nutrients to equilibrate for 10 days. The samplers were positioned at the same depth

with pore water samples colleted from 3.5 to 21.5 cm below the sediment surface. The pore-water samplers were deployed May 23, 2007, collected one at a time on June 23, 2007 and processed immediately as above.

Photosynthetic Measurements: I used a Clark-type oxygen electrode (DW1) (Hansatech, Norfolk, UK) to measure respiration and photosynthesis. Light was provided by a 50 W tungsten-halogen lamp in an LS-2 light source (Hansatech, Norfolk UK) and attenuated with neutral density filters. The electrode chamber contained 2 mL of $20^{o}C$ seawater (from the outdoor mesocosms) that was filtered through a 0.45 μm nitrocellulose membrane filter. Measurements were made under high flow rates generated by the magnetic stirrer at the bottom of the chamber set at maximum speed. Measurements were made on 3 cm long leaf sections cut 3.5 cm from the base of the third leaf (with the first leaf being the youngest). Measurements were made in darkness to estimate dark respiration (Rd), and at a saturating light level of 500 μmol photons·m^{-2}· s^{-1} for maximum photosynthesis (P_{max}) and continued until a stable oxygen flux was achieved (usually 5 to 10 min). I also studied photosynthetic responses under zero flow conditions using the rapid light curve program of a diving PAM (Pulse Amplitude Modulated Fluorometer: Walz, Effeltrich, Germany). Light levels were increased every 10 s and electron turnover rate (ETR), yield (variable light-adapted fluorescence), and incident light were used to calculate non-photochemical quenching. Absorbance was calculated by measuring light intensity of a saturating flash with, and without the leaf on the light sensor of the PAM.

DIC kinetics at pH 8 were measured by placing leaf tissue into the Hansetech chamber in DIC-free seawater (prepared according to Schmid *et al.* 1992) containing 5mM Tris-Cl at a light level of 500 µmoles m^{-2} s^{-1}. 50mM of Tris-Cl in seawater was found to reduce photosynthesis in several seagrass species (Hellblom *et al*. 2001, Uku *et al.* 2005). I used the minimum amount of Tris-Cl to prepare the DIC-free seawater (5mM), recognizing that this may have an effect on DIC kinetics by reducing the effectiveness of the proton pump. A comparison of photosynthetic rates measured without a buffer to the photosynthetic rates in the DIC kinetics experiment revealed no significant difference between treatments. Oxygen production was measured as increasing amounts of sodium bicarbonate was injected into the chamber from 0.05 to 10mM, allowing rates to stabilize before injecting additional DIC.

Dry weight:fresh weight, CHN and Total P analysis: Tissue samples (~ 0.1 g) were blotted dry and weighed to determine fresh weight prior to drying in an oven at $60^{o}C$ for 48 h. Tissue was allowed to cool in a desiccator prior to determining dry weight. Dried samples were ground to a fine powder in a mortar and pestle, redried at $60^{o}C$, and stored in tightly capped glass vials in a desiccator prior to CHN measurements using a CE-440 Elemental Analyzer from Exeter Analytical, Ltd., Coventry, UK. Total P was digested and run in a Bran and Luebbe automated colormetric analyzer (Elmsford, New York).

Carbon uptake mechanisms: External carbonic anhydrase was estimated using the method described in Mercado *et al.* (1997) at 500 µmol photons m^{2} s^{-1}. Proton extrusion was calculated by measuring photosynthesis at 500 µmol photons m^{2} s^{-1} before and after injecting 16µl of 1mM

stock of Erythrosin B, a proton-pump inhibitor, into the 2ml chamber of the Hansetech oxygen electrode.

Growth, Biomass and Morphological Measurements: Growth in the mesocosms was determined after 20 days with the hole punch method (Short and Duarte 2001). At the end of the experiment, rhizome, root and leaf tissue were separated and dried for above/below ground biomass measurements. Each leaf was measured for length and width.

Statistical Analyses: Data were analyzed using nested ANOVA with Duncans test for post-hoc multiple comparisons using Statmost3[2] (DataMost Corp, South Sandy UT).

The experimental design in the mesocosms was constrained by the availability of tanks and it was only possible to run two tanks per treatment. To avoid pseudo-replication, the analyses were done with an n = 2, with the tanks serving as replicates, and with the mean for each replicate representing the values of several individual plants from the two tubs per tank. When examining this data it is important to consider that the n= 2 increases the possibility of Type II error, and to examine trends in the data as well as significant differences. Repeating the experiment to increase replication was not feasible for logistical reasons (the tanks became too hot for *Zostera marina* in the summer), and because this would have introduced further variables associated with seasonal differences in temperature, light, nutrient and other environmental conditions.

Results

Sediment and Water Column and Porewater Characteristics:

Organic content of the sediment was 0.14% for sand and 0.98% for mud in the mesocosm. Grain size of the sand was much larger than mud, with 94% of sand grains being $\geq 250\mu m$, whereas 95% of the mud grains were ≤ 250 and 75% $\leq 125\mu m$.   Organic content of the sediment at the field sites was 12% at Bullhead Bay and 0.80 at Tyndal Point. Grain size at Bullhead Bay was smaller than at Tyndal Point with 87% of the sediment at Bullhead Bay less than 63μm whereas 92% of sand grains at Tyndal Point were $\geq 125\mu m$.

Nutrient concentrations in the water column of the mesocosm were similar, as were porewater (1.5 cm depth) concentrations of nitrate and DIC (Table 1). In contrast, pore water concentrations of sulfide, ammonium and phosphate were all approximately an order of magnitude higher in the mud than sand treatments (Table 1).   Porewater concentrations of ammonium, phosphate (Figure 2A, 2B) in the field were significantly higher at Bullhead Bay than Tyndal Point (Table 2) and increased with depth. In contrast, nutrient levels at Tyndal Point were similar at all depths. Sulfide levels at Bullhead Bay were extremely high (average 1963 μM) and similar at depth. Sulfide levels at Tyndal Point were significantly lower (average 11 μM) and similar at depth (Figure 2C). pH values were lower at Bullhead Bay (6.9 at 3.5cm), than at Tyndal Point (7.2 at 3.5cm) (Figure 2D). DIC and non particulate organic carbon were also much higher at Bullhead Bay than Tyndal Point (Table 2, Figure 3).

Table 1. Morphology, pore water and water column nutrients of *Zostera marina* under three water velocity treatments (0, 1, and 20 cm · s^{-1}) and two sediment types (sand and mud) in outdoor tanks at Horn Point Lab in May and June of 2006. Values are means $\pm$ (SE) n = 2. Data shown in figures are not included in table.

| Measurement (units)	Mud 0 cm · s^{-1}	1 cm · s^{-1}	20 cm · s^{-1}	Sand 0 cm · s^{-1}	1· cm s^{-1}	20· cm s^{-1}
Morphology						
Final leaf length cm	8.2 (0.35)	10.0 (0.21)	12.1 (1.43)	7.8 (0.29)	9.5 (0.53)	9.2 (1.43)
Final leaf width cm	0.23 (0.00)	0.20 (0.00)	0.20 (0.02)	0.24 (0.02)	0.21 (0.01)	0.22 (0.06)
Pore Water Nutrients						
Ammonium µM	547 (208)	630 (150)	321 (52.3)	55 (13.2)	78 (30.8)	100 (33.0)
Nitrate + nitrite µM	1.8 (0.27)	1.1 (0.71)	1.2 (0.06)	1.2 (0.01)	0.91 (0.25)	1.1 (0.14)
Phosphate µM	38.7 (11.6)	49.4 (12.1)	13.4 (2.03)	8.09 (1.33)	10.8 (5.92)	12.4 (5.32)
DIC mM	3.46 (13.1)	3.83 (18.8)	2.55 (8.08)	1.94 (0.97)	2.82 (14.6)	1.43 (3.11)
Sulfides µM	942 (449)	1099 (309)	280 (93.9)	10.4 (3.63)	71.4 (68.9)	4.18 (0.18)
Final Water Column Nutrients						
	0 cm · s^{-1}		1 cm · s^{-1}		20 cm · s^{-1}	
Ammonium µM	4.32 (2.16)		4.84 (1.61)		2.99 (1.59)	
Nitrate + nitrite µM	0.71 (0.09)		0.81 (0.08)		0.83 (0.09)	
Phosphate µM	0.37 (0.05)		0.30 (0.04)		0.24 (0.01)	
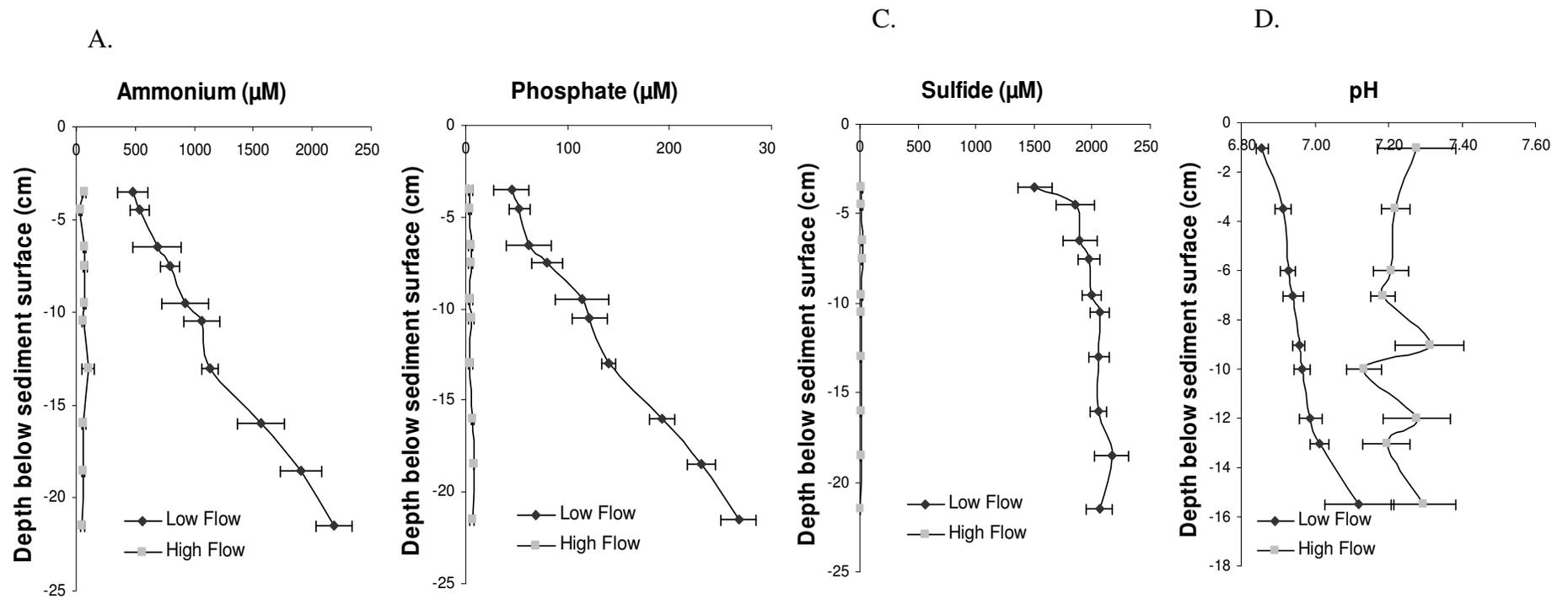
A.

Ammonium (µM)

Phosphate (µM)

C.

Sulfide (µM)

D.

pH

Figure 2. Depth profiles of NH_4 (A), PO_4 (B), SO_4 (C) and pH (D) in sediment pore water in *Zostera marina* beds at Bullhead Bay and Tyndal Point. Error bars represent SE of mean (n=4).

Table 2. Porewater nutrients (n=4), percent tissue nutrients (n=9) and physiology (n=4) of *Zostera marina* at low (Bullhead Bay) and high (Tyndal Point) sites including statistics. Values are means $\pm$ (SE).   Statistical values are p and F from one-way ANOVA.

| **Pore Water Nutrients** (3.5 cm depth)			
Ammonium (µM)	476 (130)	64.6 (13.9)	p = 0.0202 F = 9.84
NPOC (mg/l)	9.37 (2.26)	4.81 (0.341)	p = 0.0929 F = 3.99
Phosphate (µM)	45.1 (17.5)	4.58 (2.06)	p = 0.0607 F = 5.32
DIC (µM)	7018 (1229)	2365 (218)	p = 0.0247 F = 10.1
Sulfides	1504 (148)	14.9 (5.54)	p < 0.0001 F = 101
Percent Tissue Content			
Nitrogen (%)	1.65 (0.0693)	1.74 (0.0379)	p = 0.218 F = 1.65
Phosphate (%)	0.307 (0.0177)	0.273 (0.0120)	p = 0.138 F = 2.47
Carbon (%)	36.1 (0.112)	38.1 (0.274)	p < 0.0001 F = 49.9
Carbonic Anhydrase	0.657 (0.070)	0.610 (0.045)	p = 0.573 F = 0.342
Erythrosin B (% inhibition)	37.6 (8.88)	24.9 (7.38)	p = 0.306 F = 1.25
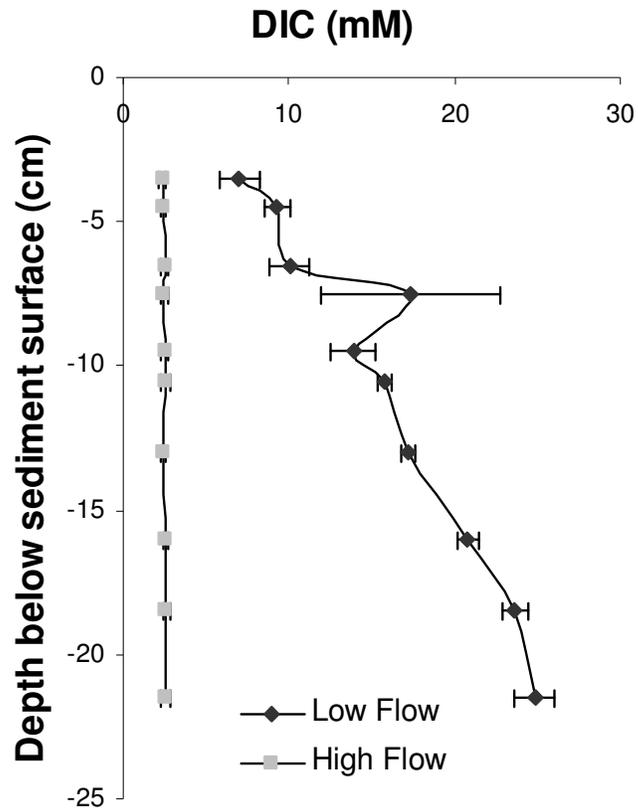
Figure 3. Depth profile of DIC in sediment pore water in *Zostera marina* beds at Bullhead Bay and Tyndal Point. Error bars represent SE of mean (n=4).

Photosynthetic metabolism: CA_{ext} and the degree of inhibition of photosynthesis by the proton pump inhibitor were both significantly affected by current velocity in the mesocosm and inversely proportional to water flow (Figure 4). In contrast, there were no significant effects of water flow on R_d and either net or gross P_{max}, measured with the oxygen electrode under high flow conditions (Table 3 and 4). Leaf light absorptance was also not affected by water flow, reflecting a constant chlorophyll content and varied from 42-50% of incident light (Table 3). DIC uptake kinetics for photosynthesis measured under high flow conditions in the electrode chamber indicated that *Zostera marina* is not DIC saturated at ambient carbon levels (~2 mM) (Figure 5). Measurements of photosynthesis (ETR) under stagnant conditions indicate an increase in photosynthetic capacity in plants grown under zero flow (Figure 6) with more pronounced differences between flows $0 > 1 > 20$ cm $\cdot$ s^{-1} in sand (Figure 6B). These large differences in ETR of leaves in the sand treatment were correlated with changes in non-photochemical quenching, which varied $20 > 1 > 0$ cm $\cdot$ s^{-1} (Figure 7).

CA_{ext} activity was not significantly different between the two field sites. There was also no significant difference in inhibition to erythrosin B (proton pump inhibitor), although percent inhibition was higher at Bullhead Bay (38) than at Tyndal Point (25) (Table 2, Figure 8).

Growth: *Zostera marina* exhibited an increase in the number of shoots, as well as above ground, below ground, and total biomass during the experiment. The final number of aerial shoots (bundle of first, second, third, and subsequent leaves) was significantly affected by sediment type
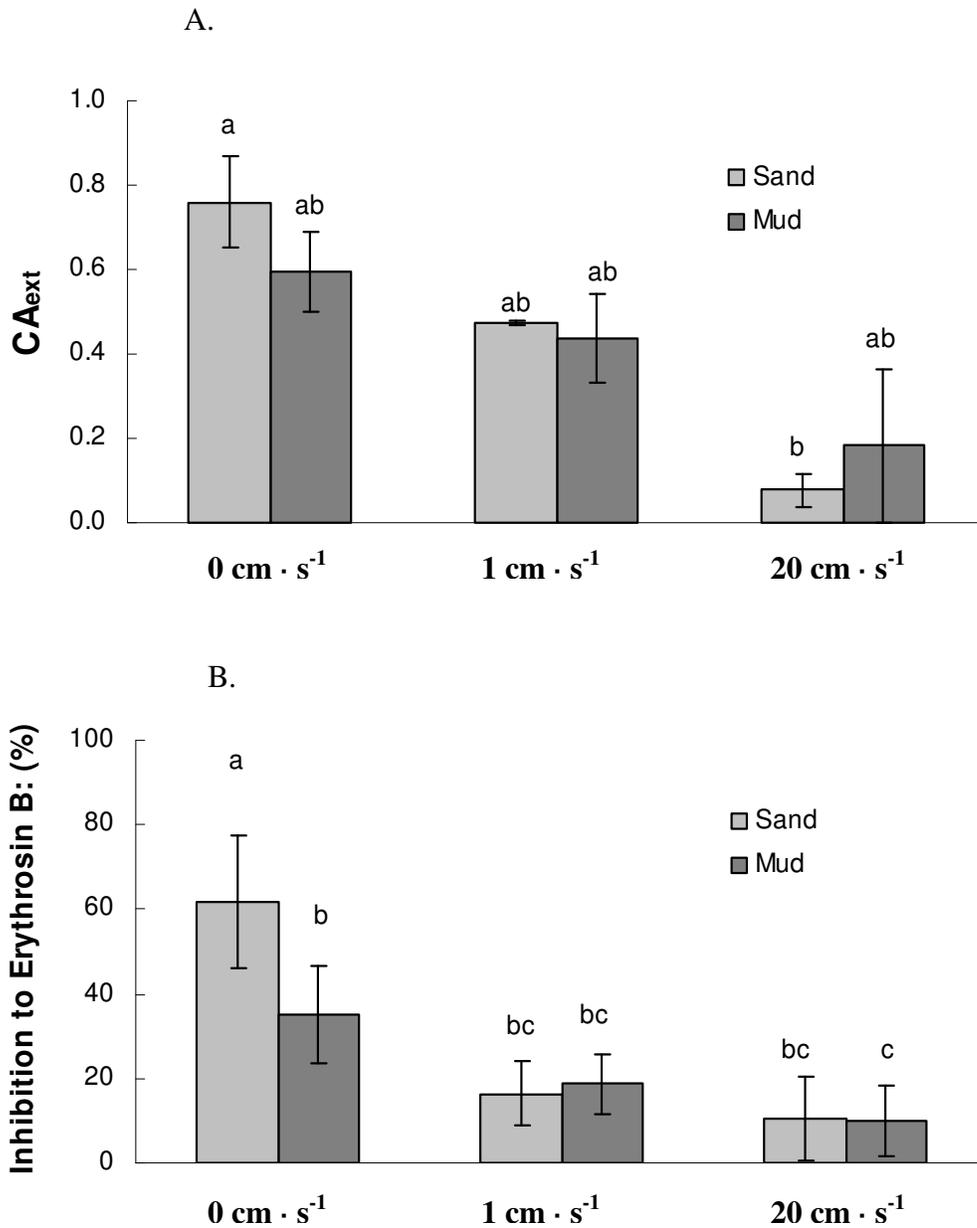
Figure 4. CA_{ext} (A) and inhibition of photosynthesis by erythrosin-B (B) of *Zostera marina* grown in different current velocities and sediment types. Error bars represent SE of mean (n=2). Significant differences between treatments ($p \leq 0.05$) are indicated by different letters.

Table 3. Physiology and biochemistry of *Zostera marina* under three water velocity treatments (0, 1, and 20 cm · s^{-1}) and two sediment types (sand and mud) in outdoor tanks at Horn Point Lab in May and June of 2006. Values are means ± (SE) n = 2. Data shown in figures are not included in table.

Measurement (units)	Mud			Sand		
	0 cm · s^{-1}	1 cm · s^{-1}	20 cm · s^{-1}	0 cm · s^{-1}	1 cm · s^{-1}	20 cm · s^{-1}
Biochemical Composition of tissue						
Nitrogen %	1.30 (0.05)	1.56 (0.04)	1.47 (0.34)	1.21 (0.09)	1.39 (0.30)	1.58 (0.33)
Phosphate %	0.135 (0.01)	0.180 (0.05)	0.180 (0.00)	0.378 (0.28)	0.520 (0.05)	0.349 (0.02)
Carbon %	39.4 (0.17)	36.9 (0.18)	38.8 (0.27)	37.6 (0.04)	37.5 (0.08)	37.7 (0.09)
Chl *a* ng cm^{-1}	2.87 (0.15)	2.65 (0.13)	2.44 (0.66)	3.61 (0.40)	9.25 (0.14)	7.38 (0.35)
Photosynthetic metabolism						
R_d µmol·g^{-1}·h^{-1}	-29.6 (2.02)	-13.0 (1.48)	-26.9 (5.72)	-29.2 (10.9)	-3.79 (1.81)	-21.7 (4.03)
P_{max} µmol· g^{-1}·h^{-1}	7.66 (2.36)	10.5 (4.25)	9.86 (0.28)	7.43 (3.81)	5.93 (5.25)	16.9 (3.62)
Gross P_{max} µmol· g^{-1}·h^{-1}	37.3 (0.34)	23.5 (2.77)	36.7 (5.45)	36.6 (7.09)	9.73 (7.06)	38.6 (7.64)
Leaf Absorbance %	0.425	0.457	0.418	0.430	0.475	0.506

Table 4. Statistics of growth, morphology, pore water nutrients, and physiology of *Zostera marina* under three water velocity treatments (0, 1, and 20 cm $\cdot$ s^{-1}) and two sediment types (sand and mud) in outdoor tanks at Horn Point Lab in May and June of 2006. Values are p and F statistics from two-way ANOVA (degrees of freedom = 1).

Measurement (units)	Statistics Sediment	Current	Interaction
Growth			
Growth cm^{-2} $\cdot$shoot^{-1} $\cdot$day^{-1}	p = 0.051 F = 5.94	p = 0.765 F = 0.281	p = 0.216 F= 2.00
Final number of shoots $\cdot$tank^{-1}	p = 0.018 F = 10.39	p = 0.103 F = 3.39	p = 0.223 F = 1.95
Above ground biomass gdw $\cdot$ original shoot^{-1}	p = 0.567 F = 0.367	p = 0.705 F = 0.370	p = 0.961 F = 0.040
Below ground biomass $\cdot$ gdw $\cdot$ original shoot^{-1}	p = 0.097 F = 3.13	p = 0.754 F = 0.288	p = 0.887 F = 0.120
Total Biomass gdw $\cdot$ original shoot^{-1}	p = 0.194 F = 1.813	p = 0.805 F = 0.219	p = 0.929 F = 0.07
Rhizome biomass gdw $\cdot$ original shoot^{-1}	p = 0.146 F = 2.79	p = 0.03 F = 7.14	p = 0.02 F = 7.34
Root biomass gdw $\cdot$ original shoot^{-1}	p = 0.042 F = 6.63	p = 0.9916 F = 0.008	p = 0.171 F = 2.41
Morphology			
Above ground biomass gdw $\cdot$ final shoot^{-1}	p = 0.567 F = 0.367	p = 0.7054 F = 0.370	p = 0.961 F = 0.040
Below ground biomass gdw $\cdot$ final shoot^{-1}	p = 0.048 F = 6.16	p = 0.100 F = 3.46	p = 0.229 F = 1.90
Root biomass gdw $\cdot$ final shoot^{-1}	p = 0.001 F = 31.6	p = 0.274 F = 1.62	p = 0.192 F = 2.20
Rhizome biomass gdw $\cdot$ final shoot^{-1}	p = 0.811 F = 0.06	p = 0.090 F = 3.68	p = 0.194 F = 2.18
Total Biomass gdw $\cdot$ final shoot^{-1}	p = 0.664 F = 0.208	p = 0.368 F = 0.184	p = 0.790 F = 0.244
Ratio above:below ground biomass	p = 0.010 F = 13.8	p = 0.566 F = 0.566	p = 0.342 F= 0.342
Leaf length cm	p = 0.219 F = 1.88	p = 0.146 F = 2.69	p = 0.539 F= 0.686
Leaf width cm	p = 0.477 F = 0.582	p = 0.542 F = 0.680	p = 0.897 F= 0.110
Root length cm	p = 0.001 F = 35.4	p = 0.015 F = 9.05	p = 0.022 F= 7.64
Leaf weight per area g f wt $\cdot$ cm^{-2}	p = 0.203 F = 2.03	p = 0.005 F = 15.1	p = 0.066 F= 4.44

Table 4. Continued.

| Pore Water Nutrients			
Ammonium μM	p = 0.003 F = 22.7	p = 0.458 F = 0.893	p = 0.337 F= 1.31
Nitrate μM	p = 0.339 F = 1.08	p = 0.350 F = 1.26	p = 0.718 F= 0.351
Phosphate μM	p = 0.009 F = 14.1	p = 0.156 F = 2.57	p = 0.105 F= 3.35
DIC mM	p = 0.174 F = 2.38	p = 0.532 F = 0.703	p = 0.809 F= 0.220
Sulfides μM	p = 0.022 F = 9.53	p = 0.172 F = 2.39	p = 0.476 F= 0.844
Biochemical composition			
Nitrogen %	p = 0.777 F = 0.087	p = 0.391 F = 1.10	p = 0.768 F= 0.276
Phosphate %	p = 8.6×10^{-5} F = 86.9	p = 0.053 F = 4.97	p = 0.105 F= 3.36
Carbon %	p = 0.0010 F = 35.4	p = 0.0003 F = 40.5	p = 0.001 F= 32.3
Chlorophyll-a $\mu g \cdot cm^{-2}$	p = 0.2425 F = 1.6806	p = 0.0133 F = 9.6790	p = 0.048 F= 5.23
Photosynthetic metabolism			
CA_{ext} Relative	p = 0.816 F = 0.059	p = 0.045 F = 15.5	p = 0.731 F= 0.331
Erythrosin B % inhibition	p = 0.314 F = 1.20	p = 0.022 F = 7.68	p = 0.316 F= 1.40
Respiration $\mu mol \cdot cm^{-2} \cdot h^{-1}$)	p = 0.308 F = 1.44	p = 0.445 F = 0.670	p = 0.926 F= 0.039
P_{max} $\mu mol \cdot cm^{-2} \cdot h^{-1}$)	p = 0.629 F = 0.259	p = 0.191 F = 2.21	p = 0.447 F= 0.923
Gross P_{max} $\mu mol \cdot cm^{-2}$ h^{-1})	p = 0.569 F = 0.364	p = 0.403 F = 1.06	p = 0.746 F= 0.308
Leaf Absorbance %	p = 0.755 F = 0.295	p = 0.486 F = 0.552	p = 0.748 F= 0.305
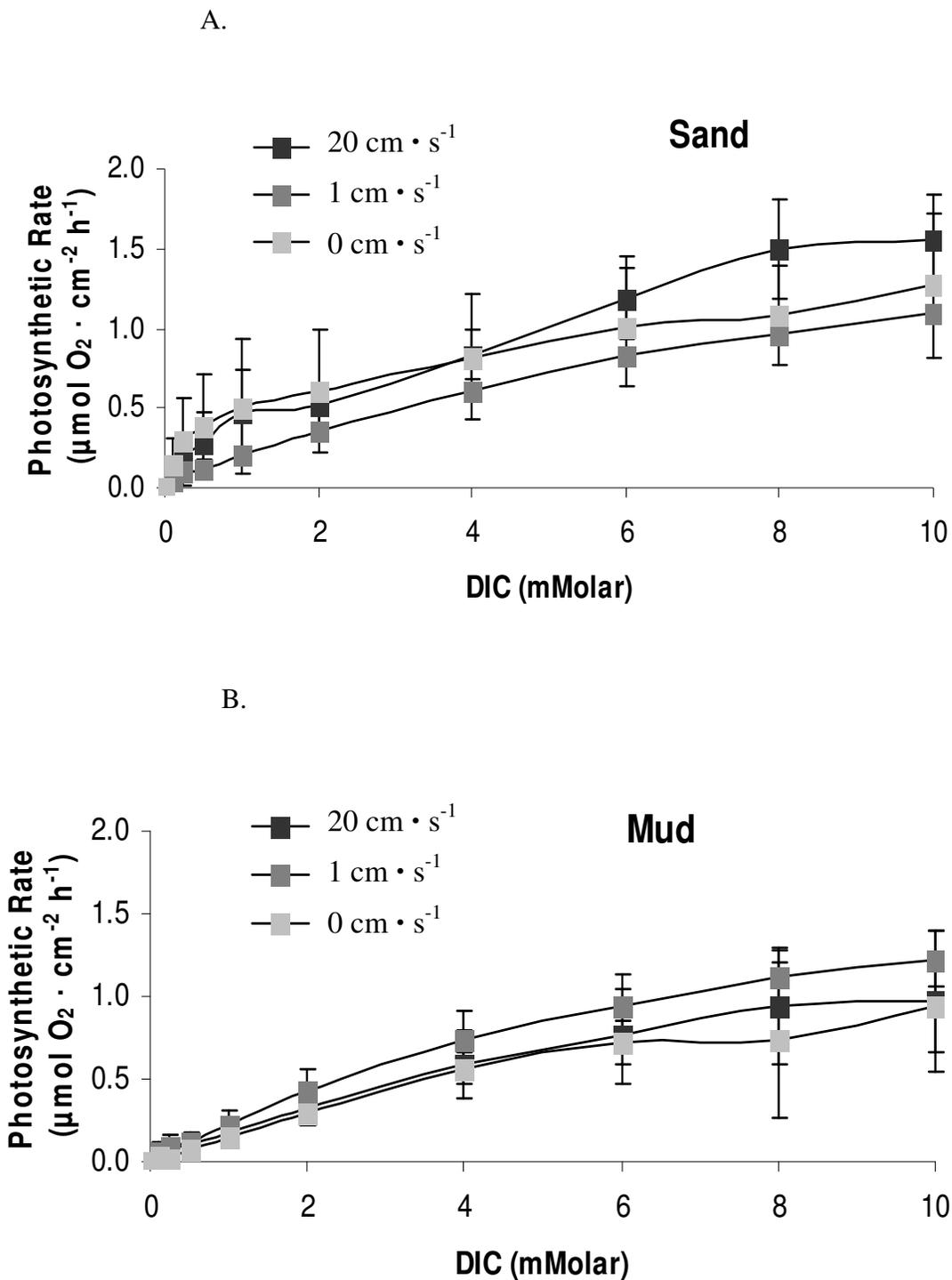
A.

B.

Figure 5. DIC-uptake kinetics of photosynthesis of *Zostera marina* grown in different current velocities and sediment types: sand (A) and mud (B). Error bars represent SE of mean (n=2).
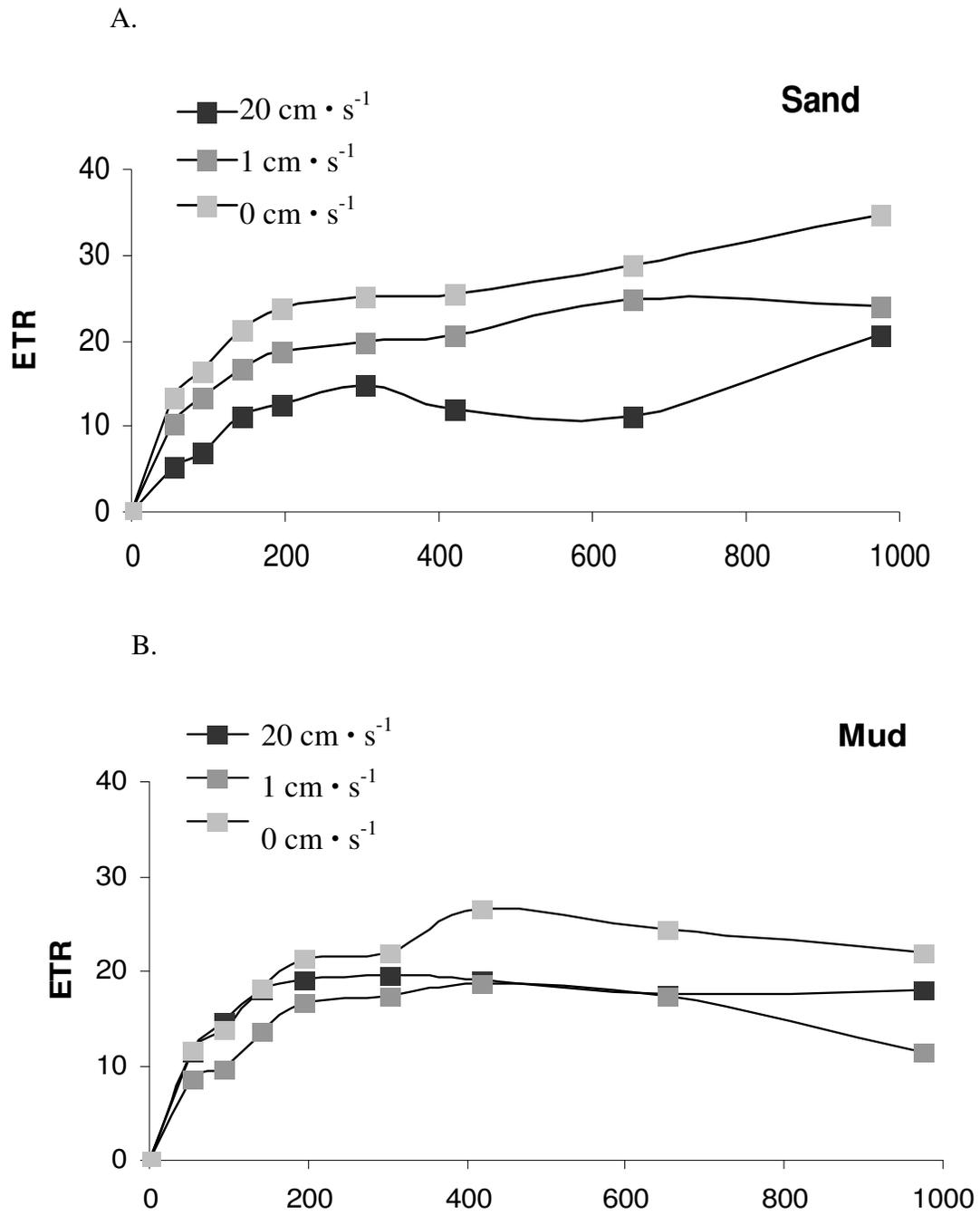
A.

B.

Figure 6. Electron turnover rate (ETR) of photosynthesis of *Zostera marina* grown in different current velocities and sediment types: sand (A) and mud (B). Error bars represent SE of mean (n=2).
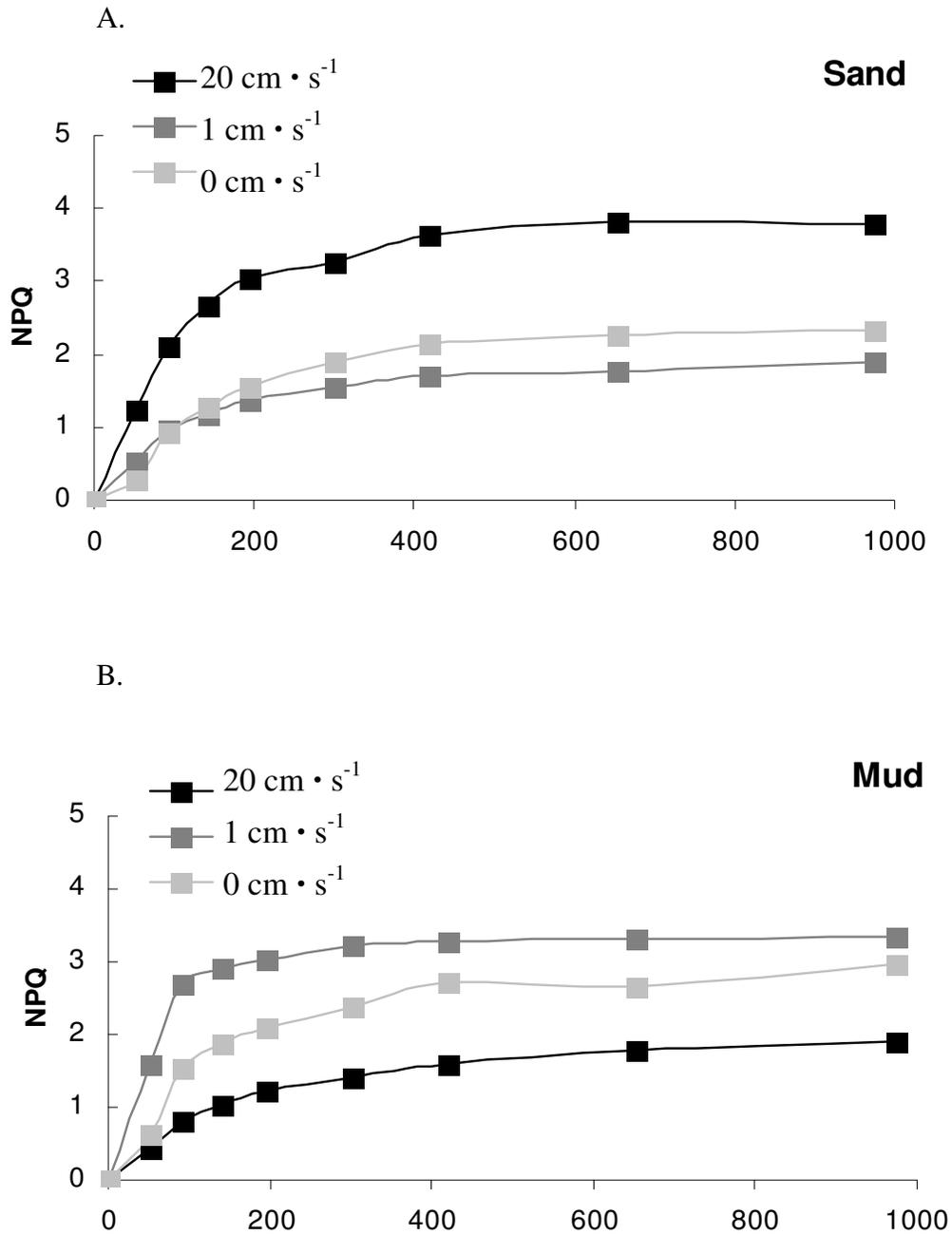
A.

Sand

B.

Mud

Figure 7. Non-photochemical quenching of photosynthesis of *Zostera marina* grown in different current velocities and sediment types: sand (A) and mud (B). Error bars represent SE of mean (n=2).
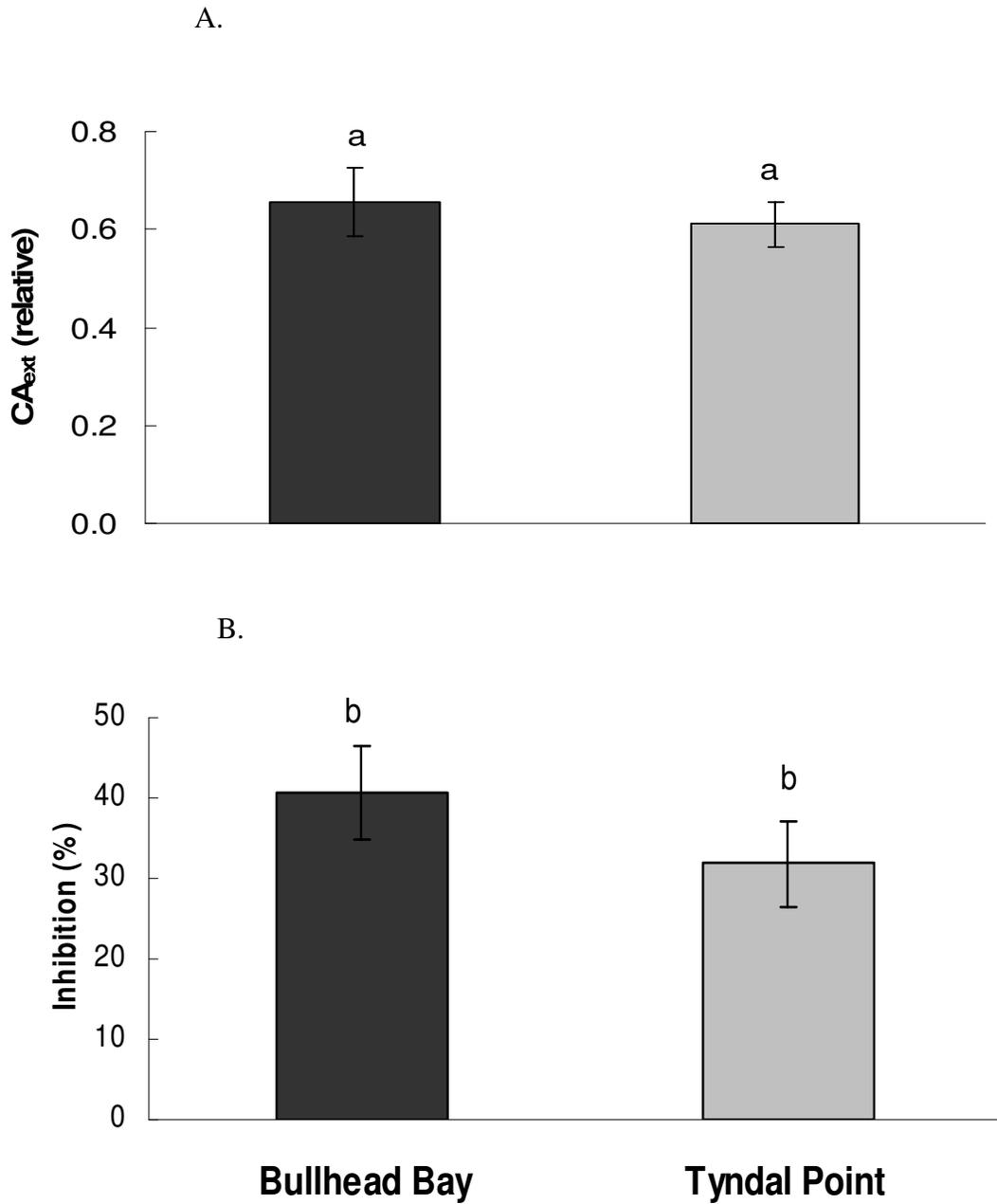
Figure 8. CA_{ext} (A) and inhibition of photosynthesis (B) by erythrosin-B in *Zostera marina* at Bullhead Bay and Tyndal Point. Error bars represent SE of mean (A: n=6; B: n=4). Significant differences between treatments ($p \leq 0.05$) are indicated by different letters.

(Table 4) and was higher in mud than sand (Figure 9A) although multiple comparisons indicated the only significant differences were between zero flow sand and 20 cm · s^{-1} mud. Measurements of leaf growth were also affected by sediment type with higher growth rates in mud at 0 and 20 cm s^{-1} (Figure 9B). The above and below ground biomass and total biomass also exhibited the same trends as shoot number, although these differences were not significant (Figure 10). The ratio of above to below ground biomass was significantly higher in mud than sand (Figure 11).

Morphology: There were no treatment effects on the total or above ground dry weights of the individual shoots at the end of the experiment in the mesocosm (Table 4) or in leaf length or width (Table 1). In contrast, the weight per unit area of the leaves was much higher in the 1 cm · s^{-1} treatment than in the other two flow regimes, with this being most pronounced in the mud (Figure 12). Below ground dry weight of each shoot was significantly affected by sediment (Table 4), with root biomass being higher in sand than mud (Figure 13A). There was no significant difference in rhizome biomass (Figure 13B). There was an interaction between current and sediment on root length (Figure 13C, Table 3). Average root length tended to be slightly higher in sand than mud, although this was much more pronounced in the 0 cm · s^{-1} treatment.

Biochemical Composition: In the mesocom **t**here was no significant effect of treatment on nitrogen content of the tissue (Tables 2 and 3). In contrast there was a significant interaction between sediment and current for carbon content and chlorophyll content
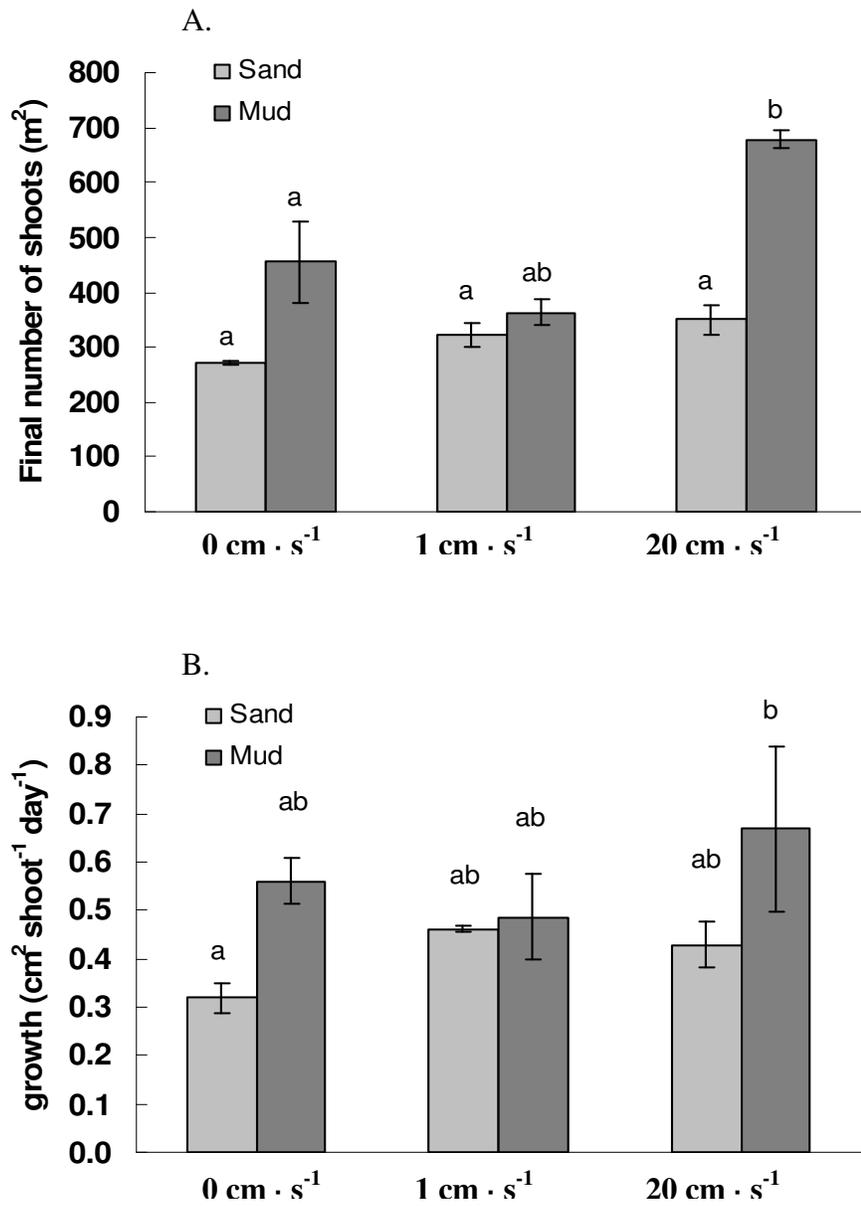
A.

B.

Figure 9. Growth rate (A) number of shoots and (B) of leaves of *Zostera marina* exposed to three current velocities for 8 weeks. Error bars represent SE of mean (n=2). Significant differences (p ≤ 0.05) between treatments are indicated by different letters.
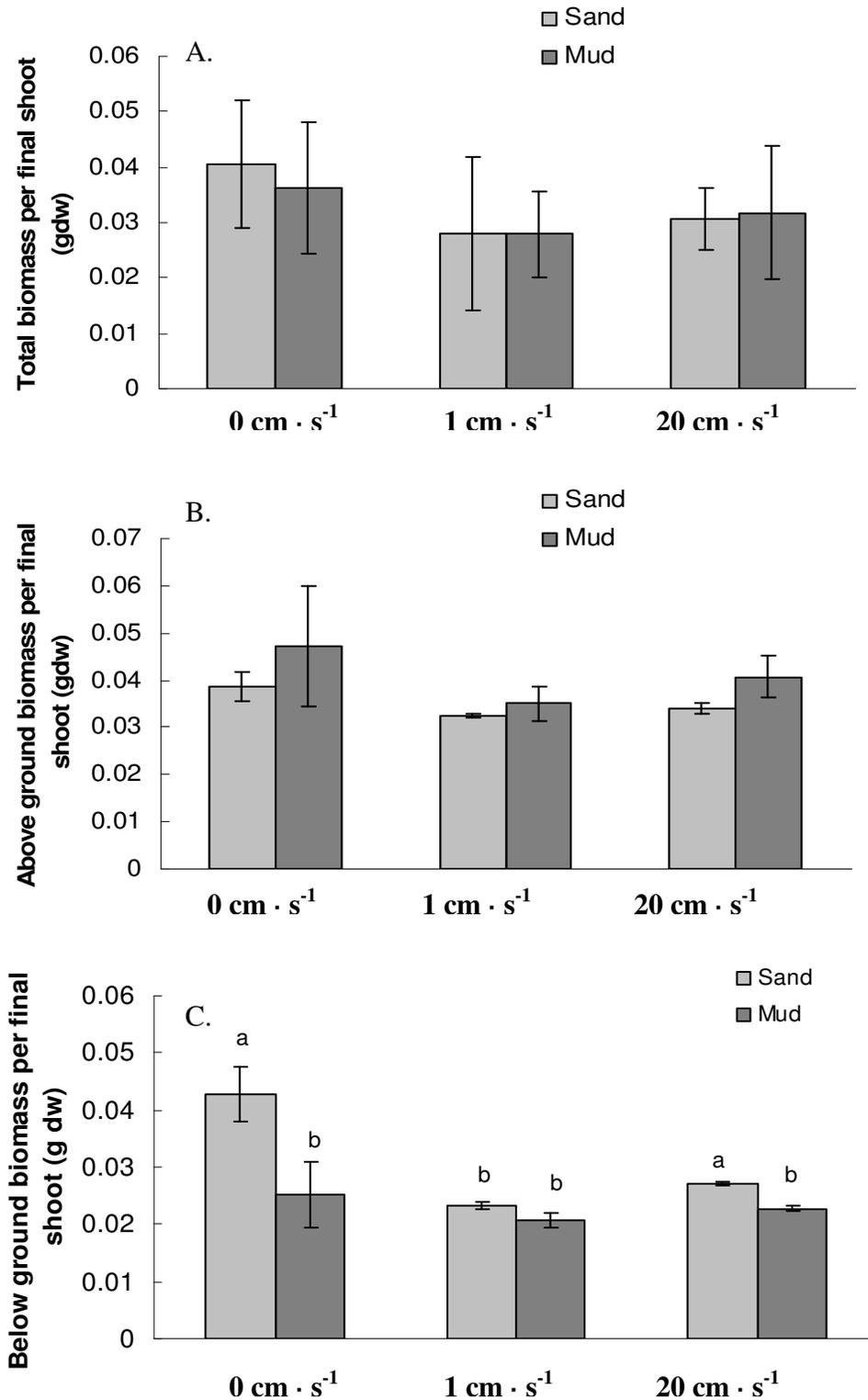
Figure 10. Total (A), above (B) and below (C) ground *Zostera marina* biomass expressed per number of final shoots per tray. Error bars represent SE of mean (n=2). Significant differences between treatments (p ≤ 0.05) are indicated by different letters.
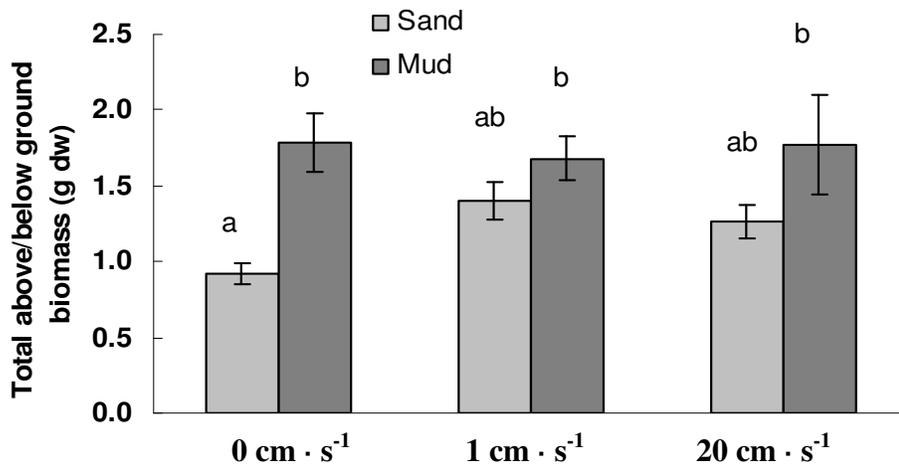
Figure 11. Ratio of above to below ground biomass in *Zostera marina* at end of an experiment that exposed this seagrass to different current velocities for 8 weeks. Error bars represent SE of mean (n=2). Significant differences between treatments (p ≤ 0.05) are indicated by different letters.
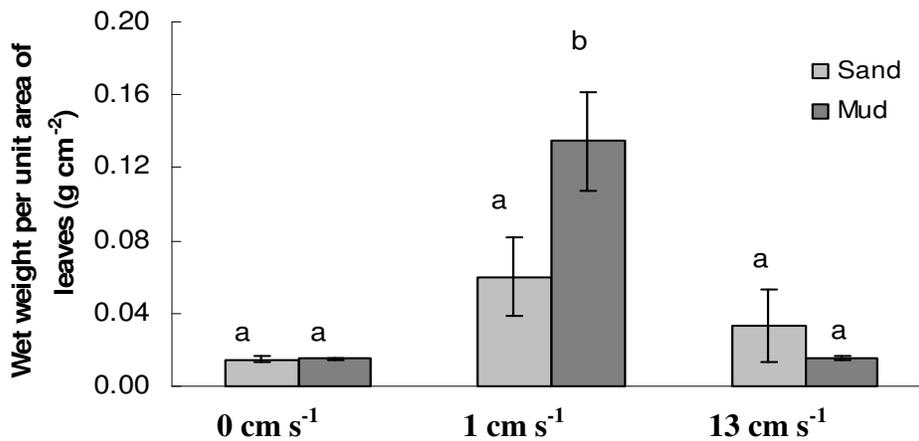

Figure 12. Fresh weight expressed on the basis of *Zostera marina* leaf area at the end of the experiment. Error bars represent SE of mean (n=2). Significant differences between treatments (p ≤ 0.05) are indicated by different letters.
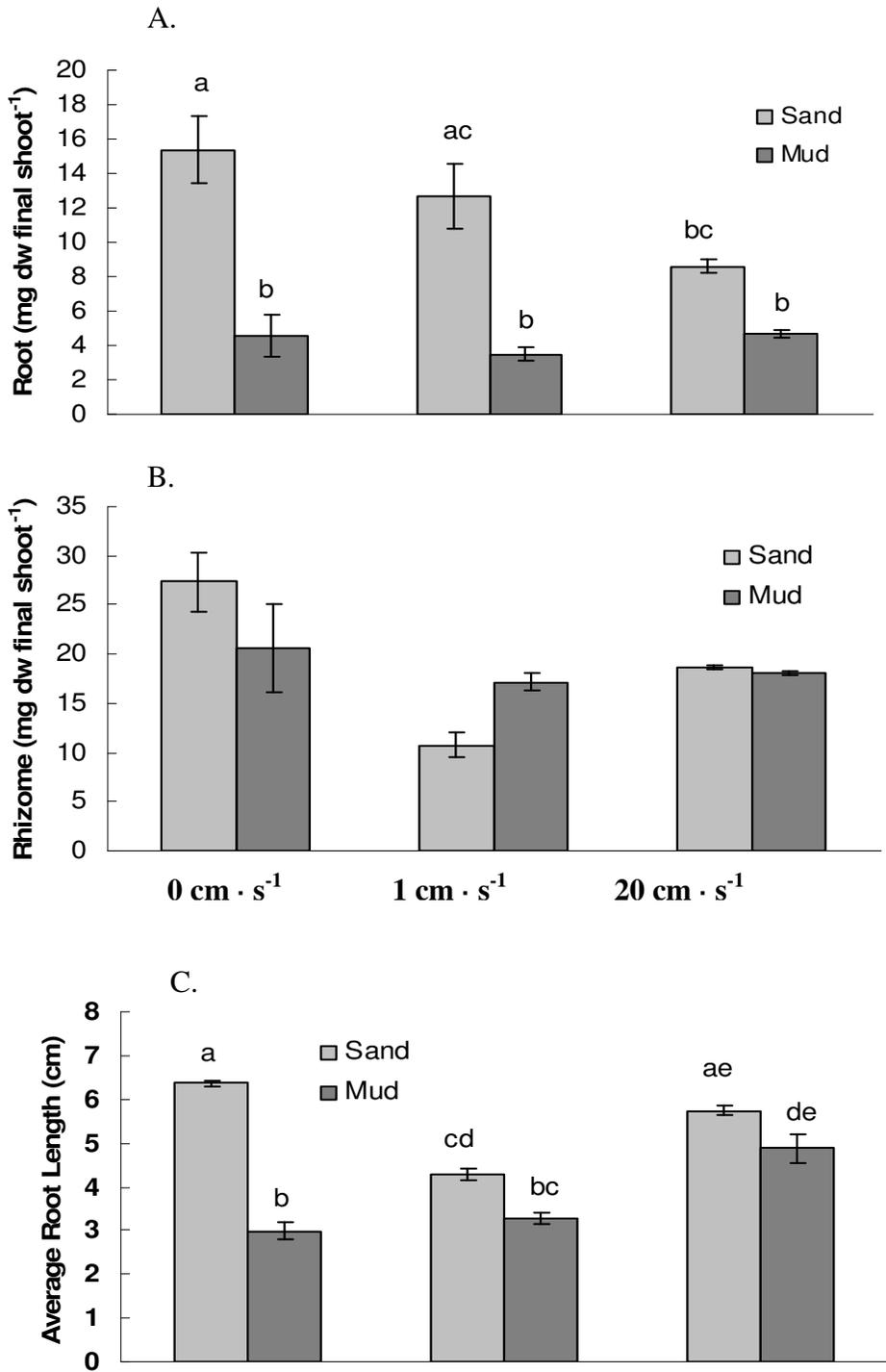
Figure 13. Root (A) and rhizome (B) biomass per final number of *Zostera marina* shoots per tray. Root length (C) at end of an experiment that exposed this seagrass to different current velocities for 8 weeks Error bars represent SE of mean (n=2). Significant differences between treatments ($p \leq 0.05$) are indicated by different letters.

(Table 3). Tissue carbon content in the sand treatments was similar at ~ 37.5%, whereas in mud treatments was higher at 0 and 20 than 1 cm · s^{-1} (Table 2). Chlorophyll content of the mud treatment was similar at ~2.65 ng cm^{-1} but the sand treatments were higher with levels in the 1 cm·s^{-1} treatment the highest (Figure 14, Table 2). Phosphate content was significantly affected by both sediment type and current velocity (Table 3), with sediment having the largest effect and concentrations in the sand treatments 2-3 times higher than the mud (Table 2).
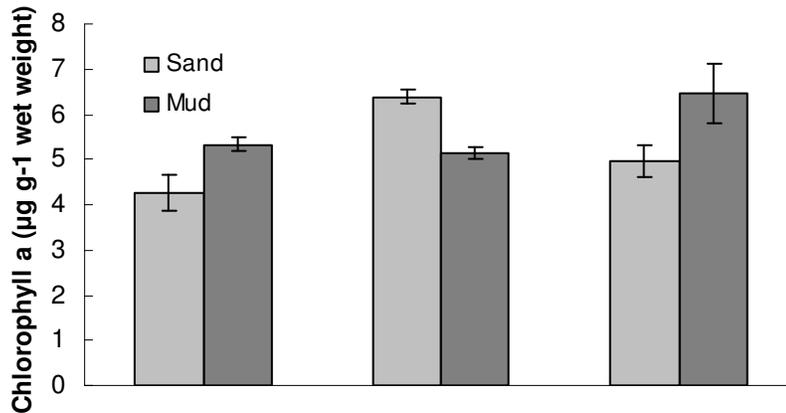
Figure 14. Chl-a expressed on the basis of *Zostera marina* leaf area at the end of the experiment. Error bars represent SE of mean (n=2). Significant differences between treatments (p ≤ 0.05) are indicated by different letters.

Discussion

My data suggest that *Zostera marina* has the ability to physiologically acclimate photosynthetic metabolism to compensate for the effect of water flow on DIC uptake. This physiological acclimation involves the two mechanisms associated with facilitated

diffusion of HCO_3^- (CA_{ext} and the proton pump). Similar growth rates across all flow regimes suggest that the metabolic compensation accomplished by modulating DIC uptake mechanism is able to provide sufficient CO_2 for photosynthesis and growth across a wide range of water currents. Although root length also responded to water flow, increasing in stronger currents, sediment composition, an indirect effect of water flow, was the dominant factor affecting root morphology and biomass.

The ability to acclimate photosynthetic metabolism to water flow is important because seagrasses inhabit a wide range of hydrodynamic conditions, ranging from high and variable current and drag forces from waves (Marba *et al.* 1994, Boller and Carrington 2006, Hepburn *et al.* 2007, Mach *et al.* 2007) to sheltered bays protected from wave action and characterized by very low current velocities. Even lower velocities, however, are likely to occur inside seagrass beds because the dense stands of plants attenuate flow (Fonseca and Koehl 2006). It is not clear, to what extent water flow affects DIC uptake *in situ* (Gerard 1982, Koehl and Alberte 1988, Hurd 2000). Seagrass photosynthesis is rate-limited by DIC concentrations in nature even at high current velocities (Thom 1995, Beer and Koch 1996, Beer and Rehnberg 1997, Björk *et al.* 1997, Schwarz *et al* 2000, Invers *et al* 2001). Consistent with Fick's first law, reduced water flow can further limit the flux of DIC to the plant surface by increasing the DBL thickness and thereby increasing the diffusional path of molecules from the water to the plant surface. As a result, seagrasses could be "starved" of the nutrients necessary for growth and photosynthesis under low flow conditions ultimately leading to compromised growth and survival. This seems contradictory as water flow within dense canopies of seagrass beds is known to be quite low (Gambi *et al.* 1990, Fonseca and Koehl 2006), especially under

unidirectional flows (Koch and Gust 1999), but seagrasses thrive under these conditions. This suggests that seagrass are likely to acclimate to low water flows just as they acclimate to reduced light levels.

Zostera marina exposed to low water flow were able to acclimate to the additional flux limitation imposed by a thicker DBL by increasing CA_{ext} and increasing proton pump extrusion. Proton extrusion acidifies the DBL, increasing the equilibrium concentration of CO_2 because the HCO_3^- - CO_2 equilibrium is pH dependent. As CO_2 in the DBL diffuses into the cell and chloroplast down the concentration gradient created by fixation by Rubisco, it is replenished by HCO_3^-. However, the conversion of HCO_3^- to CO_2 is not instantaneous; in fact the reestablishment of equilibrium after the addition of HCO_3^- requires tens of seconds (Schulz *et al.* 2006), which is extremely slow for a chemical inter-conversion. The rate limiting step for DIC uptake for photosynthesis then becomes the conversion of HCO_3^- to CO_2, catalyzed by CA_{ext}. CA_{ext} increases carbon availability by accelerating the conversion of HCO_3^- to CO_2. Acetazolamide, an inhibitor of CA_{ext}, reduced photosynthetic rates in *Zostera marina* by between 20 and 65% (Hellblom and Bjork 1999, Hellblom *et al.* 2001) demonstrating the significance of CA_{ext} in carbon uptake kinetics. These two mechanisms working in concert effectively increase CO_2 uptake. As a result, *Z. marina* was able to grow even under stagnant conditions. In contrast to the brown alga *Saccharina latissima*, *Z. marina* responded to low flows by increasing both CA_{ext} and the proton pump, which may explain the ability of the seagrass to occur in dense beds in sites with very low current velocities.

Although the acclimation to water flow via CA_{ext} and the proton pump appeared to be quite effective in *Zostera marina* in the mesocosm, as seen by similar tissue carbon contents and growth rates, photosynthesis was regulated as a function of water flow. Although R_d and P_{max}, measured at high flows in the oxygen electrode, were unaltered by current velocity in the growth conditions, maximum ETR, another measure of P_{max}, when determined under zero flow conditions, varied in inverse relationship to water flow in both sediment treatments, with a maximum in the zero flow sand treatment. The difference between P_{max} measured with the electrode under high flow and via ETR in zero flow indicates that the acclimation responses only confer an advantage when DIC transport is DBL-limited, which is consistent with the mechanism of action of CA_{ext} and the proton pump and their role in facilitated diffusion. The higher ETR in plants from stagnant conditions may reflect the increase in CA_{ext} and proton pump enhancing DIC uptake, or an up-regulation of Rubisco in the chloroplast which would have the same effect by fixing more CO_2 and increasing the diffusion gradient between the DBL and the chloroplast. My data is consistent with that of Enríquez and Rodríguez-Román (2006) who found that although the seagrass *Thalassia testudinum* decreased photosynthesis (ETR) when transferred to low water flow conditions, rates recovered after 6 days showing acclimation to water flow. If the flux of carbon is limited by the DBL and ETR is not at its maximum rate (e.g. in 20 cm s^{-1} grown plants exposed to stagnant conditions), light can also not be used as effectively. As a result, non-photochemical quenching was inversely related to the maximum ETR.

The plants acclimated to stagnant water (in higher organic sediment) were more effective in DIC uptake at low concentrations (≤ 1 mM) than the plants allowed to grow under flowing water (1 and 20 cm s-1; Figure 8B), but at DIC levels ≥ 2 mM, there were no differences in DIC uptake between treatments, although photosynthesis continued to increase up to 10 mM DIC. Although the DIC uptake kinetics were measured at high flow velocities in the oxygen electrode chamber, the data for high organic sediment suggest that acclimation to zero flow confers an increased ability for DIC uptake over low or moderate current velocities, despite the fact that CA_{ext} and the proton pump are similar between the 0 and 1 cm s^{-1} plants. However, these differences in DIC kinetics did not occur in the plants grown in lower organic sediment, which showed no difference between treatments (Figure 8A), despite the fact that these plants had higher levels of CA_{ext} and significantly greater dependence on the proton pump than the stagnant mud treatments. Although I have found both in *Saccharina* (chapter 2) and *Zostera* that physiological acclimation of DIC uptake mechanisms in response to water flow occurs regardless of nitrogen status, there may be other mechanisms, involved in the acclimation response to water flow such as up or down regulation of Rubisco that are nutrient dependent. The differences in nitrogen status of the plants (see below) could potentially affect Rubisco content and explain the differences in the DIC kinetic data between the sand and the mud treatments. Importantly, the DIC kinetics emphasize that photosynthesis in *Z. marina* is rate-limited under ambient DIC (2 mM) so that any reductions in seawater DIC at midday in a dense seagrass bed will exacerbate the effects of DBL limitation.

Although my controlled experiments demonstrate the capacity of *Zostera marina* to acclimate DIC-uptake to metabolically compensate for rate limitation of photosynthesis under low flows, I found no evidence that this occurred at the low-current field site at Bullhead Bay. There were no significant differences between the field sites in CA_{ext} or the dependence of photosynthesis on H^+ extrusion. It is important to remember that these data are for a single pair of field sites measured in early summer of 2006 and it is probable that acclimation of DIC uptake to water flow occurs in other field sites or at the Bullhead Bay site at other times of the year. Visual observation and measurements both confirmed that Bullhead Bay and Tyndal Point are close to the lower and upper limit of water flow inhabited by *Zostera marina* with water motion at Bullhead Bay being further attenuated by the large dense seagrass bed. However, continuous measurements of water flow were not possible and current velocities at Bullhead Bay may have been above the threshold where acclimation of DIC uptake takes place at different times in the tidal cycle. The mesocosm experiments indicate that the threshold for acclimation of CA_{ext} and the proton pump is between 1 and 20 cm s^{-1}.

Another possible explanation for the lack of acclimation of DIC-uptake by *Zostera marina* leaves at Bullhead Bay is the high concentration of pore water DIC in the sediment at this site. In this regard, Bullhead Bay sediment differs from the mud used in our flume experiments, which had an order of magnitude less organic content. Given the concentration difference between water and pore water at the sediment surface at Bullhead Bay of ~ 5 mM, one would anticipate a considerable efflux of DIC from the sediment into the water column that might alleviate DIC limitation. Alternatively, it is

possible that there was direct transport of DIC to the leaves from the sediment through the roots, as has been reported in *Z. marina* by Penhale and Thayer (1980).

Afternoon temperatures at Bullhead Bay were well above the optima for *Zostera marina* growth (15-20°C) and photosynthesis (23°C) (see review by Lee *et al.* 2007), and photosynthesis declines above the optimum (Santamaŕia and Vierssen 1997). An additional potential stressor at Bullhead Bay was a very high sulfide level in the porewater, which has been reported to decrease P_{max} (Mendelssohn and Seneca 1980, Morris 1980, Koch *et al.* 1990). Ammonium levels in the porewater at Bullhead Bay were just below those reported to cause die back in *Z. marina* (Van Katwijk 1997). These toxic levels of ammonium may also have reduced photosynthesis. If any of these factors became rate limiting to photosynthesis, the need for carbon would be diminished in response to the reduced photosynthetic rate and would explain the lack of acclimation of DIC uptake mechanisms.

Plant morphology responded to both sediment type and water flow. The effect of sediment can be considered to be a secondary effect of water motion because currents influence sediments, with coarser, less organic and more nutrient poor sediments being characteristic of high flow areas (Huettel *et al.* 2003). The tissue nitrogen contents in all treatments were below the value of 1.8% that differentiates nitrogen as the limiting factor for seagrass growth (Short 1987, Duarte 1990), suggesting that all treatments were nitrogen deficient. However, the data for growth and pore water ammonium suggests that nitrogen deficiency was more severe in sand than mud. Plants grown in sand had significantly longer roots and more root biomass than the mud treatments, a

morphological response commonly associated with sediment-induced nutrient-limitation (Short, 1987). This morphological response may ameliorate differences in nutrient availability by increasing the capacity for nutrient uptake, as described by Lee and Dunston (1999) who found that similar amounts of nitrogen were taken up by *Thalassia testudinum* in different sediments because of the inverse relationship between below ground biomass and pore water nutrients. Root length was also affected by current velocity presumably to increase anchorage in high flow. Duarte *et al* (1998) found that while root thickness proportional to the overall dimensions of a seagrass plant, root length is influenced by other factors, such as nutrient uptake and anchoring plants against current induced drag.

In summary, my data indicate that low water flows induce rate limitation of photosynthetic DIC uptake but that this limitation is ameliorated by acclimation of CA_{ext}, proton extrusion and possibly also changes in Rubisco. The metabolic compensation achieved by up or down-regulating the mechanisms involved in facilitated diffusion of DIC provide adequate carbon to support growth across a wide range of water flows, avoiding growth limitation by carbon. My data suggests that growth is controlled by nitrogen availability; a secondary effect of water motion mediated via its effect on sediment composition. Overall, my data indicate that the response of seagrasses to water motion is complex and depends on mass transport of DIC across the DBL, but also on factors such as sediment composition and nutrient availability.

Appendix 1. Effect of water flow on the morphology and physiology of natural populations of the marine angiosperm *Zostera marina* L.

Abstract

I examined the morphology and physiology of the marine angiosperm *Zostera marina* L. exposed to different water flow regimes *in situ*. My goal was to determine if the acclimation of DIC-uptake to water flow that was observed under controlled conditions also occurs in nature. The sites were Tyndal Point, characterized by high currents, coarse sandy substrate and low sediment organic content (0.8%) and Bullhead Bay a sheltered low current site with soft muddy sediment and high sediment organic content (12%). Porewater NH_4, PO_4 and sulfide concentrations were at least an order of magnitude greater at Bullhead Bay. Tissue nitrogen and phosphorous were similar at both sites and indicative of N-limitation, but tissue carbon was significantly higher at Tyndal Point. *Z. marina* exhibited morphological differences with those from Bullhead Bay having significantly longer, wider leaves and shorter roots than those from Tyndal Point. Although there were differences in photosynthetic metabolism, with chlorophyll-*a*, P_{max}, electron transport rate and non-photochemical quenching all being higher in plants at Tyndal Point, there were no differences in either CA_{ext} or the dependence of photosynthesis on H^+ extrusion. Thus, while our research does not negate the possibility that acclimation of DIC uptake metabolism occurs in nature under certain circumstances, it is clearly not ubiquitous and other environmental factors such as light and nutrients may be more important than flow in controlling growth and physiology of this seagrass.

Introduction

This appendix contains an extensive series of measurements, collected during my time as a student, on populations of *Zostera marina* growing at high and low flow sites in Peconic Bay, NY. Some of this data has been included in chapter 2, however, the entire data set is presented in this appendix.

The mesocosm experiments (chapter 3) indicated that *Zostera marina* has the capacity to acclimate carbon uptake mechanisms in response to water flow. The rationale for the work presented in this appendix was to ascertain if acclimation of DIC-uptake occurs in field populations of *Z. marina* exposed to low current flows. In nature *Z. marina* occurs across a wide range of current flows ranging from sheltered bays with no discernable current to tidal rips in narrow inlets. In addition, dense seagrass beds attenuate currents with water velocity decreasing both with distance downstream in the bed and depth below the canopy surface (Fonseca and Koehl 2006). My data indicated that there were no differences in either CA_{ext} or the proton pump indicative of acclimation of DIC-uptake mechanisms between a low (Bullhead bay) and a high (Tyndal Point) current site in Peconic Bay. Although these data demonstrate that acclimation of DIC-uptake in response to water flow is not ubiquitous in nature, the study is limited because I only examined two sites at a single point in time. However, I did find large differences between the morphology of *Z. marina* at these sites as well as in environmental characteristics such as temperature, sediment organic matter and pore water nutrients. Consequently, the data are included here to provide valuable information on environmental parameters affecting *Z. marina* in the field that may be useful to future researchers.

Materials and Methods

Field Sites: Two sites were selected in Peconic Bay on Long Island, N.Y. , Tyndal Point and Bullhead Bay ($43^{o}50.037$'N, $69^{o}30.926$'W and $43^{o}50.411$'N, $69^{o}33.354$'W, respectively). The sites were chosen due to obvious differences in water flow. Tyndal Point is at a narrow constriction in the bay system that experiences high current velocities at both outgoing and incoming tides. Bullhead Bay is in a sheltered cove with negligible currents at all stages of the tide. Water flow was measured with a Marsh McBurnie Flo-Mate 2000 current meter. Velocities at Tyndal Bay exceeded 40 cm s^{-1} whereas those at Bullhead Bay were below the level of detection of the instrument. Plants standing straight up also suggest that water flow was negligible. Both sites were of similar depth at about 1.0 m at high tide. The seagrass bed at Bullhead Bay was very dense, with leaves of almost 50 cm in length that form a dense canopy at the water surface at low tide. The seagrass bed at Tyndal Point was sparse and the plants were much smaller with leaves of less than 20 cm in length.

Experimental material: Entire plants of *Zostera marina* were collected from each site and immediately placed in a cooler filled with ambient seawater for the trip back to the laboratory at Cornell University Marine Environmental Center in Southold N.Y. The plants were held in a greenhouse in an indoor tank in flowing ambient seawater, ambient light, and temperature. Plants were used for experiments within 24 h of collection from the field.

Morphological characterization. I determined blade length and width with a measuring tape and ruler. Number of roots per node were counted as well as length of each root per node.

Photosynthetic Measurements: I used a Clark-type oxygen electrode chamber (DW1, Hansatech, Norfolk, UK) to measure photosynthetic parameters at 20°C as described previously (Collén and Davison 1999). Light was provided by a 50 W tungsten-halogen lamp in an LS-2 light source (Hansatech) attenuated with neutral density filters. The electrode chamber contained 2 ml of ambient seawater, filtered through a 0.45 μm nitrocellulose membrane filter. Measurements were made under high flow rates generated by the magnetic stirrer at the bottom of the chamber set at maximum speed. A 3 cm leaf section cut 4 cm from the base of the third oldest leaf was used for measurements. R_d was measured in darkness after which the tissue was exposed to progressively higher light levels to obtain photosynthesis x irradiance (PI) curves. Each light level was maintained until a stable oxygen flux was achieved (usually 5-10 min). I also studied photosynthetic responses under zero flow conditions using the rapid light curve program of a diving PAM (Pulse Amplitude Modulated Fluorometer: Walz, Effeltrich, Germany). Light levels were increased every 10 s and electron turnover rate (ETR), yield (variable light-adapted fluorescence), and incident light were used to calculate non-photochemical quenching.

Organic content and grain size: Sediment cores were obtained from both sites to a depth of 10 cm. Sediment was homogenized and samples were oven dried at 60° for

seven days and weighed. After being ashed in a furnace at 450°C for four hours the

samples were reweighed and organic content was calculated from the loss of weight.

Grain size was determined by putting a dried sediment sample through a series of sieves

according to Erftemeijer and Koch (2001).

Light and Temperature Levels: Temperature (Stowaway Tidbit, Onset Computer

Corporation, Bourne, MA) and light sensors (Odyessey, Data Flow Systems PTY

Limited, Christ Church, New Zealand) were deployed on June 23 and collected on June

26, 2007. Temperature sensors were deployed at the top and mid canopy recording every

five minutes. Light levels were measured at the water surface at Bullhead Bay, by

deploying the light sensor on a float moored to an anchor and in the middle of the canopy

at both sites (10 and 25 cm from the sediment surface, at Tyndal Point and Bullhead Bay

respectively). Surface light was not measured at Tyndal point because the current pulled

the float underwater, but was assumed to be similar to Bullhead bay.

Porewater Measurements: Porewater nutrients in the sediments were measured by

allowing nutrients to equilibrate for 10 days into 10mL deoxygenated (N_2 bubbled),

deionized water contained plastic cells covered with semi-permeable polysulfonate

membrane with openings to the sediment that were protected by nylon screening

(Hesslein 1976). The samplers were positioned at the same depth with pore water

samples colleted from 3.5 to 21.5 cm below the sediment surface. For a discussion of

equilibrium dynamics of porewater samples see Webster *et al*. 1998. The pore-water

samplers were deployed May 23, 2007, collected one at a time on June 23, 2007 and

processed immediately. Samples were filtered through a 0.45µm membrane filter

attached to a polystyrene syringe and placed into plastic vials. Samples for analysis of

NH_4, NO_3 and PO_4 were frozen in plastic vials at -20^oC. For H_2S, 1 ml of sample was

placed in a glass vial together with 80µl chilled diamine (prepared by adding 8g N-N-

dimethyl-p-phenylenediamine and 12g $FeCl_3$ to 500 mls 6M HCl for the samples from the

high flow site and 20g and 30g respectively for the samples from the low flow site).   1

ml samples for DIC analysis were stored in glass vials with 1.5 µl $HgCl_2$. H_2S and DIC

samples were stored at room temperature until processed. NH_4, NO_3 and PO_4 were

measured using Bran + Luebbe autoanalyzer, (Bran + Luebbe Inc., Delavan, WI). H_2S

and DIC samples were analyzed in a Shimazu TOC 5000A (Shimazu Company Ltd.,

Kyoto, Japan) autoanalyzer.

Dry weight:fresh weight and CHN analysis: Tissue samples (~ 0.1 g) were blotted dry

and weighed to determine the fresh weight prior to drying in an oven at 60^oC for 48 h.

Tissue was allowed to cool in a desiccator prior to determining dry weight. Dried

samples were ground to a fine powder in a mortar and pestle, redried at 60^oC, and stored

in tightly capped glass vials in a desiccator prior to CHN measurements using a CE-440

Elemental Analyzer Exeter Analytical (UK) Ltd., Coventry UK.

Carbon uptake mechanisms: External carbonic anhydrase was quantified using the

method described in Mercado *et al.* (1997) at light levels of 250 to 300 µmol m^2 s^{-1}.

Proton extrusion was determined by measuring photosynthesis at a 500 µmol m^2 s^{-1} light

level before and after injection of 16μl of 1mM stock of Erythrosin B, a proton-pump inhibitor into 2ml of seawater.

Statistical Analyses: Data were analyzed using nested ANOVA with Duncans test for post-hoc multiple comparisons using Statmost3[2] (DataMost Corp, South Sandy UT).

Results

Leaf lengths were between 14-18 cm at Tyndal Point and 37-48 cm at Bullhead Bay, where they reached the surface at low tide. The highest light level measured at the surface of Bullhead Bay was 1600 μmol m^2 s^{-1} and although the sensor was 15 cm deeper at Tyndal Point than Bullhead Bay, mid canopy light levels were greater at the high current site (highest level was 1300 μmols m^2 s^{-1}) than Bullhead Bay (highest level 1000 μmols m^2 s^{-1}) (Figure 1).

Water temperatures varied diurnally at both sites and exhibited a progressive increase from June 22 to June 27, 2007 (Figure 1). Compared to Tyndal Point, temperatures at Bullhead Bay had greater diurnal amplitude (4^oC cf.1.5^oC), were 3-5°C higher at the hottest part of the day, and increased at a faster rate reaching a maximum of 28^oC. The temperature was the same at the top and bottom of the canopy at both sites.

Organic content of the sediment was 12% at Bullhead Bay and 0.80 at Tyndal Point. Grain size at Bullhead Bay was smaller than at Tyndal Point with 87% of the sediment at Bullhead Bay less than 63μm whereas 92% of sand grains at Tyndal Point were ≥ 125μm.

Figure 1. Changes in temperature (A) in *Zostera marina* measured at the top (o, Δ) and bottom (●, ▲) of the canopy and light levels (B) at the surface (o) and mid-canopy (●, ▲) at Bullhead Bay and Tyndal Point between June 22 and 27,
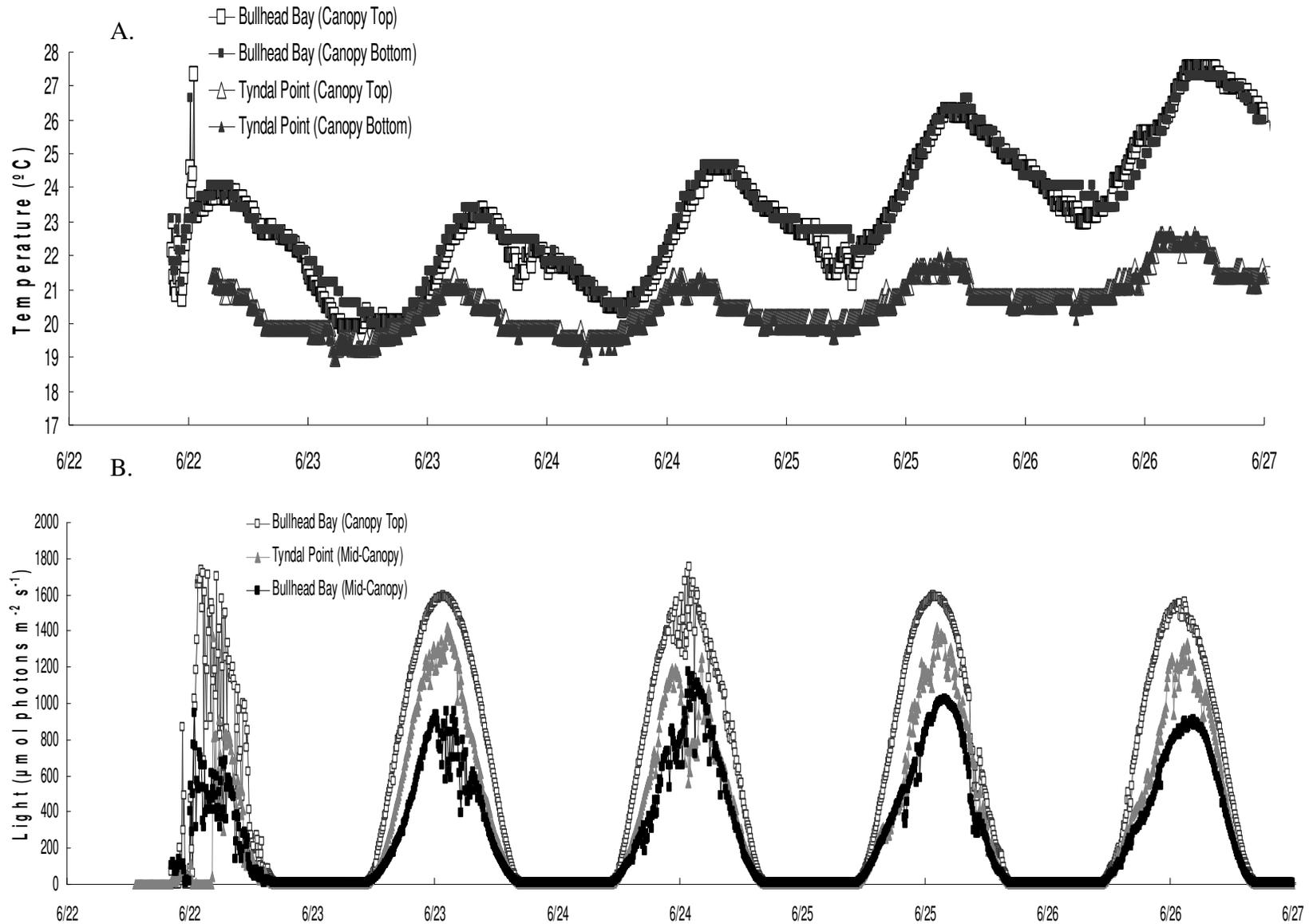
Porewater concentrations of ammonium, phosphate (Figure 2A, 2B), DIC and non particulate organic carbon were significantly higher at Bullhead Bay than Tyndal Point (Table 1) and increased with depth. In contrast, nutrient levels at Tyndal Point were similar at all depths. Sulfide levels at Bullhead Bay were extremely high (average 1963 μmM) and similar at depth. Sulfide levels at Tyndal Point were significantly lower (average 11 μM) and similar at depth (Figure 2C). pH values were lower at Bullhead Bay (6.9 at 3.5cm), than at Tyndal Point (7.2 at 3.5cm) (Figure 2D).

There were obvious morphological differences between the low flow site (Bullhead Bay) the high flow site (Tyndal Point). Leaf length and width were significantly greater at Bullhead Bay than Tyndal Point when leaf two and leaf three at both sites were compared (Table 1, Figure 3). Length of leaf four was significantly different but leaf width was not. Number of roots per node was higher at Bullhead Bay than Tyndal Point (Table 1, Figure 4A). Root length at Bullhead Bay was less than at Tyndal Point, however not significantly (Table 1, Figure 4B).

Percent tissue nitrogen ~ 1.7% and phosphorous ~ 0.30% were not significantly different (Table 1, Figure 5A); however, percent tissue carbon was significantly elevated at Tyndal Point (Table 1, Figure 5B). Chlorphyll-*a* content of Bullhead Bay plants was significantly lower (0.25 μmol $\cdot$ g^{-1}) (Table 1) than Tyndal Point plants (0.34 μmol $\cdot$ g^{-1}).

There was no significant difference in respiration between the two sites. However, P_{max} was significantly higher at Tyndal Point (Table 1, Figure 6). ETR and NPQ were both higher at Tyndal Point (Figure 7).
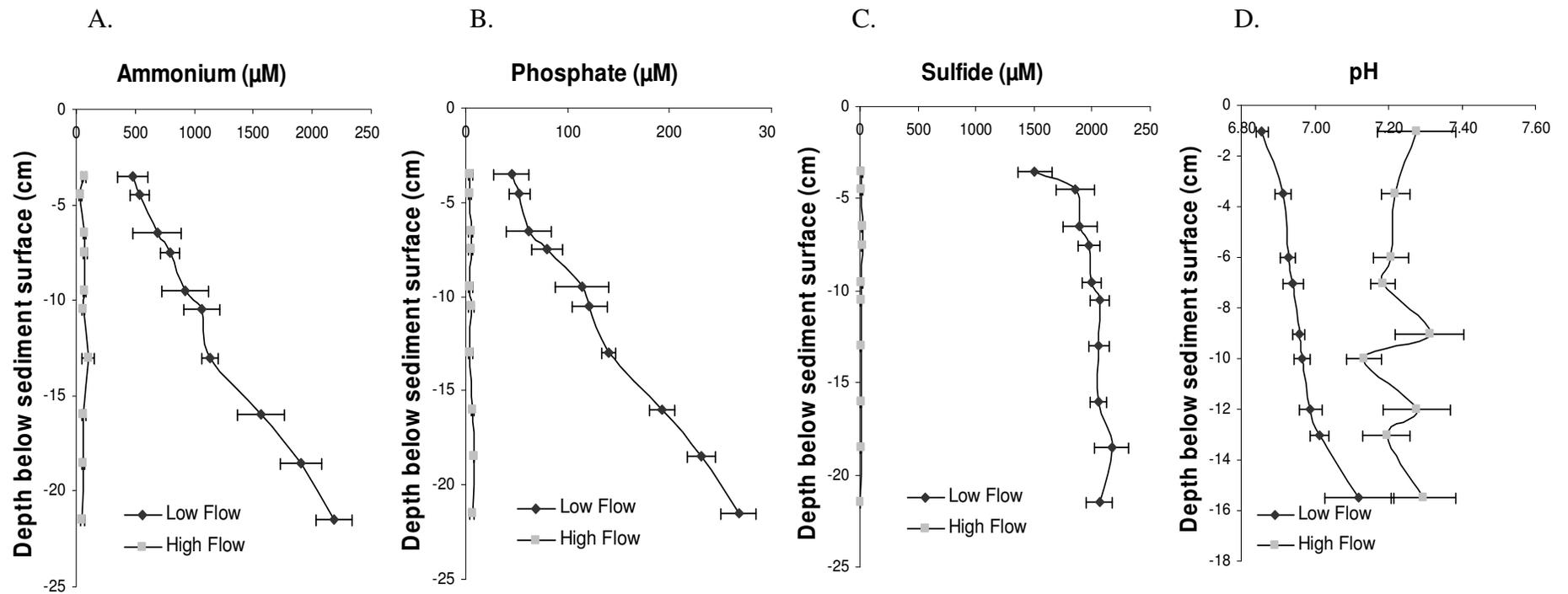
Figure 2. Depth profiles of NH_4 (A), PO_4 (B), SO_4 (C) and pH (D) in *Zostera marina* beds at Bullhead Bay and Tyndal Point. Error bars represent SE of mean (n=4).

Table 1. Morphology (n=10), pore water nutrients (n=4), percent tissue nutrients (n=9) and physiology (n=4) of *Zostera marina* at low (Bullhead Bay) and high (Tyndal Point) sites including statistics. Values are means $\pm$ (SE). Statistical values are p and F from one-way ANOVA.

Measurement (units)	Bullhead Bay	Tyndal Point	Statistics
Number of roots	17.3 (1.44)	11.5 (1.35)	p = 0.0115 F = 8.40
Root length (cm)	3.86 (0.321)	5.50 (0.706)	p = 0.0613 F = 4.09
Leaf length (cm)			
Leaf 2	44.9 (2.63)	18.0 (0.723)	p < 0.0001 F = 90.8
Leaf 3	48.3 (2.84)	15.3 (0.547)	p < 0.0001 F = 118
Leaf 4	37.6 (3.05)	14.4 (0.722)	p < 0.0001 F = 50.9
Leaf width			
L2	0.515 (0.0211)	0.393 (0.0167)	p < 0.0001 F = 26.1
L3	0.543 (0.0242)	0.411 (0.0217)	p = 0.0009 F = 16.1
L4	0.490 (0.0485)	0.400 (0.0247)	p = 0.882 F = 3.44
Pore Water Nutrients (3.5 cm depth)			
Ammonium (µM)	476 (130)	64.6 (13.9)	p = 0.0202 F = 9.84
NPOC (mg/l)	9.37 (2.26)	4.81 (0.341)	p = 0.0929 F = 3.99
Phosphate (µM)	45.1 (17.5)	4.58 (2.06)	p = 0.0607 F = 5.32
DIC (µM)	7018 (1229)	2365 (218)	p = 0.0247 F = 10.1
Sulfides	1504 (148)	14.9 (5.54)	p < 0.0001 F = 101
Percent Tissue Content			
Nitrogen (%)	1.65 (0.0693)	1.74 (0.0379)	p = 0.218 F = 1.65
Phosphate (%)	0.307 (0.0177)	0.273 (0.0120)	p = 0.138 F = 2.47
Carbon (%)	36.1 (0.112)	38.1 (0.274)	p < 0.0001 F = 49.9

Table 1 (continued)

|			
Carbonic Anhydrase	0.657 (0.070)	0.610 (0.045)	p = 0.573 F = 0.342
Erythrosin B (% inhibition)	37.6 (8.88)	24.9 (7.38)	p = 0.306 F = 1.25
Respiration (μmol g^{-1} h^{-1})	-4.48 (0.345)	-8.14 (2.09)	p = 0.135 F = 2.97
P_{max} (μmol g^{-1} h^{-1})	12.3 (0.986)	36.3 (7.13)	p = 0.0160 F = 11.0
Gross P_{max} (μmol g^{-1} h^{-1})	16.9 (1.20)	44.5 (6.43)	p = 0.0056 F = 17.7
Respiration (μmol cm^{-2} h^{-1})	-0.110 (0.0192)	-0.155 (0.0399)	p = 0.316 F = 1.199
P_{max} (μmol cm^{-2} h^{-1})	0.294 (0.00354)	0.665 (0.108)	p = 0.0138 F = 10.9
Gross P_{max} (μmol cm^{-2} h^{-1})	0.404 (0.0126)	0.820 (0.0950)	p = 0.0049 F = 18.8
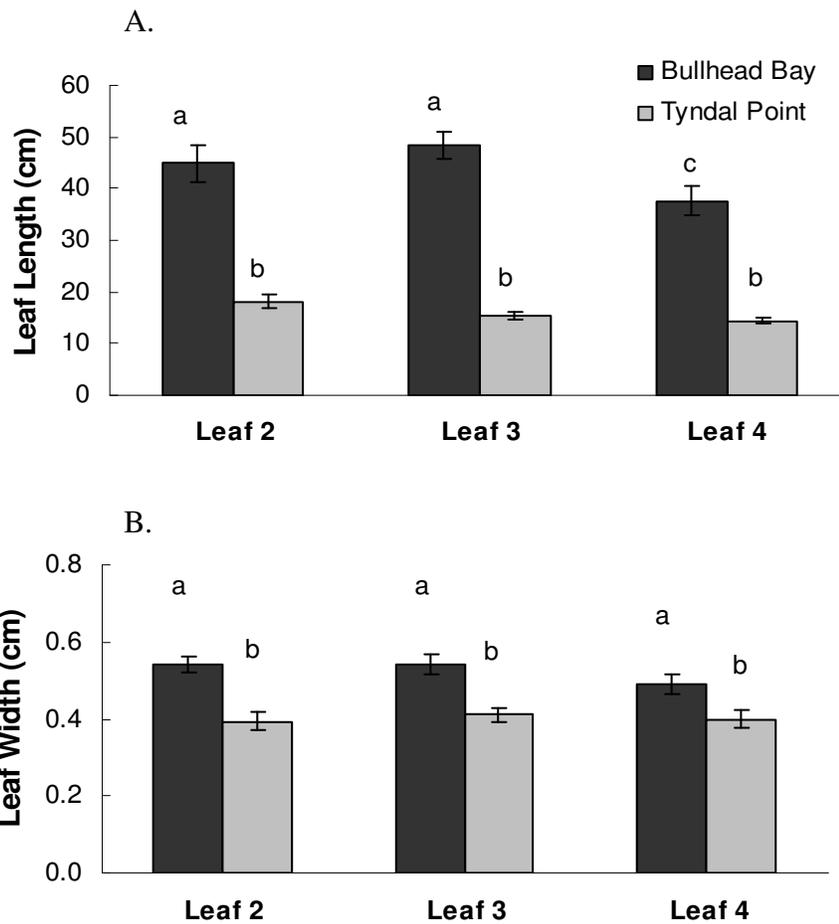
Figure 3. Length (A) and width (B) of *Zostera marina* leaves 2 through 4 (oldest) at Bullhead Bay and Tyndal Point. Error bars represent SE of mean (n=10). Significant differences between treatments ($p \leq 0.05$) are indicated by different letters.
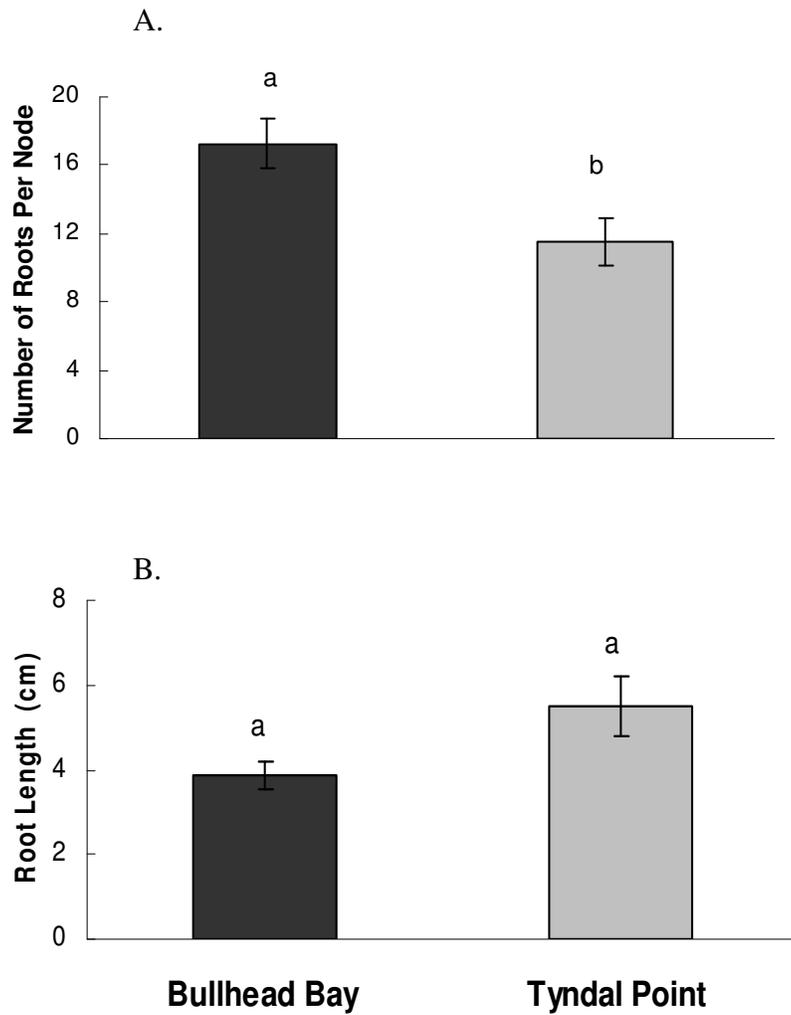
Figure 4. Number of *Zostera marina* roots per node (A) and average root length (B) at Bullhead Bay and Tyndal Point. Error bars represent SE of mean (n=10). Significant differences between treatments (p ≤ 0.05) are indicated by different letters.
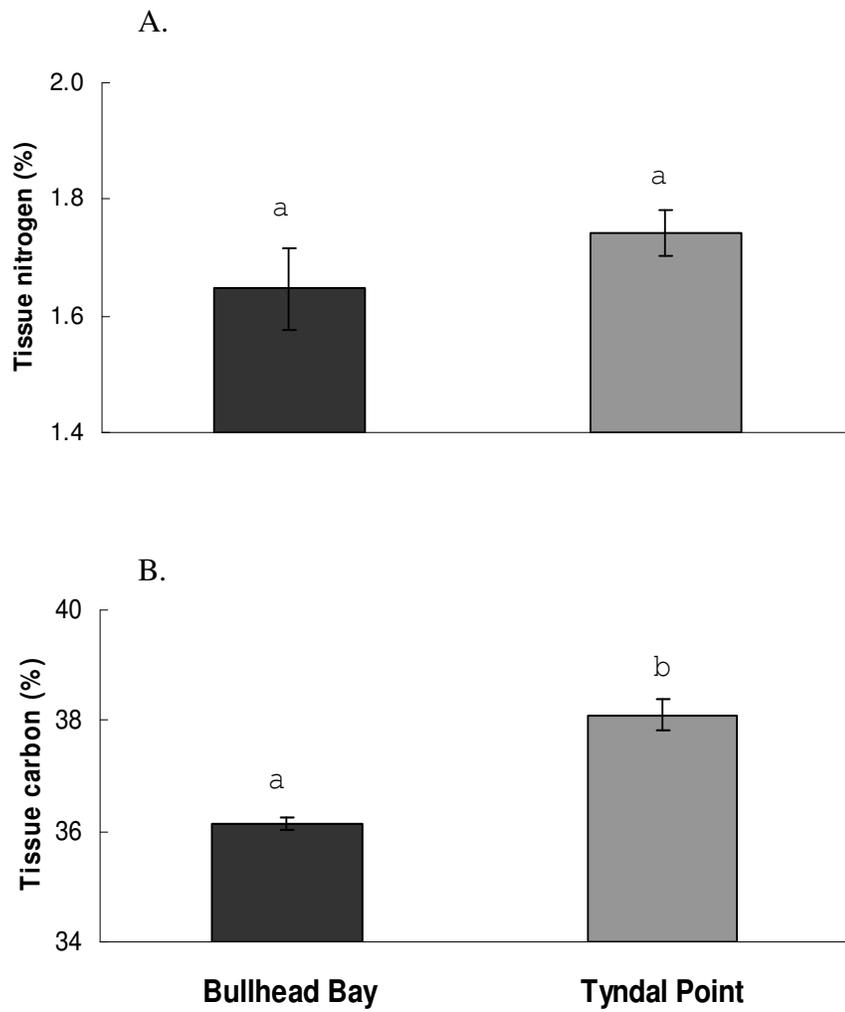
Figure 5. *Zostera marina* tissue nitrogen (A) and tissue carbon (B) at Bullhead Bay and Tyndal Point. Error bars represent SE of mean (n=9). Significant differences between treatments ($p \leq 0.05$) are indicated by different letters.
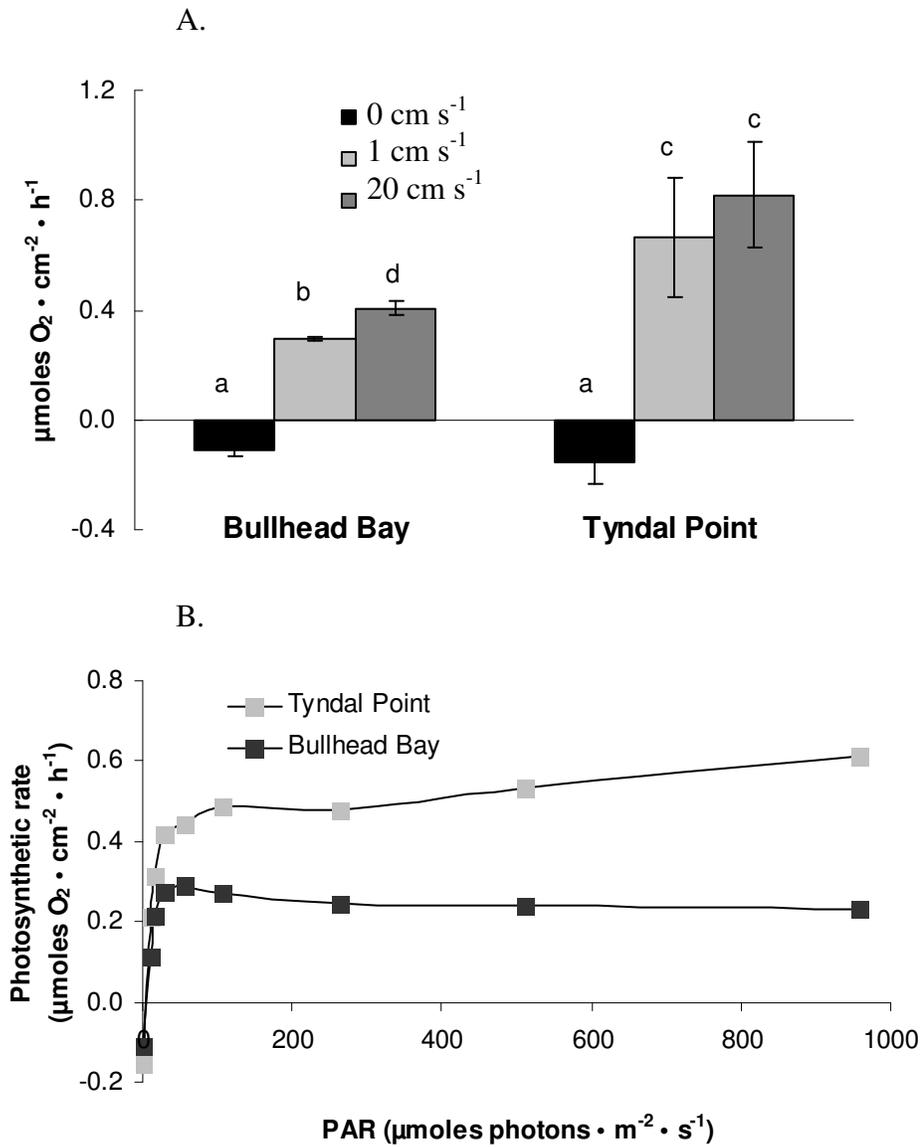
Figure 6. Respiration, P_{max}, and Gross P_{max} (A) of *Zostera marina* and oxygen flux vs photosynthetically active radiation (B) at Bullhead Bay and Tyndal Point. Error bars represent SE of mean (n=4). Significant differences between treatments ($p \leq 0.05$) are indicated by different letters.

A.

B.

Figure 7. Electron turnover rate (ETR) (A) and non-photochemical quenching of photosynthesis (B) in *Zostera marina* at Bullhead Bay and Tyndal Point. Error bars represent SE of mean (n=5). Significant differences between treatments ($p \leq 0.05$) are indicated by different letters.
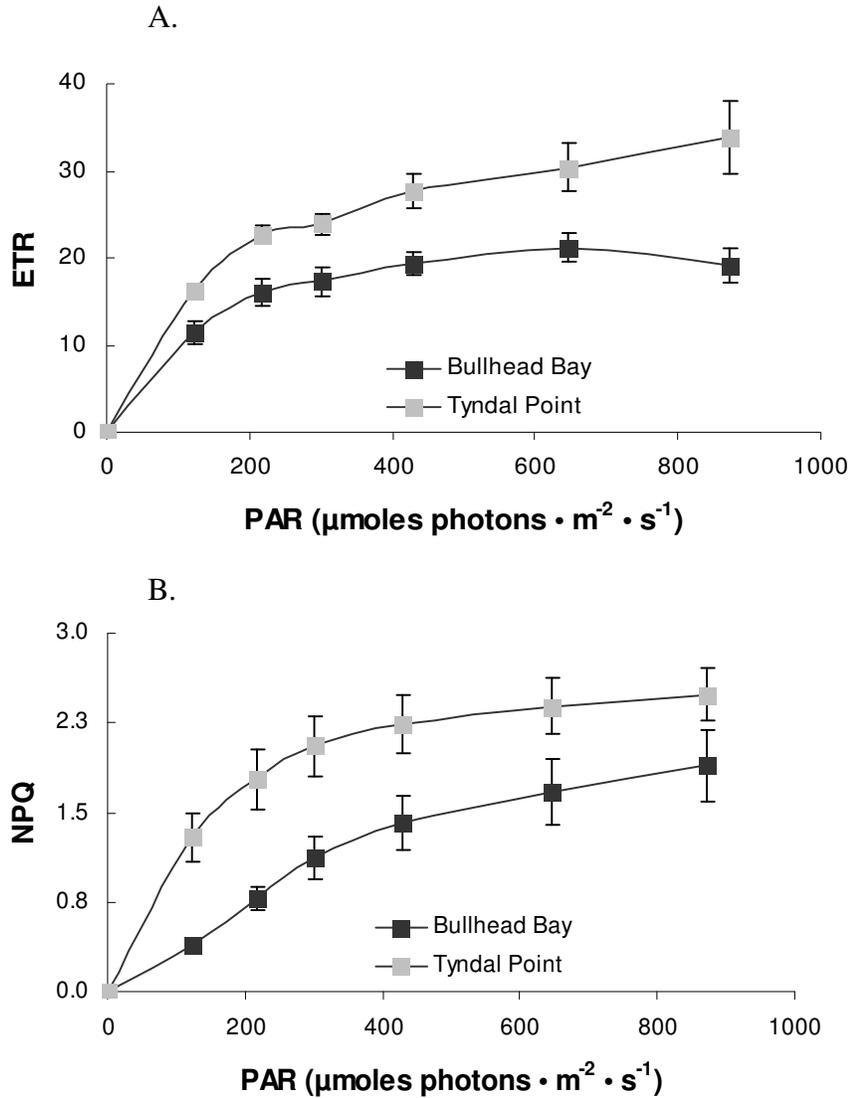
External CA activity was not significantly different at the two sites. As with CA_{ext}, there was no significant difference in inhibition to Erythrosin B (proton pump inhibitor), although percent inhibition was higher at Bullhead Bay (38) than at Tyndal Point (25) (Table 1, Figure 8).
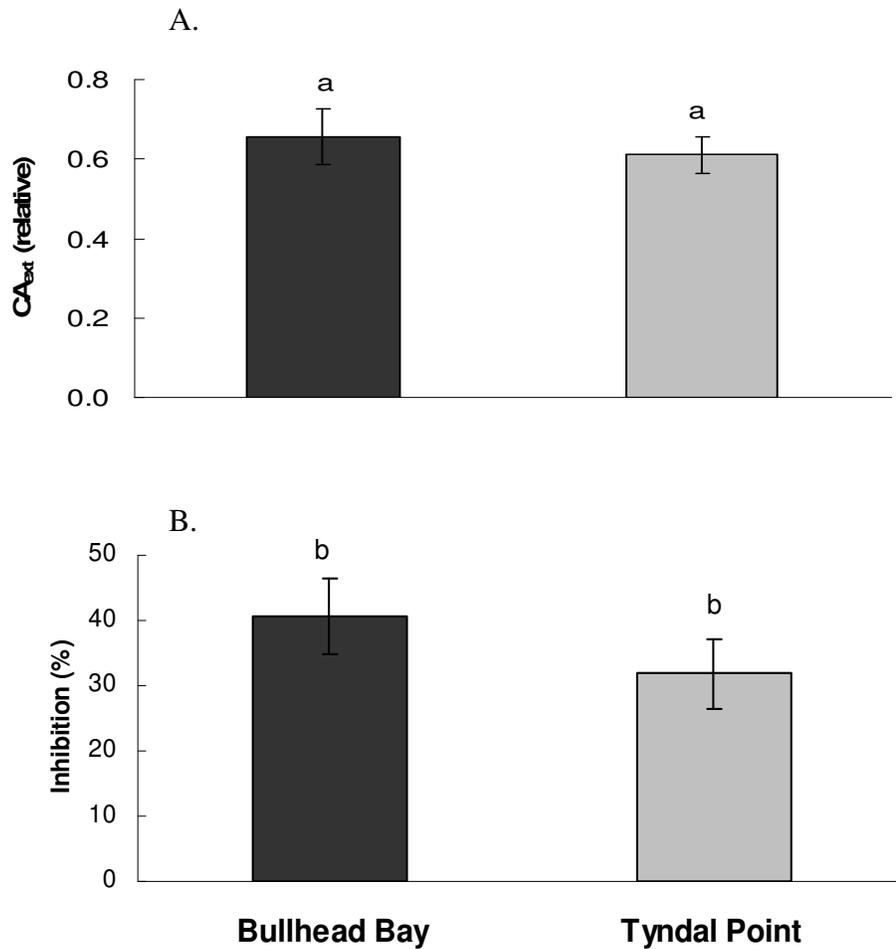

Figure 8. CA_{ext} (A) and inhibition of photosynthesis (B) by erythrosin-B in *Zostera marina*. Error bars represent SE of mean (A: n=6; B: n=4). Significant differences between treatments ($p \leq 0.05$) are indicated by different letters.

References

Almasi, M. N., Hoskin, C. M. Reed, J. K. and Milo, J. (1987). Effects of natural and artificial *Thalassia* on rates of sedimentation. *Journal of Sediment Petrology*. **57:** 901-906.

Andria, J. R., Brun, F.G., Perez-Llorens, J. L. and Vergara, J. J. (2001). Acclimation Responses of *Gracilaria* sp. (Rhodophyta) and *Enteromorpha intestinalis* (Chlorophyta) to Changes in the External Inorganic Carbon Concentration. *Botanica Marina* **44:** 361-370.

Axelsson, L., Larsson, C. and Ryberg, H. (1999). Affinity, capacity and oxygen sensitivity of two different mechanisms for bicarbonate utilization in *Ulva lactuca* L. *Plant, Cell and Environment* **22:** 969-978.

Axelsson, L., Mercado, J. M. and Figueroa, F. L. (2000). Utilization of HCO_3^- at high pH by the brown macroalga *Laminaria saccharina*. *European Journal of Phycology* **35:** 53-59.

Bandeira, S.O. (2000). Diversity and ecology of seagrasses in Mozambique: emphasis on *Thalassodendron ciliatum* structure, dynamics, nutrients and genetic variability. PhD Thesis, Göteborg University, Sweden.

Beer, S. and Shragge, B. (1987) Photosynthetic carbon metabolism in *Enteromorpha compressa* (Chlorophyta)**.** *Journal of Phycology* **23:** 580-584.

Beer, S. and Isreal, A. (1990). Photosynthesis of *Ulva fasciata* IV. pH, carbonic anhydrase and inorganic carbon conversions in the unstirred layer. *Plant Cell and Environment* **13:** 555-560.

Beer, S. and Koch, E. (1996). Photosynthesis of marine macroalgae and seagrasses in globally changing CO_2 environments. **Marine Ecology Progress Serices 141**: 199-204.

Beer, S. and Rehnberg, J. (1997). The acquisition of inorganic carbon by the seagrass *Zostera marina*. *Aquatic Botany* **56:** 277-283.

Beer, S. (1998). The acquisition of inorganic carbon in marine macrophytes. *Isreal Journal of Plant Sciences* **46:** 83-87.

Beer, S., Björk, M., Hellblom, F. and Axelsson, L. (2002). Inorganic carbon utilization in marine angiosperms (seagrasses). *Functional Plant Biology* **29:** 349-354.

Bell, E. C. and Denny, M. W. (1994). Quantifying wave exposure – A simple device for recording maximum velocity and results of its use at several field sites. *Journal of Experimental Marine Biology and Ecology* **181 (1):** 9-29.

Bjork, M., Weil, A., Semesi, S. and Beer, S. (1997). Photosynthetic utilization of inorganic carbon by seagrasses from Zanzibar, East Africa. *Marine Biology* **129:** 363-366.

Blanchette, C. A. (1997). Size and survival of intertidal plants in response to wave action: A case study with *Fucus gardneri*. *Ecology* **78 (5):** 1563-1578.

Boese, B. L., Robbins, B. D. and Thursby, G. (2005). Desication is a limiting factor for eelgrass (*Zostera marina* L.) distribution in the intertidal zone of a northeastern Pacific (USA) estuary. *Botanica Marina* **48:** 274-283.

Boller, M. L. and Carrington, E. (2006). The hydrodynamic effects of shape and size change during reconfiguration of a flexible macroalga. *Journal of Experimental Biology* **209 (10):** 1894-1903.

Bostrom, C. and Bonsdorff, E. (1997). Community structure and special variation of benthic invertebrates associated with *Zostera marina* (L) beds in the northern Baltic Sea. *Journal of Sea Research*. **37 (1-2)**: 153-166.

Bozzo, G. G. and Colman, B. (2000). The induction of inorganic carbon transport and external carbonic anhydrase in *Chlamydomonas reinhardtii* is regulated by external CO_2 concentration. *Plant, Cell and Environment* **23:** 1136-1144.

Brouns, J. J. (1994). Seagrasses and climate change. *Marine conservation and development report* 59-72.

Caldeira, K. and Wickett, M. E. (2003). Oceanography: Anthropogenic carbon and ocean pH. *Nature* **425:** 365.

Cayabyab N. M. and Enriquez, S. (2007). Leaf photacclimatory responses of the tropical seagrass *Thalassia testudinum* under mesocosm condition: a mechanistic scaling-up study. *New Phytologist* **176 (1):** 108-123.

Chapman, V. J. and Chapman, D. J. (1980). Seaweeds and their uses. London: Chapman and Hall 334 pp.

Chapman, A. R. O. and Craigie, J. S. (1978). Seasonal growth in Laminaria longicruris – Relations with reserve carbohydrate storage and production. *Marine Biology* **46 (3):** 209-213.

Collén, J. and Davison, I.R. (1999). Reactive oxygen metabolism in intertidal Fucus spp (Phaeophyceae). *Journal of Phycology* **35:** 62-69.

Collier, C. J., Lavery, P. S. and Ralph, P.J. (2008). Physiological characteristics of the seagrass Posidonia sinuosa along a depth-related gradient of light availability. *Marine Ecology Progress Series*. **353:** 65-79.

Conley, D. J. (1999). Biogeochemical nutrient cycles and nutrient management strategies. *Hydrobiologia* **410:** 87-96.

Cotton, A. (1912). Marine algae. Clare Island Survey, Part 15. *Proc. Roy. Irish Academy* **31:** 1-178.

Crossley, M., Dennison, W., Williams, R. and Wearing, A. (2002). The interaction of water flow and nutrients on aquatic plant growth. *Hydrobiologia* **489:** 63-70.

Davis, R. (1985). *Coastal Sedimentary Environments*. Springer Verlag, New York, pp. 716.

Davison, I. R., Andrews, M. and Stewart, W. D. P. (1984). Regulation of growth in *Laminaria digitata* – Use of invivo nitrate reductase activities as an indicator of nitrogen limitation in field populations of Laminaria spp. *Marine Biology* **84 (2):** 207-217.

Davison, I.R., Greene, M. and Podolak, E. (1991). Temperature acclimation of respiration and photosynthesis in the brown alga *Laminaria saccharina. Marine Biology* **110:** 449-454.

Demming-Adams, B. and Adams, W.W. (1992). Photoprotection and other responses of plants to high light stress. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **43:** 599-626.

Denny M. and Roberson, L. (2002). Blade motion and nutrient flux to the Kelp, *Eisenia arborea. Biological Bulletin* **203 (1):** 1-13.

Drechsler, Z. and Beer, S. (1991). Utilization of Inorganic Carbon by *Ulva lactuca*. *Plant Physiology* **97:** 1439-1444.

Duarte, C. M., Merino, M., Agawin, N. S. R., Uri, J., Fortes, M. D., Gallegos, M. E. Narbá, N. And Hemminga, M. A. (1998). Root production and belowground seagrass biomass. *Marine Ecology Progress Series* **171:** 97-108.

Dudgeon, S. and Johnson, A. (1992). Thick vs. thin: Thallus morphology and tissue mechanics influence differential drag and dislodgement of two co-dominant seaweeds. *Journal of Experimental Marine Biology and Ecology* **165:** 23-43.

Duffy, J. E. (2006). Biodiversity and functioning of seagrass ecosystems. *Marine Ecology Progress Series* **311**:233-250.

Duncan, M. J. and Harrison, P. J. (1982). Comparison of solvents for extracting chlorophylls from marine macrophytes. Bontanica Marina 25 (9): 445-447.

Enríquez, S. and Rodríguez-Román, A. (2006). Effect of water flor on the photosynthesis of three marine macrophytes from a fringing-reef lagoon. *Mar Ecol Prog Ser* **323:** 119-132.

Erftemeijer, P. L. A. and Koch, E. W. (2001). Sediment geology methods for seagrass habitat In: Short, F. T and Coles, R.G. (eds) *Global Seagrass Research Methods.* Elsevier Science, pp 156-158.

Evans, A. S., Webb, K. L. and Penhale, P. A. (1988). Photosynthetic temperature-acclimation in 2 coexisting seagrasses, *Zostera marina* L. and *Ruppia maritima* L. *Aquatic Botany* **24 (2):** 185-197.

Fisher, T. R., Peele, E. R., Ammerman, J. W. and Harding L. W. (1992). Nutrient limitation of phytoplankton in Chesapeake Bay. *Marine Ecology-Progress Series* **82 (1)**: 51-63.

Flores-Moya, A. and Fernández, J. (1998). The role of external carbonic anhydrase in the photosynthetic use of inorganic carbon in the deep-water algae *Phyllariopsis purpurascens* (Laminariales, Phaeophyta). *Planta* **207:** 115-119.

Fonseca, M.S. and Kenworthy, W.J. (1987). Effects of current on photosynthesis and distribution of seagrasses. *Aquatic Botany* **27:** 59-78.

Fonseca, M. S. (1989). Sediment stabilization by *Halophila decipiens* in comparison to other seagrasses. *Estuarine, Coastal and Shelf Science.* **29 (5):** 501-507.

Fonseca, M.S. and Koehl, M.A.R. (2006). Flow in seagrass canopies: The influence of patch width. *Esturine, Coastal and Shelf Science* **67:** 1-9.

Forster, R. M. and Dring, M. J. (1992). Interactions of blue-light and inorganic carbon supply in the control of light saturated photosynthesis in brown-algae. *Plant, Cell and Environment* **15 (2):** 241-247.

Franklin, L. A., Seaton, G. G. R., Lovelock, C. E. and Larkum, A.W. D. (1996). Photoinhibition of photosynthesis on a coral reef. *Plant Cell and Environment* **19:** 825-836.

Gacia, E., Duarte, C. M., Marbá, Terrados, J., Kennedy, H. Fortes, M.D. and Tri, N. H. (2003). Sediment deposition and production in SE-Asia seagrass meadows. *Estuarine, Coastal and Shelf Science*. **56 (5-6):** 909-919.

Gambi, M. C., Nowell, A. R. M. and Jumars, P. A. (1990). Flume observations on flow dynamics in *Zostera marina* (eelgrass) beds. ***Marine Ecology Progress Series*** **61:** 159-169.

Gerard, V.A. and Mann, K. (1979). Growth and production of *Laminaria longicruis* (Phaeophyta) populations exposed to different intensities of water movement. ***Journal of Phycology*** **15:** 33-41.

Gerard, V.A. (1982). *In situ* water motion and nutrient uptake by the giant kelp *Macrocystis pyrifera.* ***Marine Biology*** **69:** 51-54.

Gerard, V.A. (1987). Hydrodynamic streamlining of *Laminaria saccharina* L in response to mechanical stress. ***Journal of Experimental Marine Biology and Ecology*** **107:** 237-44.

Gevaert, F., Creach, A., Davoult, D., Migne, A., Levavasseur, G., Arzel, P., Holl, A. and Lemoine, Y. (2002). *Laminaria saccharina* photosynthesis measured in situ: Photoinhibition and xanthophylls cycle during a tidal cycle. ***Marine Ecology Progress Series*** **247**: 43-50.

Gonen, Y., Kimmel, E. and Friedlander, M. (1995). Diffusion boundary layer transport in *Gracilaria conferta* (Rhodophyta). ***Journal of Phycology*** **31:** 768-773.

Gordillo, F. J. L., Niell, F. X. And Figueroa, F. L. (2001). Non-photosynthetic enhancement of growth by high CO_2 levels in the nitrophillic seaweeks *Ulva rigida* C. Agardh (Chlorophyta). ***Planta*** **213(1):** 64-70.

Grice, A. M., Loneragan, N. R. and Dennison, W. C. (1996). Light intensity and the interaction between physiology, morphology and stable isotope ratios in five species of seagrass. ***Journal of Experimental Marine Biology and Ecology*** **195:** 91-110.

Gunstheimer, S. and Jahreis, G. (1998). Marine macroalgae. ***Ernahrungs-Umschau.*** **45 (12):** 424.

Gutierrez, L., and Fernández, C. (1992). Water motion and morphology in *Chondrus crispus* (Rhodophyta). ***Journal of Phycology*** **28:** 156-162.

Haglund, K., and Pedersén, M. (1992). Growth of the red alga *Gracilaria tenusitipitata* at high pH- Influence of some environmental factors and correlation to an increased carbonic anhydrase activity. ***Botanica Marina*** **35 (6):** 579-587.

Harker, M., Berkaloff, C., Lemoine, Y., Britton, G., Young, A. J., Duval, J. C., Rmiki, N.E., Rousseau, B. (1999) Effects of high light and desiccation on the operation of the xanthophyll cycle in two marine brown algae. ***European Journal of Phycology*** **34 (1):** 35-42.

Harris, L. A., Buckley B., Nixon, S.W. and Allen, B.T. (2004). Experimental studies of predation by bluefish *Pomatomus saltatrix* in varying densities of seagrass and macroalgae. *Marine Ecology-Progress Series* **281:** 233-239.

Haugan, P.M. and Drange, H. (1996). Effects of CO2 on the ocean environment. *Energy Conservation Management* **37:** 1019-1022.

Heck Jr., K. L., Hays, G. and Orth, R. J. (2003). Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology-Progress Series* **253:** 123-136.

Hellblom, F. and Bjork, M. (1999). Photosynthetic responses of *Zostera marina* to salinity, inorganic carbon content and osmolality. *Aquatic Botany* **65:** 97-104.

Hellblom, F., Beer, S., Bjork, M. and Axelsson, L. (2001). A buffer sensitive inorganic carbon utilization system in *Zostera marina*. *Aquatic Botany* **69:** 55-62.

Hemminga, M. A. (1998). The root/rhizome system of seagrasses: an asset and a burden. *Journal of Sea Research.* **39 (3-4):** 183-196.

Hemminga, M. A. and Duarte, C. M. (2000). **Seagrass ecology: an introduction.** Cambridge, UK, Cambridge University Press, pp 289.

Hepburn C. D., Holborow, J.D., Wing, S. R., Frew, R. D. and Hurd, C. L. (2007). Exposure to waves enhances the growth rate and nitrogen status of the giant kelp *Macrocystis pyrifera*. *Marine Ecology-Progress Series* **339:** 99-108.

Hesslein, R. H. (1976). *In situ* samples for close interval pore water studies. *Limnology and Oceanography* **21(6):** 912-914.

Holbrook, G. P., Beer, S., Spencer, W. E., Reiskind, J.B., Davis, J. S. and Bowes, G. (1988). Photosynthesis in marine macroalgae: evidence for carbon limitation. *Canadian Journal of Botany* **66**: 577-583.

Hurd, C. L., Harrison, P. J. and Druehl, L. D. (1996). Effect of seawater velocity on inorganic nitrogen uptake by morphologicall distinct forms of *Macrocystis integrifolia* from wave sheltered and exposed sites. *Marine Biology* **126:** 205-214.

Hurd, C. L. and Stevens, C. L. (1997) Flow Visualization around single and multiple bladed seaweeds with various morphologies. *Journal of Phycology* **33**: 360-367.

Hurd, C. (2000). Water motion, marine macroalgal physiology and production. *Journal of Phycology* **36:** 453-472.

Huettel, M., Roy. H., Precht, E. and Ehrenhauss, S. (2003). Hydrodynamic impact on biogeochemical processes in aquatic sediments. *Hydrobiologia* **494:** 231-236

Invers, O., Zimmerman, R., Alberte, R., Pérez, M. and Romero, J. (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:** 203-217

Jensen H. S., McGlathery, K. J., Marino R, and Howarth, R. W (1998). Forms and availability of sediment phosphorus in carbonate sand of Bermuda seagrass beds. *Limnology and Oceanography* **43 (5):** 799-810.

Johnson, K. (1982). Carbon dioxide hydration and dehydration kinetics in seawater. *Limnology and Oceanography* **27:** 849-855.

Kirkman, H. and Kendrick, G.A. (1997). Ecological significance and commercial harvesting of drifting and beach-cast macro-algae and seagrassses in Australia: a review. *Journal of Applied Phycology* **9 (4):** 311-326.

Klenell, M., Snoeijs, P. and Pedersen, M. (2002). The involvement of plasma membranes H$^+$–ATPase in the blue-light enhancement of photosynthesis in *Laminaria digitata* (Phaeophyta). *Journal of Phycology* **38:** 1143-1149.

Klenell, M., Snoeijs, P. and Pedersen, M. (2004). Active carbon uptake in *Laminaria digitata* and *Laminaria saccharina* (Phaeophyta) is driven by a proton pump in the plasma membrane. Hydrobiologia 514 (1-3): 41-53.

Koch, E.W. (1994) Hydrodynamics, diffusion-boundary layers and photosynthesis of the seagrasses *Thalassia testudinum* and *Cymodocea nodosa*. *Marine Biology* **118:** 767-776.

Koch, E. W. and Gust, G. (1999). Water flow in tide and wave dominated beds of the seagrass Thalassia testudinum. Marine Ecology Progress Series 184: 63.72.

Koch, E.W. (2001). Beyond light: physical, geological, and geochemical parameters as possible submersed aquatic vegetation habitat requirements. *Estuaries* **24:** 1-17.

Koch, E.W., Ackerman, J. D., Verduin, J. and van Keulen, M. (2005). Fluid dynamics in seagrass ecology – from molecules to ecosystems in Larkum, A. W. D., Orth, R. J. and Duarte, C. M. (eds) *Seagrasses: Biology, Ecology and Conservation.* Springer, pp 193-225.

Koehl, M. and Alberte, R. (1988). Flow, flapping, and photosynthesis of Nerocystis luetkeana: a functional comparison of undulate and flat blade morphologies. *Marine Biology* **99:** 435-444.

Kűbler, J.E. and I.R. Davison 1993 High-temperature tolerance of photosynthesis in the red alga Chondrus crispus. *Marine Biology* **117:** 327-335.

Kuehl, M., Glud, R., Borum, J., Roberts, R. and Rysgaard, S. (2001). Photosynthetic performance of surface-associated algae below sea ice as measured with a pulse amplitude-modulated (PAM) Fluorometer and O_2 microsensors. *Marine Ecology Progress Series* **223:** 1-14.

Kyung-sil, C., Snoeijs, P. and Pedersén, M. (2002). Uptake of inorganic carbon by *Cladophora glomerata* (Chlorophyta) from the Baltic Sea. *Journal of Phycology* **38:** 493-502.

La Loggia, G., Calvo, S., Ciraolo, G., Mazzola, A., Pirrotta, M., Sarå, G., Tomasello and Vizzini, S. (2004). Influence of hydrodynamic conditions on the production and fate of Posidonia oceanica in a semi-enclosed shallow basin. *Chemistry and Ecology* **20 (3):** 183-201.

Larkum, A. (1976). Ecology of Botany Bay Part 1: Growth of *Posidonia australis* in botany bay and other bays of the Sydney Basin, Australia. *Australian Journal of Marine and Freshwater Research* **27:** 117-127.

Larkum A. W. D., Roberts, G., Kuo, J. and Strother, S. (1989). Gaseous movement in seagrasses. In: Larkum, A. W. D., McComb, A. J., Shepherd, S. A. (eds) **Biology of Seagrasses** – a treatise on the biologyyof seagrasses with special references to the Australian region, Elsevier, New York. pp. 691-715.

Larsson, C., Axelsson, L., Ryberg, H. and Beer, S. (1997). Photosynthetic carbon utilization by *Enteromorpha intestinalis*) (Chlorophyta) from a Swedish rockpool. *European Journal of Phycology* **32:** 49-54.

Lavavasseur, G. Edwards, G.E. and Osmond, C.B. (1991). Inorganic carbon limitation of photosynthesis in *Ulva rotundata* (Chlorophyta). *Journal of Phycology* **27 (6):** 667-672.

Lee, K. S., and Dunton, K. H. (1999). Influence of sediment nitrogen-availability on carbon and nitrogen dynamics in the seagrass *Thalassia testudinum*. *Marine Biology* **134 (2):** 217-226.

Lee, K. S. and Dunton, K.H. (1999). Inorganic nitrogen acquisition in the seagrass *Thallasia testudinum*: development of a whole plant nitrogen budget. *Limnology and Oceanography* **44 (5):** 1204-1215

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

Lesser, M., Weis, V., Patterson, M. and Jokiel, P. (1994). Effects of morphology and water motion on carbon delivery and productivity in the reef coral, *Pocillopora*

damicornis (Linnaeus): diffusion barriers, inorganic carbon limitation, and biochemical plasticity. ***Journal of Experimental Marine Biology and Ecology* 178:** 153-179.

Lippsett, L. (2005). Beyond warming the planet, buildup of CO_2 threatens the shells of ocean organisms. ***Oceanus* 44:** 7.

Luning, K and Pang, S.J. (2003). Mass cultivation of seaweeds: current aspects and approaches. ***Journal of Applied Phycology* 15 (2-3):** 115-119.

Mach, K. J., Hale, B. B. Denny, M. W. and Nelson, D. V. (2007). Death by small forces: a fracture and fatigue analysis of wave-swept macroalgae. ***Journal of Experimental Biology* 210 (13):** 2231-2243.

Machalek, K. M., Davison, I. R. and Falkowski, P. G. (1996). Thermal acclimation and photoacclimation of photosynthesis in the brown alga *Laminaria saccharina*. ***Plant Cell and Environment* 19 (9):** 1005 – 1016.

Madsen, T.V. and Sand Jensen, K. (1991). Photosynthetic carbon assimilation in aquatic macrophytes. ***Aquatic Botany* 41 (1-3)**: 5-40.

Madsen, T. V., Maberly, S. C. and Bowes, G. (1996). Photosynthetic acclimation of submersed angiosperms to CO_2 and HCO_3^-. ***Aquatic Botany* 53:** 15-30.

Marba, N., Gallegos, M.E., Merino, M. and Duarte, C. M. (1994). Vertical Growth of *Thalassia testudinum* seasonal and interanual variability. ***Aquatic Botany* 47 (1):** 1-11.

Mathieson, A., Norton, T., and Neushul, M. (1981). The taxonomic implications of genetic and environmentally induced variations in seaweed morphology. ***Botanical Review* 47:** 313-47.

McRoy, C.P. and Goering, J.J. (1974). Nutrient transfer between the seagrass *Zostera marina* and its epiphytes. ***Nature* 248**: 173-174.

Mercado, J. M., Figueroa, F, L, Xavier Niell, F. and Axelsson L. (1997). A new method for estimating external carbonic anhydrase activity in macroalgae. ***Journal of Phycology* 33:** 999-1006.

Mercado, J. M., Niell, F. X., Silva, J. and Santos, R. (2003). Use of light and inorganic carbon acquisition by two morphotypes of *Zostera noltii* Hornem. ***Journal of Experimental Marine Biology and Ecology* 297 (1):** 71-84.

Mendelssohn, I. A. and Seneca, E.D. (1980). The influence of soil drainage on the growth of salt march cord grass *Spartina alterniflora* in North Carolina. ***Estuarine Coastal Marine Science* 11:** 27-40.

Miller, A.G. (1985) in **Carbon Uptake by Aquatic Photosynthetic Organisms** by the American Society of Plant Physiologists pp 17-29.

Morris, J. T. (1980). The nitrogen uptake kinetics of *Spartina alterniflora* in culture. *Ecology* **61:** 1114-1121.

Nejrup, L. B. and Pedersen, M. R. (2008). Effects of salinity and water temperature on the ecological performance of *Zostera marina*. *Aquatic Botany* **88 (3):** 17-29.

Norton, T., Mathieson, A. and Neushul, M. (1982). A review of some aspects of form and function in seaweeds. *Botanica Marina* **25:** 501-510.

Olesen, B. and Madsen, T.V. (2000). Growth and physiological acclimation to temperature and inorganic carbon availability by two submerged aquatic macrophyte species, *Callitriche cophocarpa* and *Elodea canadensis*. *Functional Ecology* **14:** 252-260.

Ostenfeld, C. H. (1908). On the ecology and distribution of the grass-wrack (Zostera marina) in Danish waters. *Rep. Dan. Biol. Stn.* **16:** 1-62.

Penhale, P. A. and Thayer, G. W. (1980). Uptake and transfer of carbon and phosphorus by eelgrass (*Zostera marina* L.) and its epiphytes. *Journal of Experimental Marine Biology and Ecology*. **42 (2):** 113-123.

Peralta, G., Brun, F. G., Hernåndez, Vergara, J. J. and Pérez-Lloréns (2005). Morphometric variations as acclimation mechanisms in *Zostera noltii* beds. *Estuarine, Coastal and Shelf Science* **64:** 347-356.

Porter, E. T., Sandford, L. P. and Suttles, S. E. (2000). Gypsum dissolution is not a universal indicator of 'water motion'. *Limnology and Oceanography* **45 (1):** 145-158.

Provasoli, L. (1968). Media and prospects for cultivation of marine algae. *In* Watanabe, A and Hattori, A. [Eds.] **Cultures and Collections of Algae** Japanese Society of Plant Physiology pp 47-74.

Ralph, P.J., Gademann, R. and Dennison, W. C. (1998). *In situ* seagrass photosynthesis measured using a submersible, pulse-amplitude modulated fluorometer. *Marine Biology*. **132 (3):** 367-373.

Ralph, P.J., Polk, S. M., Moore, K. A., Orth, R. J. and Smith, W.O. (2002). Operation of the xanthophyll cycle in the seagrass *Zostera marina* in response to variable irradiance. *Journal of Experimental Marine Biology and Ecology*. **271 (2):** 189-207.

Raven, J. A., Osborne, B. A. and Johnston, A. M. (1985). Uptake of CO_2 by aquatic vegetation. *Plant, Cell and Environment* **8:** 417-425.

Raven, J.A. and Osmond, C.B. (1992). Inorganic C acquisition processes and their ecological significance in inter- and sub-tidal macroalgae of North Carolina. *Functional Ecology* **6:** 41-47.

Raven, J. A. (1999). Photosynthesis in the intertidal zone: Algae get an airing. *Journal of Phycology*. **35 (6):** 1102-1105.

Roberson, L. M. and Coyer, J. A. (2004). Variation in blade morphology of the kelp *Eisenia arborea*: incipient speciation due to local water motion? *Marine Ecology Progress Series* **282:** 115-128.

Rodrigues, M. A. and Pereira dos Santos, C. 2002. A smaller and impaired xanthophyll cycle makes the deep sea macroalgae *Laminaria abyssalis* (Phaeophyceae) highly sensitive to daylight when compared with shallow water *Laminaria digitata. Journal of Phycology* **39**: 939-947.

Ryther, J. H. and Dunston, W. M. (1971). Nitrogen, phosphorus and eutrification in the coastal marine environment. *Science, New Series*. **171 (3975):** 1008-1013.

Sabine C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K, Bullister, J. L. Wanninkhof, R., Wong, C. S., Wallace D. W. R., Tilbrook, B., Millero, F. J., Peng, T. H., Kozyr, A., Ono, T. and Rios, A. F. (2004). The oceanic sink for anthropogenic CO_2. *Science* **305 (5682):** 367-371.

Sand-Jensen, K. (1977). Effects of epiphytes on eelgrass photosynthesis. *Aquatic Botany* **3:** 55-63.

Santamaŕia, L. and van Viersson, W. (1997). Photosynthetic temperature responses of fresh and brackish water macrophytes: A review. *Aquatic Botany* **58:** 135-150.

Schulz, K. G., Riebesell, U., Rost, B., Thoms, S. and Zeebe, R. E. (2006). Determination of the rate constants for the carbon dioxide to bicarbonate inter-conversion in pH-buffered seawater systems. *Marine Chemistry* **100**: 53-65.

Schwarz, A., Bjork, M., Buluda, T., Mtolera, M., and Beer, S. (2000). Photosynthetic utilization of carbon and light by two tropical seagrass species as measured *in situ. Marine Biology* **137:** 755-761

Seely, G. R., Vidaver, W. E. and Duncan, M. J. (1972). Preparative and analytical extraction of pigments from brown algae with dimethy sulfoxide. *Marine Biology* **12 (2):** 184.

Short, F. T., Davis, M. W., Gibson, R. A. and Zimmermann, C. F. (1985). Evidence for phosphorus limitation in carbonate sediments of the seagrass *Syringodium filiforme*. *Estuarine, Coastal and Shelf Science* *20:* 419-430

Short, F.T. (1987). Effects of sediment nutrients on seagrasses - literature review and mesocosm experiment. *Aquatic Botany* **27:** 41-58.

Short, F. T. and Neckles, H. A. (1999). The effects of global climate change on seagrasses. *Aquatic Botany* **63:** 169-196.

Short, F. T. and Duarte, C. M. (2001). Methods for the measurement of seagrass growth and production In: Short, F. T and Coles, R.G. (eds) *Global Seagrass Research Methods.* Elsevier Science, pp 156-158.

Stapel, J., Aarts, T. L., van Duynhoven, B. H. M., de Groot, J. D., van den Hoogen, P. H. W. and Hemminga, M. A. (1996). Nutrient uptake by leaves and roots of the seagrass *Thalassia hemprichii* in the Spermonde archipelago, Indonesia. *Marine Ecology Progress Series* **134**: 195-206.

Sundene, O. (1964). The ecology of *Laminaria digitata* in Norway in view of transplant experiments. *Nytt Mag. Bot.* **11:** 83-107.

Tanka, Y. and Nakaoka, M. (2007). Interspecific variation in photosynthesis and respiration balance of three seagrasses in relation to light availability. *Marine Ecology Progress Series*. **350**: 63-70.

Terrados, J. and Duarte, C. M. (2000). Experimental evidence of reduced particle resuspension within a seagrass (*Posidonia oceanica* L.) meadow. *Journal of Experimental Marine Biology and Ecology* **243 (1):** 45-53.

Thom, R. M. (1995). CO_2 enrichment effects on eelgrass and bull kelp. *Water Air Soil Pollution* **82**:1-9

Tomascik, T. (1997). **The ecology of the Indonesian seas.** Periplus Editions, Hong Kong. pp. 1388.

Tubbs, C.R. (1995). The Meadows in the Sea. *British Wildlife* **6(6):** 351-355.

Uka, J., Beer, S. and Björk, M. (2005). Buffer sensitivity of photosynthetic carbon utilization in eight tropical seagrasses. Thesis, Stockholm University.

Urquhart, J. T. 1984). On the preparation of the *Zostera* or seagrass in Orkney. Prize essays and transactions of the Highland Society of Scotland. 6: 590.

Van Heukelem, L. and Thomas, C. S. (2001). Computer-assisted high-performance liquid chromatography method development with applications to the isolation and analysis of phytoplankton pigments. Jouranl of Chromatography A. 910 (1): 31-49.

Van Katwijk, M. M., Vegeer, L. H. T., Schmitz, G. H. W., Roelofs, J. G. M. (1997). Ammonium toxicity in eelgrass *Zostera marina*. ***Marine Ecology Progress Series*** **157:** 159-173.

Van Tussenbroek, B. I. (1996). Leaf dimensions of transplants of *Thalassia testudinum* in a Mexican Caribbean reef lagoon. ***Aquatic Botany*** **55:** 133-138.

Viera, M. P., Pinchetti, J. L. G., de Vicose G. C., Bilbao, A. Suarez, S., Haroun R. J. And Izquierdo, M. S. (2005). Suitability of three red macroalgae as a feed for the abalone *Haliotis tuberculata coccinea* Reeve. ***Aquaculture*** **248 (1-4):** 75-82.

Ward, L. G., Kemp, W. M. and Boyton, W. R. (1984). The influence of waves and seagrass communities on suspended particulates in an estuarine embayment. ***Marine Geology*** **59:** 85-1-3.

Webster, I. T., Teasdale, P. R. and Grigg, N. J. (1998). Theoretical and experimental analysis of peeper equilibrium dynamics. ***Environmental Science and Technology*. 32 (11):** 1727-1733.

Wheeler, W. (1980). Effect of boundary layer transport on the fixation of carbon by the giant kelp *Macrocystis pyrifera*. ***Marine Biology*** **56:** 103-110.

Wheeler, W. (1982). Nitrogen nutrition of *Macrocystis*. In Srivastava, L. M. (Ed.) **Synthetic and Degradative Processes in Marine Macrophytes**. Walter de Gruyter, Berlin, pp. 121-137.

Wicks, E. C. (2005) Thesis: The effect of sea level rise on seagrasses: Is sediment adjacent to retreating marshes suitable for seagrass growth? University of Maryland, Horn Point Laboratory.

Williams, S. L. and Herbert, S. K. (1989). Transient photosynthetic responses of nitrogen-deprived *Petalonia fascia* and *Laminaria saccharina* (Phaeophyta) to ammonium resupply. ***Journal of Phycology*** **25 (3):** 515-522.

Wium-Anderson, S. (1971). Photosynthetic uptake of free CO_2 by roots of *Lobelia dortmanna*. ***Physiologia Plantarum*** **25:** 245.

Wyllier-Echeverria, S. and Cox, P. A. (1999). The seagrass (Zostera marina [Zosteraceae]) industry of Nova Scotia (1907-1960). ***Economic Botany*** **53 (4):** 419-426.

Zimmerman, R. C., Kohrs, D.G., Steller, D. L. and Alberte, R. S. (1997). Impacts of CO_2 enrichment on productivity and light requirements of eelgrass. ***Plant Physiology*** **115 (2):** 599-607.