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Survival strategies of eelgrass in reduced light

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SURVIVAL STRATEGIES OF EELGRASS IN REDUCED LIGHT

BY

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DISSERTATION

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DEDICATION

This dissertation is dedicated to my grandmother, Lea Okello Omullo, also known as “Mama ma Nyakach”, whose faith has influenced my life.

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ABSTRACT

SURVIVAL STRATEGIES OF EELGRASS IN REDUCED LIGHT

by

Caroline A. Ochieng

University of New Hampshire, September, 2008

Light reduction due to anthropogenic impacts is the most widespread cause of worldwide decline of eelgrass, an ecologically important marine angiosperm whose role in supporting overall coastal ecosystem productivity has been widely recognized. Understanding eelgrass plant and meadow responses to light reduction has therefore received significant research interest over the last 30 years, while managers have sought tools to identify critical thresholds for light availability and predict impacts of human-induced disturbances in order to prevent further eelgrass loss. In the present thesis, a review of some of the literature on light reduction and its effects on eelgrass (i) summarized the importance of light as a requirement for eelgrass growth, (ii) highlighted factors that reduce light availability to eelgrass, (iii) summarized eelgrass responses to light reduction to understand its sensitivity to reduced water clarity, and (iv) documented worldwide losses of eelgrass caused by light reduction (Chapter II). An outdoor mesocosm experiment explained eelgrass response to a gradient of light conditions, improving the understanding of the relationship between light availability, growth and survival (Chapter III). The study concluded that at temperatures between 18°C and 23°C, eelgrass plants can thrive at light levels of 58% surface irradiance (SI) and above, and are light-limited at 34% SI and below, but that the minimum light required for long-term eelgrass growth and survival is greater than 11% SI. Finally, a field study at the maximum depth limit of eelgrass colonization was carried out to understand the mechanisms of eelgrass plant adaptation to extreme light reduction (Chapter IV). The results showed that deep edge eelgrass plants were chronically light-limited and that the plants exhibited a greater degree of morphological acclimations to further light reduction in winter than plants at shallower depths. Winter survival of deep edge plants depended largely on wintertime photosynthesis with mobilization of stored reserves playing only a minor role. The thesis concludes with a synthesis (Chapter V) linking all chapters.

CHAPTER 1

GENERAL INTRODUCTION

Seagrasses are marine flowering plants (angiosperms) found in all of the world's coastal waters except Antarctica, extending from the intertidal down to 70 m depth (Green and Short, 2003). Seagrasses have unique physiological, morphological and ecological characteristics that allow them to exist completely submersed in the marine environment. The total number of seagrass species in the world has been variously reported as 59 (Green and Short, 2003), 66 (den Hartog and Kuo, 2006), and 60 (Short et al., 2007). The exact nomenclature is currently under taxonomic review, based on additional information generated by new genetic techniques.

Seagrass plants form extensive monospecific or multispecific meadows, which together with associated species such as macroalgae and epiphytic algae, form highly productive ecosystems that support complex food webs. The canopy structure of these plants provides breeding grounds and nurseries for important shellfish and finfish populations (Heck et al., 1997; Nagelkerken et al., 2000; Beck et al., 2001; Heck et al., 2003), while some herbivores such as dugong, manatee, sea turtles and waterfowl have seagrass as their critical food source (Heck and Valentine, 2006). Seagrass canopies also trap suspended solids and reduce wave impacts (Ward et al., 1984; Fonseca and Calahan, 1992), while the below-ground structures bind sediment, stabilizing shorelines and thereby reducing erosion (Fonseca, 1989; Hemminga and

Nieuwenhuize, 1990). The role of seagrass in filtering nutrients as well as heavy metals, that in turn helps cleaning the water column has been acknowledged (Short and Short, 1984; Hoven et al., 1999). Seagrass plants recycle nutrients and oxygenate surrounding waters through photosynthetic oxygen production and, in this way, improve water quality and support overall ecosystem productivity (Short and Wyllie-Echeverria, 1996; Constanza et al., 1997).

Seagrass communities are dynamic systems that are shaped by competition, resource availability, physical disturbance, species-specific stress tolerance levels and life-history strategies. Despite various scales of disturbance affecting seagrass meadows, the plants exhibit the capacity for acclimation and plasticity, which allow them to continue to exist or recover from disturbances. However, when disturbance levels are extreme, chronic or irreversible, the plants' coping mechanisms may be insufficient, leading to seagrass decline or loss (Hemminga and Duarte, 2000).

Seagrass plants support an extensive network of below-ground non-photosynthetic tissues (roots and rhizomes) that are often embedded in highly reduced sediments. During periods of light, photosynthesis releases oxygen which can diffuse to the roots and rhizomes, oxidizing the rhizosphere (the zone surrounding the roots) and preventing root mortality (Smith et al., 1984; Hemminga, 1998). Because of the plants' reliance on photosynthetic oxygen production to meet the respiratory demand of these below-ground tissues, seagrasses require relatively high amounts of light (11 - 30% of surface irradiance compared with, for example, 1% SI for phytoplankton). Such high light requirements imply that seagrasses are sensitive to environmental disturbances, that impact water clarity and thereby reduce light available to these bottom-dwelling plants (Lee et al., 2007).

Seagrass meadows are affected by disturbances of varying scales. Such disturbances include partial and total herbivory (Tubbs and Tubbs, 1983; Madsen, 1988; Jacobs et al., 1981), ice scouring (Robertson and Mann, 1984), bioturbation from animal burrows (Stapel and Erftemeijer, 2000), boat anchoring and propeller scaring (Creed and Amado Filho, 1999), dredge and fill operations (Onuf, 1994), shade from boat docks (Burdick and Short, 1999), floods (Preen et al., 1995), storms (Cabello-Pasini et al., 2002), disease (Muehlstein et al., 1991) and fishing operations (Neckles et al., 2005). Die-back of some seagrass meadows can be explained by extreme environmental conditions associated with hot El-Nino summers (Johnson et al., 2003) and sulphide stress (Koch and Erskine, 2001). There is growing evidence that seagrass meadows are presently experiencing worldwide decline, mainly because of anthropogenic disturbance (Short and Wyllie-Echeverria, 1996; Hemminga and Duarte, 2000; Duarte, 2002; Green and Short, 2003; Orth et al., 2006). Yet the most ubiquitous and pervasive cause of seagrass decline is the reduction of light availability caused particularly by increased sediment loading and nutrient runoff as a result of human activities in the adjacent watersheds, and by activities disturbing bottom sediments such as boating, land reclamation, dredging and some fishing methods (Walker and McComb, 1992; Cambridge and McComb, 1984; Short and Wyllie-Echeverria, 1996; Hauxwell et al., 2003; Orth et al., 2006).

The seagrass *Zostera marina* L. (eelgrass) is mainly distributed in temperate coastal estuarine environments often bordering heavily industrialized and developed areas of the northern hemisphere (Short and Burdick, 1996; Moore and Short, 2006). Its distribution range on the Western Atlantic coast extends from Canada to North Carolina, while on the Eastern Atlantic coast eelgrass extends from Norway to the

Mediterranean (Green and Short, 2003). In the Pacific, eelgrass stretches from the Alaskan coast to the Sea of Cortez and Baja Peninsula to as far south as Japan on the Western Pacific coast (Green and Short, 2003). Eelgrass grows from the intertidal zone down to depths of as much as 12 m below mean sea level (Green and Short, 2003). The general morphology of a typical eelgrass plant is presented in Figure 1.1.

Eelgrass is one of the most studied species of seagrass to date, with the earliest reported research on anatomy and taxonomy going back to more than a century ago (Hofmeister, 1861). Ecological studies on eelgrass started at the beginning of the twentieth century (Ostenfield, 1908), when the ecological importance of eelgrass was first put forward by Petersen and Boysen-Jensen (1911). In the 1930s, eelgrass populations along the Atlantic Coast of North America and Europe were dramatically reduced by "wasting disease" caused by the infection of a marine slime mold-like protist, *Labyrinthula zosterae* Porter and Muehlstein (Muehlstein, 1989; Muehlstein, et. al., 1991). Following the epidemic, eelgrass recovered in most locations of North America and parts of Europe, but not in the Dutch Wadden Sea. It is also one of the first seagrass species for which the relationship between depth distribution and underwater light attenuation was modeled (Verhagen and Nienhuis, 1983; Nielsen et al., 1989; Duarte, 1991). Recent research and monitoring has shown that over vast ranges of its distribution, eelgrass is showing decline (Orth et al., 2006; Short et al., 2006).

In this thesis, it is hypothesized that because eelgrass occurs under a wide distribution range spanning temperate areas with differing light regimes, the plant has considerable acclimative ability and plasticity, which enables it to tolerate and survive extreme light reduction.

The bulk of the work presented in the thesis is based on field research in the Great Bay Estuary, and mesocosm and laboratory work at the Jackson Estuarine Laboratory, University of New Hampshire, USA, carried out between 2003 and 2005.

Chapter II of this thesis presents the outcome of a literature review on eelgrass and light reduction. The chapter evaluates light as a requirement for eelgrass survival, natural and anthropogenic factors that reduce light availability, and the response and sensitivity of eelgrass to reduction in light availability.

In Chapter III, the results of an outdoor mesocosm experiment are described, in which eelgrass seedlings and mature plants were subjected to various levels of shading in order to understand the relationship between light availability, growth and survival. By monitoring the photosynthetic activity of eelgrass plants over time during the summer growth season, and assessing the morphology of individual seed-generated eelgrass plants at the end of the experiment, the study was able to explain eelgrass plant response to a gradient of light conditions: at what light level does eelgrass become light limited, and after how long does eelgrass respond significantly to the effect of shading?

Chapter IV of the present thesis seeks to understand the mechanisms of eelgrass plant adaptation to extreme light reduction during the winter. The aim of the study was achieved by quarterly evaluating the photosynthetic and morphological plant parameters and carbohydrate storage in eelgrass plants growing (i) at the maximum depth limit (the "deep edge"), where eelgrass plants are believed to be growing at or near their minimum light requirements and small changes in light availability can result in large changes in growth and photosynthesis, and (ii) along a depth gradient in Great Bay Estuary. In addition, the study tries to answer the question: what is the relative contribution of carbohydrate storage and wintertime photosynthesis to allow winter survival?

Finally, in Chapter V, the results of the different studies are integrated and summarized in light of the general ecology of seagrasses and resource management. By providing a better understanding of some of the mechanisms of eelgrass survival under light reduction, the thesis gives insight into the potential consequences of increased light attenuation in coastal waters. The results thus contribute significantly to a better understanding of the response of seagrass ecosystems to a changing environment, and the information generated can be used to refine critical thresholds for light limitation, and predict short-term human perturbations, thereby helping in efforts to manage water quality.

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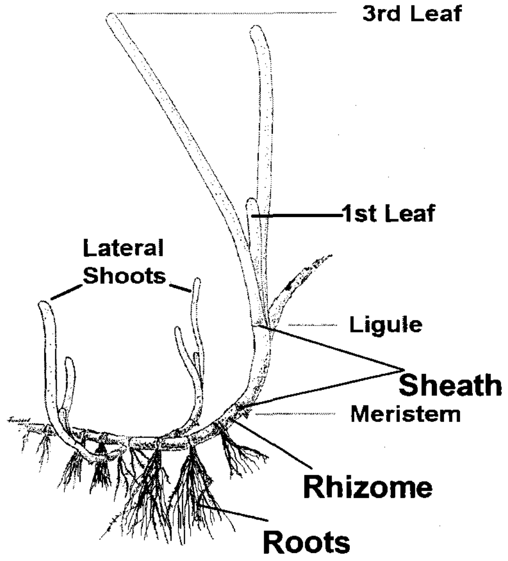

Figure 1.1. Morphology of *Zostera marina* L. (eelgrass). Drawing modified from Gaeckle (2006).

CHAPTER II

REVIEW:

LIGHT REDUCTION AND ITS EFFECTS ON EELGRASS

Light is widely regarded as one of the most important environmental factors controlling the distribution and abundance of the temperate seagrass species, *Zostera marina* L. (eelgrass). In recent years, substantial scientific research on eelgrass has focused on the role of light availability on growth and survival of this ecologically important marine angiosperm, ranging from molecular/physiological studies of eelgrass photosynthesis and studies of eelgrass growth responses to experimental shading, to predictive modelling of eelgrass depth limits as a function of water transparency in order to provide guidance for water quality management. The present chapter reviews some of the literature on light reduction and its effects on eelgrass and is organized into four sections. The first section covers the importance of light as a requirement and limiting factor for eelgrass growth. Factors that reduce light availability to eelgrass are addressed in the second section. The third section reviews eelgrass responses to light reduction to understand its sensitivity to reduced water clarity, and documents worldwide losses of eelgrass caused by light reduction. The fourth and final section draws some general lessons and conclusions from the review. The present review focuses attention on light reduction, a factor that is implicated in widespread eelgrass decline. Other aspects of light (e.g. photoinhibition, light quality and effects of UV) or other environmental factors that may limit eelgrass growth and production such as nutrients, temperature, etc., are not addressed by this review, but reference is made to Hemminga and Duarte (2000), Larkum et al. (2006), and Lee et al. (2007) for further recent information on these topics.

LIGHT AS A REQUIREMENT AND A LIMITING FACTOR FOR EELGRASS GROWTH

Light and eelgrass - an introduction

Seagrasses require light for photosynthesis, which provides chemically fixed energy and carbon skeletons for metabolic processes that permit growth. Light availability (i.e., the quantity of photosynthetically active radiation (PAR); 400-700 nm) is the most important factor limiting the survival, growth and depth distribution of the temperate seagrass species *Zostera marina* L. (eelgrass) (Ostenfield, 1908; Olesen, 1996). Light availability to eelgrass is generally described as irradiance[1] (I), which can be measured directly as photosynthetically active radiation (PAR, in μmol photons $m^{-2}\ d^{-1}$). Light within an eelgrass meadow can also be expressed as daily light periods in hours (e.g. H_{sat}), or as a relative measure (e.g. in % of surface irradiance). Light in the sea is reduced with increasing depth, ultimately to zero. Several studies have shown that light limitation affects the maximum depth distribution of eelgrass. For example, eelgrass transplants that were planted below the deep edge of a natural eelgrass meadow in Great Harbor (Massachusetts, USA) died, and eelgrass seedlings that newly established themselves at these depths naturally during summer did not persist through winter (Dennison and Alberte, 1986). In San Francisco Bay estuary,

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[1] For consistency, irradiance (the flux of radiant energy on a defined surface area in mol photons $m^{-2}\ s^{-1}$) is denoted by the symbol I instead of E (Falkowsky and Raven, 1997) throughout this thesis.

eelgrass transplant survival was found to be strongly depth-dependent (Zimmerman et al., 1995a). In turbid areas, colonization of eelgrass is often limited to very shallow depths (Zimmerman et al., 1995a; Cabello-Pasini et al., 2003). In the Wadden Sea, reduced water clarity has markedly reduced the potential vertical distribution range of eelgrass over the last century (Ostenfield, 1908; Giesen et al., 1990; Bostrom et al., 2003). But what is the depth limit to which eelgrass can grow? How much light is still enough for eelgrass plants to persist?

The minimum light requirement concept

The minimum light requirement (MLR) is defined as the light level necessary for eelgrass to maintain basic metabolism and below which plants cannot sustain growth. It has been considered as the primary habitat requirement of eelgrass (Kemp et al., 2004). The ability of eelgrass plants to cope with light reduction depends on their minimum light requirements for survival and growth (Dennison et al., 1993). Minimum light requirements have been expressed in a number of ways, the most common of which is percent of surface irradiance. Reported estimates of MLR for eelgrass range between 11- 30% (Table 2.1), a much higher light level than that of the photic zone of many species of phytoplankton and algae (1 – 5%) (Kenworthy and Fonseca, 1996). The MLR for seagrasses is higher because, unlike algae and phytoplankton, seagrass plants must maintain the metabolism of, and support the net productivity of, non-photosynthetic tissues (roots and rhizomes) growing in anoxic sediments. Metabolism of below-ground parts of eelgrass alone can generate a daily carbon demand equivalent to 1 - 2 hours of irradiance saturated photosynthesis (Zimmerman, 2006). Eelgrass plants also have some above-ground non-photosynthetic tissues and cell structures (e.g. parts of leaf sheaths, parts of reproductive organs, aerenchyma and vascular tissues) that through the additional carbon demand further contribute to the differences in light requirements between eelgrass and algae.

From the results of a light manipulation experiment, Bintz and Nixon (2001) suggested that rapid expansion of seedling patches could only occur at irradiance levels greater than 7.9 mol photons m^{-2} d^{-1}. Gatusso et al. (2006) concluded, based on a review of 45 primary research papers on the topic, that eelgrass requires between 1.2 and 12.6 mol photons m^{-2} d^{-1} for survival, with a mean of 6.0 mol photons m^{-2} d^{-1} (corresponding to an estimated 69 μmol photons m^{-2} s^{-1}, which falls within the range of values reported in literature for the minimum irradiance for photosynthetic saturation I_k; see Table 2.2). At the deepest end of the depth distribution of eelgrass in Woods Hole, Massachusetts, plants received between 1.1 and 13 mol photons m^{-2} d^{-1} (Dennison, 1987). Light stress experiments showed that eelgrass mortality occurred when irradiance fell below 3 mol photons m^{-2} d^{-1} for approximately one week. Long-term *in situ* growth measurements revealed major reductions in leaf growth at light levels of 4 – 5 mol photons m^{-2} d^{-1} (Shafer, 1999). These data reveal a wide range of values (1 – 13 mol photons m^{-2} d^{-1}) for the daily amount of PAR required by eelgrass. The underlying cause of such variations is not well understood. I conclude that there seems to be agreement, however, among the various studies that the minimum daily irradiance required by eelgrass is at least above 1 mol photons m^{-2} d^{-1}.

Approaches to investigating minimum light requirements

There are three different approaches that address the minimum light requirements of eelgrass: depth limits model for survival, light compensation point model for plant metabolism and hours of saturating light (H_{sat}) model for photosynthesis. Each of theses approaches, along with their limitations, will be described below.

Depth limits model for plant survival

It is generally understood that the light conditions at the maximum depth at which eelgrass occurs are indicative of the minimum light requirements (MLR) for eelgrass. In this approach, the depth limits model describes the *in situ* minimum light requirements. An *in situ* MLR can be calculated on the basis of an annual percentage of surface irradiance reaching the eelgrass canopy at the maximum depth limit of eelgrass growth. The amount of light that reaches the sediment surface is dependent on the transparency of the water, which in turn depends on the amount of suspended organic matter (including phytoplankton) and sediment particles in the water column as well as the color of the water itself. The vertical light penetration through the water column is generally described using the so-called light attenuation coefficient (K_d), which is an index of the rate of light loss with increasing depth. An average annual K_d can be used to calculate the percentage of surface irradiance reaching the maximum 0depth of eelgrass growth (Z_{max}), using the Beer-Lambert equation:

$$I_{Zmax} = Ie^{-Kd\ Zmax}$$

where I = irradiance at the surface, I_z = irradiance at depth z in m, K_d = attenuation coefficient (m^{-1}), and z_{max} = maximum depth of eelgrass growth in m.

Since water transparency differs from place to place, the maximum depth limit of eelgrass distribution differs accordingly, even when the minimum light requirement may be the same. Values reported in the literature for the minimum light required for eelgrass survival and growth, however, vary considerably (11 to 30% of surface irradiance) (Table 2.1). The wide range of values reported in the literature for MLR has been attributed in part to differences in methodologies used to arrive at the values (Erftemeijer and Lewis, 2006), which included field, mesocosm and model analyses of light levels. Phenotypic and genotypic differences, on the other hand, might have arisen in different populations, especially since light is a strong limiting factor, which might also have contributed to this wide range of MLR values.

Recent efforts to manage and protect seagrass resources have focused on the development of easily monitored water column criteria (e.g. light attenuation), which can provide resource managers with habitat quality parameters that predict tolerance limits and ensure the survival of submerged aquatic vegetation (Kenworthy and Haunert, 1991; Batiuk et al., 2000). Based on light requirement studies in Chesapeake Bay, this approach has been used in the development of water quality models from which water clarity standards have been established (Dennison, 1987; Dennison et al., 1993; Batiuk et al., 2000). In restoration projects, the MLR can be instrumental in establishing a potential depth goal whereby the difference between the amount of light reaching the target depth and the minimum light requirement of the species denotes the level of water clarity improvement needed to meet the target.

The advantage of the depth limits approach is that it is "practical" because it makes direct correlations between surface irradiance, water column light attenuation (K_d), and the maximum depth of seagrass colonization (Z_{max}). Managers can then make predictions about the changes in eelgrass distribution with depth based on measurements of attenuation coefficient (K_d).

Limitations of the depth limits model

The depth limits approach only works in situations where light is the limiting factor. The percentage of light reaching the surface of an eelgrass leaf can be reduced further due to epiphytic load. The bulk of scientific studies used to derive water column light targets do not incorporate the shading effects of epiphytes. However, epiphyte cover on eelgrass leaves can be considerable, especially in areas under significant eutrophication, and the amount of light attenuation by epiphytes in such areas can be as much as 36% to 60% (Borum, 1985; Dixon, 2000; Drake et al., 2003). Computations of MLR in which the amount of light that actually reaches the leaf is accounted for (Kemp et al., 2004) are rare and therefore current estimations of MLR for eelgrass may be overestimating the actual values in areas where epiphyte growth (or the settlement of solids) on leaves are significant.

There is a general difficulty of obtaining long-term, continuous K_d measurements, making realistic determination of MLR using the depth limits approach problematic. Eelgrass survival and depth distribution often appears to be determined by short periods of extreme light attenuation instead of the mean light condition (Zimmerman et al., 1991; Moore et al., 1997). Such short periods of extreme light attenuation can be especially found in wind-exposed areas, where rapid and frequent changes in suspended solid concentrations in the water column can reduce light availability to near zero (Banas et al., 2005). In such areas, simple correlation of colonization depth with mean values of K_d taken at low frequencies tends to be an oversimplification of a complicated subject because periodic episodes of intense light attenuation that characterize these areas are often missed.

Furthermore, daily tidal fluctuations, especially in areas with large tidal variations, may make the approach less applicable because there is no easy way to adjust K_d for variations in tidal range. Calculations of light attenuation in the water column (K_d) with depth that do not account for tidal amplitude can easily under- or over-estimate the maximum depth to which eelgrass can grow, depending on the timing of the K_d measurements within the tidal cycle, thereby over- or under-estimating the minimum light requirements (Koch, 2001). In addition, K_d tends to vary over the tidal cycle due to resuspension effects of tidal currents. The use of different reference depths (sub surface irradiance vs. incident irradiance above the surface) can also be a source of disparity between MLR values (e.g. Dunton, 1994; Kenworthy and Fonseca, 1996). As such, the depth limits model only works well for subtidal eelgrass populations.

Based on a review of reported literature values, Duarte (1991) found that the depth limit for seagrasses (all species considered) varies with water column light attenuation according to the relationship: $Z_{max} = 1.86/K_d$. In a more recent review, this relationship has now been shown to overestimate the actual colonization depths, especially in turbid areas where the maximum depth limit is < 5 m, suggesting that plants colonizing such turbid shallow waters have higher apparent light requirements than those growing in clearer waters (Duarte et al., 2007). The MLR in such turbid areas'can vary even within a single estuary due to the considerable spatial and temporal dynamics of phytoplankton biomass and suspended matter (Höjerslev, 1978; Zimmerman et al., 1991). Higher light requirements may also reflect differences in the quality of light available to the plants (Duarte et al., 2007). Turbid coastal waters are often eutrophic, with seasonal blooms of phytoplankton. Chlorophyll *a* in phytoplankton removes light at the same wavelengths as required by the plants and may thus result in more severe light attenuation. Other constraints in shallow waters, such as high carbon losses due to intense wave action and grazing (Zimmerman et al., 1996), and higher temperatures (Marsh et al., 1986) may also increase light requirements of seagrasses. The differences in the relationship between the light attenuation coefficient and water transparency between shallow turbid waters and clear deep waters have been suggested to require separate equations are required to predict the maximum depth of seagrass plants growing in turbid waters and clearer water (Duarte et al., 2007).

Although light is often the most important parameter regulating eelgrass depth limits as assumed in the depth limits model, other parameters such as maximum water depth, sediments, N concentration and minimum oxygen concentration, which can vary from year to year, can sometimes influence depth limits (Greve and Krause-Jensen, 2005a; Steward et al., 2005).

The complexity that the aforementioned issues bring to the prediction of maximum depth limits is clearly an area that demands further research attention. There is also little information to predict the rate of retreat or expansion of eelgrass at depth in general (but see: Rivers, 2006). In addition, there is limited understanding of the time scales involved in the response of seagrasses to changing water quality conditions (Kenworthy and Fonseca, 1996; Greve and Krause-Jensen, 2005a; Zimmerman, 2006).

Light Compensation Point model for plant metabolism

In another approach, MLR has been equated to the compensation irradiance (I_c), which is defined as the light level (in µmol photons m^{-2} s^{-1}) at which photosynthesis equals respiration (Fourqurean & Zieman, 1991). At compensation irradiance, the rate of photosynthesis exactly balances the rate of (whole plant) respiration, so that the plant is not consuming or building any biomass. It corresponds to a net carbon balance where the total gross leaf productivity equals respiratory demand of all plant tissues. Net photosynthesis is achieved only above that point and a positive carbon balance is achieved only when light levels are higher than compensation irradiance. In a model that assumed below-ground tissues functioned anaerobically, the amount of carbon consumed by respiration in below-ground eelgrass tissues was found to represent up to 15% of the total carbon fixed, and this percentage increased to 25% at the deepest edge of an eelgrass meadow (Kraemer and Alberte, 1993). Thus, the light compensation irradiance for photosynthesis depends (partly) on the biomass and respiratory needs (aerobic vs anaerobic) of below-ground tissues.

The light compensation point model for plant metabolism is largely based on production-irradiance *(P-I)* curves (Figure 2.1), which describe the response relationship of the photosynthetic rate (measured as oxygen evolution) as a function of light. These curves indicate the efficiency with which plants use light energy to accumulate biomass. To generate such curves, whole plants, leaves or leaf or stem sections are exposed to varying light intensities and the photosynthetic rates are measured based on oxygen evolution or the consumption of carbon dioxide, while respiration (oxygen consumption) is estimated in the dark. Compensation irradiance is usually estimated indirectly through extrapolation from laboratory measurements of production at different light levels and measurements of respiration. Analytical (non-iterative) models of *P-I* require repeated measures of irradiance within a day (Zimmerman et al., 1994).

Estimates of compensation irradiance for eelgrass, I_c, reported in the literature generally range between 0.9 – 36 μmol photons m^{-2} s^{-1} (McRoy, 1974; Sand-Jensen, 1977; Mazzella et al., 1980; Dennison and Alberte, 1982; Dennison and Alberte, 1985; Evans et al., 1986; Marsh et al., 1986; Zimmerman et al., 1991), although one study reported an unusually high value of 417 μmol photons m^{-2} s^{-1} (Wetzel and Penhale, 1983). The wide variation may be due partly to experimental conditions such as water temperature (as demonstrated by Marsh et al.(1986)), pH and tissue age, as well as the depth taken to represent the eelgrass meadow (sediment surface or canopy height). The light compensation point (I_c) thus gives an indication of the minimum light required to maintain basic plant metabolism. For new growth, however, light reaching the plant must exceed the compensation irradiance and be of sufficient duration. For a given eelgrass area, estimates of I_c may be used to validate estimates obtained through the depth limits approach when light at the surface is known.

Theoretically, compensation depth would be the depth at which light penetrating the water allows gross daily photosynthetic carbon fixation to balance plant respiratory losses over a day. This, however, implies that for growth and expansion of eelgrass (which implies photosynthesis beyond the level required to meet respiratory demand), compensation irradiance is insufficient. Thus, the maximum depth limit where an eelgrass meadow has enough light to persist year-round is likely to be shallower than the depth at which the light level equals the compensation point.

The compensation depth of eelgrass has been predicted by empirical models which used irradiance at depth, maximum gross photosynthesis and respiration (R), as well as shoot-to-root ratios, and has been found to range from 4.2 to 11.6 m at shoot P_{net}:R ratios of 11 to 4.5 (Kraemer and Alberte, 1995), which tallies closely with the actual global depth distribution of eelgrass (Green and Short, 2003).

Limitations of the light compensation point model

A number of factors must be considered in evaluating the usefulness of the I_c model for determining minimum light requirements. The use of short-term photosynthesis-light experiments to estimate light-growth relationships and depth penetration, especially when plants are not pre-acclimated to experimental conditions and when plant segments instead of whole plants are used, has been questioned (Zimmerman et al., 1989; (Herzka and Dunton, 1997). Such measurements underestimate the amount of light required to support the whole plant (Fourqurean and Zieman, 1991) and might overestimate I_c if plants become acclimated. Even when whole plants are used in determining I_c, laboratory conditions may not represent the real situation in the field, where environmental factors other than light present additional challenges in accurately determining the I_c required by eelgrass plants. I_c estimated using laboratory P-I data generally tend to be lower than those made using P-I data collected in the field (Herzka and Dunton, 1997; Batiuk et al., 2000).

Furthermore, I_c values vary with the light history of the plant and tissue age as well as with ambient light intensity gradients (Mazzella and Alberte, 1986; Goodman et al., 1995), while I_c does not incorporate the duration of light reductions. Accurate determination of I_c for management purposes requires continuous, time-series data to be collected. Together these drawbacks may limit the usefulness of the I_c model for management purposes.

H_{sat} model for carbon balance

Another concept that has been used to characterize the eelgrass light environment to try to estimate the plant's minimum light requirements is the daily period of hours of photosynthesis saturating irradiance or the part of the day that quantum irradiance is greater than saturating irradiance in hours (H_{sat}) (Dennison and Alberte, 1986; Dennison, 1987; Zimmerman et al., 1994). H_{sat} is based on *P-I* curves (Figure 2.1) that determine the minimum irradiance for photosynthetic saturation (I_k) and adequate irradiance data over the daily photoperiod and throughout the year.

H_{sat}, typically expressed as a mean value per day, has been defined as the period (hrs) when irradiance is equal to or greater than I_k (i.e., the time period during a day when photosynthesis of a section of a leaf in question is at light saturation and maximum carbon fixation is occurring; see Table 2.2 for values). A shorter H_{sat} suggests that the period of maximum carbon fixation and oxygen production is reduced, resulting in less oxygen diffusion to the plant roots. Depending on the rates of photosynthesis and respiration, prolonged anoxia may result in a negative carbon balance that eventually could lead to eelgrass plant mortality (Dennison and Alberte, 1985; Kraemer and Alberte, 1995). H_{sat}, derived from instantaneous measurements of photosynthesis over a light gradient that exceeds ambient light conditions, is the basis of this H_{sat} model of carbon balance. H_{sat} represents the daily period of irradiance-saturated photosynthesis defined as $I > I_k$, and it involves the relationship between photoperiod and photosynthesis only. In the carbon balance model, the H_{sat} requirement is a theoretical concept that has been used to describe the number of hours of saturating light required to maintain a positive carbon balance in the plant. Carbon balance calculations (Zimmerman et al., 1995) are based on the ratio of daily whole-plant gross production (P_g) to respiration (R), according to:

$$P_g{:}R = \frac{H_{sat} \times P_{max} \times F_L}{[(24-H_{sat}) \times R_L \times F_L] + (H_{sat} \times R_R \times F_R) + [(24 - H_{sat}) \times 0.65R_R \times F_R]}$$

where F_L is the the fraction of total plant biomass represented by the leaves, R_L is leaf respiration, R_R is respiration of below-ground tissue, and F_R is the fraction of total plant biomass represented by the below-ground tissue. Minimum H_{sat} requirement is then calculated by determining H_{sat} values that result in a production to respiration ratio of 1 ($P_g{:}R = 1$); that is, no net change in C content on a 24 h basis (Zimmerman et al., 1995; Zimmerman et al., 1996).

By integrating daily carbon gain, daily carbon loss, respiration, maximum photosynthesis and the proportion of photosynthetic to non-photosynthetic tissues, various authors have attempted to quantify the H_{sat} requirement of eelgrass (Table 2.3). A major constraint has been the difficulty in determining photosynthesis for all of the plant tissue, which cannot be adequately determined from laboratory-based photosynthetic measurements on small sections of leaf.

H_{sat} is influenced by a range of factors including, but not limited to, things that reduce light availability such as latitude, water depth, and turbidity. Latitude can significantly affect the light regime in eelgrass areas through its influence on light intensity and day length. Summer day length is longer in higher latitudes, resulting in longer daylight periods and higher light intensities during the growing season, especially around summer solstice. Photosynthetically active radiation is reduced with increasing depth and with increased turbidity, and this reduction is accompanied by a shortening in H_{sat}. For example, Dennison and Alberte (1985) found that H_{sat} was 3 hrs longer at shallow sites than at deep sites and attributed higher summer productivity observed in high latitude eelgrass beds reported by McRoy and McMillan (1977) to longer periods of daylight. Koch and Beer (1996) found that the period of saturating light was shorter with greater light attenuation and with greater tidal range.

In addition to the influence on H_{sat} of factors that affect light availability, increased water temperature can depress the carbon balance of eelgrass because respiration is temperature-dependent (Marsh et al., 1986; Lee et al., 2007). Since H_{sat} required for photosynthesis to balance carbon demand decreases as the gross production to respiration ratio ($P:R$) increases, a reduction in $P:R$ (due to increased respiration) with increasing temperature would theoretically increase the required hours of H_{sat}. Eelgrass plants are, however, able to acclimate to changing temperature by stabilizing P_{net}:R ratios relative to changing temperatures as demonstrated by Zimmerman et al. (1989). Depending on timing, seasonal changes in ambient temperature may not affect the light requirements and whole-plant carbon balance because of this thermal acclimation (Zimmerman et al., 1989).

The ratio of photosynthetic to non-photosynthetic tissues can also influence H_{sat} requirement. Below-ground tissues can significantly contribute to the carbon (and oxygen) demand of the whole eelgrass plant. Therefore as light decreases, shoot-to-root ratio (S:R) increases as part of a morphological acclimation process in which plants maintain growth by increasing the photosynthetic biomass and reducing respiration of non-photosynthetic tissues (Olesen and Sand-Jensen, 1993). Consequently, the H_{sat} requirement which maintains a positive carbon balance becomes shorter as S:R increases, although beyond a S:R of 2, no more reductions were observed by Zimmerman et al. (1989). Grazing away of the chlorophyll-rich epidermis by limpets induced carbon limitation in eelgrass grown in mesocosms despite sufficient light, resulting in a longer H_{sat} required to maintain a positive carbon balance by up to 8 hours (Zimmerman et al., 1996). Thus, factors that induce carbon limitation may also influence H_{sat} requirement of plants as shown by a laboratory experiment in which CO_2 enrichment of the water resulted in a shorter H_{sat} requirement of eelgrass plants from 7 to 2.7 hrs (Zimmerman et al., 1997).

Limitations of the H_{sat} carbon balance model

The primary concern with the H_{sat} carbon balance model lies in the way in which production figures are typically derived. Measurements on a small section of leaf material made in the laboratory fail to adequately describe productivity at whole-plant scale and meadow-scale. Thus, H_{sat} requirement estimates based on such measurements may prove unreliable.

Estimates of H_{sat} for eelgrass from photosynthetic studies as reported in the literature (Table 2.3) range from 3 to 12 hours. The large variation in reported H_{sat} values may reflect the effect of different factors that directly or indirectly influence H_{sat}, as discussed above, in addition to photosynthetic and morphological adjustments of plants to local light regimes (*sensu* Lee at al., 2007).

A secondary issue of concern is that the H_{sat} carbon balance model, which uses respiratory demand and P_{max} to calculate minimum light requirements for individual eelgrass plants, fails to account for carbon losses due to herbivory, sloughing and fragmentation as well as reproductive requirements (e.g. flowering) at the meadow scale (Ralph et al., 2007). Although photosynthesis-irradiance curves are used to estimate whole plant carbon gains and losses, the H_{sat} model does not incorporate the contribution of plant carbohydrate reserves, which can be mobilized during times of negative carbon balance (i.e. $P_g:R < 1$).

A further limitation of the application of the H_{sat} model lies in the seasonality of light and temperature. Dennison and Alberte (1985) suggested that the minimum H_{sat} for growth and survival of eelgrass is 6 h, a value reiterated in some later studies (Alcoverro et al., 1999; Cabello-Pasini et al., 2003; Boese et al., 2005). However, the application of H_{sat} is tied to the value of I_k (Zimmerman et al., 1994). I_k is a photosynthetic parameter that varies with seasonally changing light availability and temperature (Kirk, 1994), and it is generally highly variable in eelgrass (Table 2.2). Thus, eelgrass plants saturate at a lower light level in the winter as part of photosynthetic acclimation to seasonal light reduction. Furthermore, in winter, P_{max} is reduced as a result of adaptive shifts in metabolic performance (Dennison, 1987; Lee et al., 2007). These seasonal changes in metabolic activity suggest that the daily H_{sat}

requirement may also vary with season. Zimmerman et al. (1995a), for example, found that eelgrass transplants in San Fransisco Bay (USA) had a 2-3 times longer H_{sat} requirement in winter compared to summer, and attributed this to photosynthetic acclimation of eelgrass plants to light reduction (Zimmerman et al., 1995a).

Different authors have used different I_k values, with some authors using fixed I_k values (e.g. Dennison and Alberte, 1985: 100 μ mol photons m^{-2} s^{-1}) or ranges (e.g. Alcoverro et al., 1999: 30 – 50 μ mol photons m^{-2} s^{-1}) for varying H_{sat}, others using changing I_k with changing season (e.g. Zimmerman et al., 1995a). Based on photosynthetic acclimations discussed above, there cannot be a single critical value that adequately describes true H_{sat} requirements of eelgrass in a given area. Furthermore, for the H_{sat} model to be widely useful, calculations of H_{sat} requirement must incorporate annual carbon budgets that incorporate seasonal variations in photosynthetic parameters, which are expensive and difficult to acquire.

While some studies show that integrated daily irradiance may not be a reliable predictor of daily production and that H_{sat}, instead, is much more reliable and agrees closely with analytical models of *P-I* (Dennison and Alberte, 1985; Zimmerman et al., 1994), other studies have demonstrated the limitations in the applicability of the H_{sat} model in estimating eelgrass productivity, and cautioned against its use in predicting production, citing the source of *P-I* data (laboratory leaf segments vs. *in situ* whole plants), water transparency, the type of sensor used and the proportion of photosynthetic to non-photosynthetic tissues as the main sources of disparity (Herzka and Dunton, 1997; Herzka and Dunton, 1998). Furthermore, the H_{sat} model, like the I_c model, may be inaccurate in predicting whole carbon balance because estimates of I_k (as with I_c) measured *in situ* tend to be higher than those measured in the laboratory

(Fourqurean and Zieman, 1991; Dunton and Tomasko, 1994; Herzka and Dunton, 1997).

The biggest issue that limits the use of the H_{sat} model is that the model assumes zero production whenever underwater irradiance is less than I_k, and in this way does not take into account the hours of light-limited photosynthesis and consequently the contribution of light-limited photosynthesis to the overall carbon balance. The majority of photosynthesis in field crops occurs at non-saturating light levels (Ort and Baker, 1988), stressing the importance of photosynthetic efficiency at low light. Similarly, eelgrass photosynthesis continues even under low light levels as shown by low light compensation and saturation, while photoacclimation processes increase efficiency and light harvesting. Fourqurean and Zieman (1991) reported significant production of *Thalassia testudinum* in Florida Bay at depths where the daily H_{sat} period was zero, suggesting that discounting photosynthesis at low light leads to an underestimation of production, further complicating the usefulness of models based on *P-I* for assessing light requirements.

Minimum light requirements – concluding remarks

The minimum light requirements concept offers managers a tool to identify critical thresholds and can be useful in impact predictions of short-term human perturbations, thereby helping to manage water quality. Practical applications of minimum light requirement models for eelgrass management are few. Examples include the use of H_{sat} for monitoring of dredging effects on eelgrass in San Fransisco Bay (USA) (Langis et al., 2000), the use of H_{sat}, %SI and I_c for setting water quality and habitat-based requirements and restoration targets for submerged aquatic vegetation in Chesapeake Bay (Batiuk et al., 2000), and the use of %SI to predict impacts of dredging on seagrasses (Erftemeijer and Lewis, 2006) and assess the habitat suitability of estuarine areas in the Dutch Wadden Sea for eelgrass restoration (De Jong et al., 2005). In my opinion, the fact that there are at least three different models to assess minimum light requirements of eelgrass makes a clear understanding of MLR difficult and may have contributed to the limited application of these models by managers to date.

Each of the approaches investigating the minimum light requirements has its advantages and disadvantages. Calculations of percent surface irradiance using the depth limits model are based on optical properties of water, making the percent surface irradiance the most straightforward approach as long as light is measured continuously to capture its variability, including incidental pulses of high turbidity. However, Peralta et al. (2002) suggested that it is more important to give light requirements in absolute values (e.g., time-integrated) rather than relative units (e.g., percent surface irradiance) because differences in latitude, tides and/or meteorological characteristics, rather than integrated light, can cause small differences in percent surface irradiance. MLR as percent surface irradiance can be directly estimated from the light compensation point but not vice versa. Furthermore, such conversions may not give an accurate representation of the true MLR, as I_c is dependent on several factors including the depth at which plants are collected, seasonal water temperature changes and S:R.

Because the daily period of light-saturated photosynthesis (H_{sat}) directly affects carbon transport and anoxic stress in root tissues by influencing the daily period of root aerobiosis, it has been suggested as a more important parameter describing eelgrass light environment than instantaneous PAR. H_{sat} requirement for growth, however, is dependent on the metabolic performance of the plants and, like I_c, varies with season and depth. Furthermore, the minimum H_{sat} required to sustain eelgrass over annual cycles is not known (Ralph, et al., 2007). Because of difficulties in translating laboratory-based experiments to field conditions, light requirement models based on photosynthesis-irradiance measurements present the biggest disadvantage to the application of I_c and H_{sat} models, and imply that the depth limits model might be the most reliable. So far, no studies have attempted a relative comparison of the various models of estimating minimum light requirements.

Having studied the available literature, I would like to point out two major issues that deserve greater attention in the further development and refinement of MLR models. Firstly, there seems to be no clear agreement on what constitutes the 'maximum depth limit'. It makes a lot of difference, when talking about light reaching eelgrass plants at the maximum depth limit, whether this is interpreted as light reaching the top of the canopy, or as light reaching the bottom (or somewhere in between), especially when plants are tall. Secondly, '*for what*' the minimum light is required remains unclear and is open to interpretation. For example, is compensation irradiance sufficient for the year-round persistence of an eelgrass plant or meadow?

Thus, different approaches with different underlying assumptions result in different outcomes in MLR for eelgrass as shown by the wide variation in MLR values reported in the literature. Factors such as temperature influence the outcome of MLR calculations; therefore, taking a single light value estimated in one area cannot accurately translate to the MLR for eelgrass throughout its range.

While it is recognized that eelgrass light requirements are higher than those of, for example, algae, information concerning the exact quantity required for long-term survival, including recovery from storms and herbivory, and allowing for meadow expansion and flowering, is still scarce. In addition, due to carbohydrate storage reserves, eelgrass might be able to survive temporarily below its minimum light requirements, suggesting that besides the minimum light requirements, the period and variability of light deprivation events can also limit eelgrass distribution (Peralta et al., 2002). Our understanding of the response of eelgrass plants to such periods of light deprivation is far from complete.

Overall, the difficulty in accurately quantifying light availability in the eelgrass environment, with measurements conducted over short periods not accounting for the effect of seasonal or pulsed turbidity changes in light availability, presents a challenge to defining and quantifying the minimum light required by eelgrass for growth.

FACTORS REDUCING LIGHT AVAILABILITY TO EELGRASS

The nature of light reduction

Any reduction in light penetration or change in the optical properties of water (absorption and scattering coefficients) can affect the quantity and quality of light reaching eelgrass leaves and in this way may ultimately compromise eelgrass survival. The natural water column is itself a source of light attenuation. In addition, suspended particles reduce water clarity by increasing turbidity. Water color and increased nutrient loading, which stimulates the growth of phytoplankton, epiphytes and other nutrient-limited algae further contribute to light attenuation, reducing the incoming light energy reaching eelgrass leaves. Reductions in light availability to eelgrass beds can either be chronic (Giesen et al., 1990), or episodic, i.e. of a temporary nature, such as events caused by seasonal pulses of turbidity (Moore et al., 1997). Depending on their duration and intensity, even short-term turbidity increases can limit eelgrass survival (Backman and Barilotti, 1976; Dennison and Alberte 1985; Moore et al., 1997).

Several factors, both natural and anthropogenic, play a role in determining the amount of light reaching eelgrass plants. Natural factors include seasonal forcing (e.g. seasonality in wind and storms, river plumes, seasonal changes in the sun's altitude, and seasonal phytoplankton blooms), factors associated with weather (e.g. cloud cover and hours of sunshine), physical characteristics of the water column (e.g., waves, tides, depth and currents) and geographic position (latitude) as well as plant parameters (e.g. self-shading and leaf orientation). Anthropogenic factors that may reduce light availability to eelgrass include maritime construction and reclamation works (including dredging), poor watershed management, and activities associated with boating and fishing. These factors contribute to a complex and extremely variable submarine light environment.

The following sections will review each of these natural and anthropogenic factors that contribute to light reduction in more detail, and draw a number of conclusions with regard to the relevance for eelgrass growth and survival and the implications for impact assessment and monitoring.

Natural factors that influence light availability

Seasonality and weather

Light reaching eelgrass beds can vary substantially as a consequence of year-to-year and seasonal fluctuations in a wide range of factors such as incident irradiance, water level, sediment resuspension, and organic matter concentration in the water column (Dennison, 1987; Zimmerman et al., 1994; 1995a; Cabello-Pasini et al., 2003). Due to seasonal changes in the sun's altitude and the amount of cloud cover, solar radiation reaching the surface of the water varies widely. As a consequence, the differences in the mean and total irradiance striking the ocean surface over different times of year can be significant. For example, between 1931 and 1934, the annual total hours of sunshine over the Wadden Sea were above average compared with the preceding decades, with excess of sunshine (+26%) in March and a deficiency (-26% and -13%) in May and July respectively (Giesen, 1990). In addition, some of the factors that reduce water transparency also vary with season, further modifying the light regime.

Whilst the elevation or altitude of the sun and day length determine the maximum possible light intensity, atmospheric conditions such as cloud cover and haze may directly reduce the quantity (intensity) and quality (spectrum) of light hitting the water surface and eventually reaching plants at the bottom. Clouds reflect, absorb and transmit the incoming solar radiation, affecting the quality and quantity of light reaching the water surface. The loss of intensity varies depending on the thickness and type of cloud cover. Cloud cover causes greater attenuation of longer wavelengths (yellow, orange, red and infrared) compared to shorter wavelengths (UV, violet, blue and green) (Odum, 1971).

Long term effects of hourly and seasonal variations in irradiance, day length and atmospheric conditions result in an annual cycle of the amount of light reaching the ocean surface and subsequently eelgrass areas (Cabello-Pasini et al., 2003; Kaldy and Lee 2007). Underwater light, however, can sometimes show unpredictable patterns, with no clear seasonal trends (Lee et al., 2005). In Chesapeake Bay, increases in turbidity during the summer months sometimes resulted in declining underwater light, even though solar PAR continued to increase (Moore et al., 1997).

Winds, storms and floods

Sediment discharge by rivers after storms and floods can cause substantial temporary increases in water column turbidity in shallow coastal areas. Seasonally changing winds and coastal currents play an additional role in determining the extent and direction of river plumes. For example, rapid changes in suspended solids in the water column were found to be significantly correlated with wind conditions in a wind-exposed lagoon in France (Banas et al., 2005). Wind may also cause substantial resuspension of fine sediments in shallow coastal areas (Lawson, 2004), greatly influencing light availability to eelgrass (Orth and Moore, 1986). Turbidity significantly increases both absorption and scattering of light, resulting in more light attenuation within the water column, and can also differentially affect the wavelengths of light that are absorbed and scattered, changing the spectra of available light at different depths (Kenworthy and Fonseca, 1996). For example, light attenuation coefficients at a lagoon and open coastal area in Baja California increased six-fold as a result of sediment resuspension caused by storms (Cabello-Pasini et al., 2002). Plankton blooms, associated with elevated nutrient input from watersheds after stormy weather, may also affect the transmittance of light. The mixing of the water column during a hurricane in Cape Cod, USA, released major amounts of nutrients held within the macroalgal canopy and upper sediments into the upper layers of the water column, prompting a short-lived (2–3 d) phytoplankton bloom (Valiela et al., 1998).

Waves and tides

Waves cause surface water movement that can act like a lens, especially in shallow water, focusing the light from the sun and resulting in flashes or specks of very high intensities (light-flecks) when compared to the intensity of light transmitted through a smooth surface (Wing et al., 1993). Eelgrass plants growing in shallow waters may benefit from such light-flecks as has been shown for macroalgae (Greene and Gerard, 1990; Wing and Patterson, 1993). Leaves that lie on top of each other impose self-shading, but when waves induce leaf flapping, fragments of light penetrate through the canopy such that in wave-dominated systems, productivity has been found to increase (Koch et al., 2006). It has also been speculated that waves and currents can lead to reduced epiphytic growth on seagrass leaves, thereby reducing the adverse effects of epiphytes on light availability, but there are little data to confirm this hypothesis (Koch et al., 2006). Waves can increase sediment resuspension, and increase mixing of the water column especially during storm events. Such processes can cause greater light attenuation by the water column resulting in lower light availability for bottom-dwelling eelgrass. Shoreline erosion caused by wave action contributed between 13% and 53% of the total suspended matter in parts of Chesapeake Bay (Biggs, 1970). Since waves can both increase and decrease light availability to eelgrass, the overall effect of wave activity on light reaching eelgrass plants will depend on local conditions (e.g. sediment composition, wave height, shoot density, plant height, etc.).

Tides and maximum depth are confounding factors since water depth is a function of tides (Koch and Beer, 1996). It is the tidal range which affects underwater light regime, and thereby light availability for eelgrass by influencing the depth of light penetration (Carter and Rybicki, 1990). The adequacy of light will depend on the timing of high and low tide, the actual height of the tide, day length and even cloud cover (Carter and Rybicki, 1990). Koch and Beer (1996) showed that the number of hours of saturating light (about 300 μmol photons m^{-2} s^{-1}) was smaller as tidal range increased. Thus, in areas with high tidal amplitude, the maximum depth of eelgrass growth may be shallower due to low light availability (Koch, 2001).

Plant parameters

Eelgrass shoot density, leaf orientation, and variable epiphyte cover on eelgrass leaves can have an effect on the intensity and spectral composition of the adjacent light field, creating an additional light gradient besides that created via water column light attenuation.

Shoot density

Self-shading by seagrasses increases with increasing shoot density. In densely vegetated, shallow *Zostera noltii* meadows in the Netherlands, self-shading - especially during low tides – was found to reduce light by more than 60% (Vermaat and Verhagen, 1996). A study that modelled the actual light climate within an eelgrass bed illustrated that light attenuation in eelgrass beds is modified by depth-related changes in biomass, density and shoot height. In a dense eelgrass bed in Danish waters, with shorter plants at shallower depths, the amount of light available was found to be half of that measured in adjacent bare areas (Krause-Jensen et al., 2000). On the contrary, in sparse beds with taller plants (deeper, close to depth limit), the amount of light available was comparable to light in adjacent bare areas (Krause-Jensen et al., 2000). These results demonstrate that self-shading in eelgrass meadows can sometimes be considerable in areas where the density of the vegetation is particularly high. The effects of such self-shading can however be reduced by wave action (Koch et al., 2006).

Differential pigmentation within leaf sections, with higher pigment concentrations at the basal end of seagrass leaves has been attributed to the self-shading effect imposed by overlying leaves in *Posidonia* meadows (Via et al., 1998). Light intensity (PAR) in an eelgrass meadow in Great Harbor, MA (USA) was found to be reduced by 50% from the top of the canopy to the leaf base. Corresponding to this gradient of light availability, leaf chlorophyll content, and consequently photosynthetic factors, varied along leaf axes from leaf tips to the base of the plants (Mazzella and Alberte, 1986). These studies show how seagrass plants can adapt to gradients of light availability and self-shading within their canopies when the vegetation is particularly dense, by adjusting the chlorophyll contents of their leaves.

Eelgrass meadows can locally increase light availability through the filtering of sediments and particulate matter from the water column, especially when shoot density is high (Van der Heide et al., 2007). The fact that eelgrass is a clonal plant implies that shoots at the maximum depth may be supported in part by connected shoots growing at shallower depths.

Leaf orientation

Light availability within eelgrass canopies is also affected by the distribution and orientation of plants relative to the incident light field (Zimmerman 2003). Light levels reaching the top of the eelgrass canopy can be substantially greater than those measured at the bottom of the canopy (sediment surface). Leaves are not always vertically oriented, and "the fractional amount of leaf biomass within the canopy is greatest near the seafloor, decreasing non-linearly toward the top of the canopy" (Zimmerman, 2006). These plant properties result in the further absorption and scattering of a significant portion of the already-dimmed incident light as light passes through the eelgrass canopy, changing the angular distribution of light and resulting in differential light quality and quantity especially near the sea floor in dense meadows. Thus, leaf orientation and shoot density determine self-shading within the eelgrass canopy (Zimmerman 2003).

Epiphyte cover

Variable epiphyte cover can further reduce the percentage of light reaching the surface of an eelgrass leaf. Epiphyte cover on eelgrass leaves can be considerable, especially in areas under significant eutrophication. The main effect of eutrophication on eelgrass has been shown to be light reduction by increased algal abundance, including epiphytes (Short et al., 1995). The amount of light attenuation by epiphytes in eutrophied areas can be as much as 36% to 60% (Borum, 1985; Dixon, 2000;

Drake et al., 2003). Epiphyte cover, however, is not evenly distributed among the different leaves on an eelgrass shoot, with no epiphytes on the youngest leaf resulting in a differential effect of epiphytes on light reaching the leaves.

Anthropogenic factors that affect light availability

Sediment loading

Light availability is influenced to a large extent by turbidity, and therefore by the sediment concentration in the water column. Increased sediment loading to estuaries from rivers, deforestation, land clearing and increased impervious surfaces lead to increased net export of sediment from watersheds into coastal waters, where it causes light reduction (Miliman and Meade, 1993). River discharge and sediments resuspended by wind energy and tidal currents are the main sources of high particle concentrations (Ward et al., 1984; Gabrielson and Lukatelich, 1985; Campbell and Spinrad, 1987; Carter et al., 1994). Within 10 different basins in the Limfjord, Denmark, Olesen (1996) showed that Secchi depth, a measure of water clarity, was highly influenced by suspended particle concentrations, accounting for up to 67% of the variability in Secchi depth. Various anthropogenic activities thus enhance concentrations of suspended sediment, which contribute to increased turbidity in coastal waters resulting in reduced light availability to eelgrass.

Eutrophication

Eutrophication refers to the increases in nutrients on land or in water, mainly nitrate and phosphate, that actually impact the environment. Anthropogenic activities in coastal watersheds often form the main source of such nutrients in the nearshore marine environment. Nutrients (particularly nitrate), end up in coastal waters through storm drains, other forms of surface run-off and sewage pipes as well as through groundwater. Such increased nitrogen loading may favour prolific growth of fast-growing algae (phytoplankton, filamentous macroalgae), reducing light availability to eelgrass and potentially leading to severe light limitation (Short et al., 1995; Valiela et al., 1997; Moore and Wetzel., 2000; Hauxwell et al., 2003; Domin et al., 2004). Consequently, depth limits for eelgrass can be highly sensitive to total nitrogen concentrations, particularly in coastal and estuarine waters. In 27 heavily eutrophied Danish fjords and coastal waters, total nitrogen was found to explain up to 73% of variability in eelgrass depth limits (Nielsen et al., 2002). Besides controlling depth limits, eutrophication has led to the decline of eelgrass in many areas. For example, in Akkeshi-ko Estuary, Hokkaido, Japan, eelgrass production declined dramatically, coinciding with an epiphyte bloom which reduced light availability in the eelgrass bed (Hasegawa et al., 2007). Nitrogen loading via groundwater, associated with housing development, was linked to rapid decline of eelgrass in Waquoit Bay (Short and Burdick, 1996). Similarly, Keser et al. (2003) attributed die-offs of entire eelgrass beds recorded in the Niantic River, Connecticut, to nutrient loading from surface run-off and groundwater sources. Den Hartog (1994) described how the growth of a dense blanket of *Ulva radiata* (approx. 10 cm thick) in Langstone Harbour (UK) in 1991 resulted in severe light limitation and loss of 10 ha of *Zostera marina* and *Zostera noltii*. These examples confirm that the mechanism by which eutrophication affects eelgrass involves light limitation, although direct impacts of nitrate on eelgrass have also been reported (Burkholder et al., 1992).

Boating

Activities associated with boating can also reduce light availability to eelgrass. Dock structures within eelgrass beds directly shade the plants from available light (Burdick and Short, 1999; Shafer, 1999; Fresh et al., 2006). Propeller wash, boat moorings (Walker et al., 1989; Hastings et al., 1995) and boaters anchoring over shallow eelgrass beds can scar eelgrass beds in addition to causing temporary resuspension of bottom sediments. Boat wakes in areas frequented by motor boats can cause repetitive resuspension of bottom sediments, thus reducing light availability to eelgrass. Based on data indicating decreased light penetration associated with boat traffic, Kenworthy et al. (1988) found a possible cause-effect relationship between boating activities and increased turbidity. Besides physically removing eelgrass plants, fishing activities (e.g., trawling, clam shell and mussel dredgers and cockle fishing) can reduce water clarity through repetitive disturbance of bottom sediment and in this way impact eelgrass survival and recovery (de Jonge and de Jong, 1992; Neckles et al., 2005).

Dredging/landfill or land reclamation

Dredging, which entails the removal of substratum from the seafloor, as well as the transport and disposal of dredged material can result in a temporary but significant decreases in water transparency, increased concentrations of suspended material and increased sedimentation rates in seagrass beds (Erftemeijer and Lewis, 2006). Increased light attenuation due to suspended material in the water column and sediment that settles on or partially buries eelgrass blades both result in reduced light available to eelgrass. De Jonge (2000) reported a two-fold increase in light attenuation over the period 1950 – 1983 in the Ems estuary due to a proportional increase in maintenance dredging and disposal activities in the estuary during the same period. Major light attenuation after a dredging operation in Laguna Madre, Texas, USA was directly attributed to dredging, with elevated levels of suspended sediments extending more than a year after dredging (Onuf, 1994). Dredge and fill operations can also significantly alter hydrological conditions, e.g., current velocities and wave conditions in an area, with significant indirect consequences for turbidity and light availability to eelgrass. For example, the closure of the Zuiderzee (3200 km^2) from the Dutch Wadden Sea in 1932 caused a significant increase in tidal range and current velocities (de Jonge and de Jong, 1992). Additional maintenance dredging, sand extraction and mussel culture in later years increased the turbidity of the Dutch Wadden Sea (Giesen et al., 1990; de Jonge et al., 1996), which – along with hysteresis (i.e. higher turbidity due to the absence of filtering plants) – has been suggested to be one of the main reasons for a lack of natural recovery of former eelgrass meadows in the Dutch Wadden Sea (van der Heide et al., 2007).

Climate change

An indirect human factor that might impact eelgrass through its effects on light reduction is climate change. Potential effects of climate change on light availability to eelgrass may include increased seasonal river plumes due to changes in precipitation patterns, increased likelihood of substantial cloud cover, increased incidents of storms and increases in water motion and tidal circulation (Short and Neckles, 1998; Duarte, 2002). Most of these climatic changes are likely to affect water transparency and nutrient inputs from terrestrial sources, thus impacting the amount of light reaching the eelgrass canopy. Furthermore, sea level rise which may be related to the warming of oceans will increase the mean sea level as well as the tidal variation (Short and Neckles, 1998). Since light attenuates with depth, and light availability controls the depth distribution of eelgrass (Dennison and Alberte, 1986; Duarte, 1991), an elevated sea level will increase water depth which in turn will reduce the amount of light reaching bottom-dwelling eelgrass, although this may be party compensated by a landward migration of eelgrass. Changes to the tidal range may exacerbate or confound the effects of increased water depth on light availability depending on the local coastal geomorphology and the extent of tidal restriction (de Jonge and de Jong, 1992)

Concluding remarks

It is clear that several and sometimes overlapping natural and anthropogenic factors associated with the physical characteristic of the water, meteorological events, and biological factors affect light availability to eelgrass. These factors, which may vary in scale and magnitude, can result in unpredictable patterns of light availability (Lee et al., 2005), and can reinforce each other leading to greater light reduction. The result is that together these factors contribute to a complex and extremely variable submarine light environment that presents a challenge to monitoring programs For example: how to guarantee sufficient temporal and spatial resolution in order to capture actual variability and extreme events? Besides natural factors, light availability to eelgrass is also affected by a range of anthropogenic factors that increase the amount of suspended sediment in the water column, enhance phytoplankton, algal and epiphyte growth through nutrient enrichment of the water, or affect light conditions through climate change. Light reduction caused by these human activities may interact and/or coincide with light reduction caused by natural events, making it a difficult challenge to precisely monitor and identify the major factors contributing to light reduction. Furthermore, detecting and evaluating effects or trends associated with anthropogenic disturbances against a background of large natural variability in turbidity can be a major challenge (detection limits) and may leave the questions of what constitutes a "significant" increase in turbidity and/or what is the turbidity threshold above which significant impacts to eelgrass can be expected. Based on the literature as presented in this chapter, I postulate that anthropogenic factors pose a threat to eelgrass only if the enhanced light attenuation they cause exceeds the magnitude, duration and frequency of the light attenuation caused by natural factors.

RESPONSES OF EELGRASS TO LIGHT REDUCTION

Introduction

Light is one of the most critical factors affecting the growth, community structure, depth distribution and long-term survival of eelgrass, and yet its availability can show considerable variability at many scales under the influence of natural and anthropogenic factors. We can therefore expect that by the nature of its light environment, eelgrass will have developed a certain degree of plasticity, that is the ability to adapt, to the varying light environment, either physiologically or morphologically as a plant, as well as through storage and mobilization of reserves and vegetative recruitment, and through the ability to recover as a population by recolonization from seed. Greater understanding of the responses of eelgrass plants and meadows to light reduction and the extent of their plasticity to recover from turbidity events will help in managing the effects of anthropogenic causes of turbidity (when these cannot be avoided) to minimize decline and loss of eelgrass populations. In this section, I explore on the basis of literature findings, what is currently known about the responses of eelgrass to light reduction, and examine the degree of plasticity displayed by eelgrass meadows. I also present evidence from the literature showing that when the magnitude and/or duration of the light reduction exceeds what eelgrass plants can tolerate, widespread decline and loss of eelgrass occurs.

Physiological responses

Eelgrass physiological changes are among the earliest responses to light reduction and can be seen as photoacclimatory processes exhibited by the plant to enhance its light harvesting efficiencies and maximize carbon gain. Physiological responses to short-term light reduction that have been observed in eelgrass include low and steady rate of electron transport, a lowering of the light saturation point (I_k), a lowering of the light compensation point (I_c), reduced maximum photosynthetic rates ($P_{max(net)}$), reduced respiration rates, a reduced chlorophyll *a/b* ratio, increased chlorophyll *a* on a leaf area basis, and reduced density but increased size of photosynthetic units (PSU) of Photosystem Two (PSII) (Dennison and Alberte, 1985; Loomis and Amthor, 1999; Ralph and Gademan, 2005; Lee et al., 2007; Ralph et al., 2007).

A lack of response in photosynthesis, respiration, PSU density and size, and chlorophyll to reduced light is sometimes reported (Dennison and Alberte, 1982; Dennison and Alberte, 1985; Kraemer and Alberte 1995). Such a lack of response could be an issue of accurate determination of light levels and/or the timing of experimental manipulations and seasonal sampling. For example, in turbid waters, where substantial scattering occurs (Kirk, 1994), spherical light sensors tend to underestimate the total light field (Moore et al., 1997) as attenuation of wavelengths most useful for photosynthesis increases. Furthermore, turbid environments generate large gradients in light availability (Herzka and Dunton, 1997). These patterns underscore the importance of accurate measurement of underwater PAR, which is crucial to the interpretation of photosynthetic response to reduced light.

Eelgrass plants also exhibit physiological acclimation with depth, in which shade acclimation is often accompanied by increased light utilization efficiency and reduced respiratory rates of leaf tissue (Dennison and Alberte, 1985; 1986). Such a response pattern is typically achieved by the combined influence of higher pigment content and larger leaf area per unit leaf biomass.

Growth responses

Leaf growth shows a strong positive relationship with light, increasing linearly with increasing light (Kraemer and Alberte, 1995; Short et al., 1995), although some studies shave shown a saturating-type relationship (Fourqurean and Zieman, 1991; Dennison and Alberte, 1985; Olesen and Sand-Jensen, 1993), and yet others, a maximum growth at intermediate light levels (Peralta et al., 2002 for *Zostera noltii*). Areal leaf production, which combines density and growth per shoot, and biomass are also positively related to light. Shortening of H_{sat} led to up to 50% reduction in leaf biomass of eelgrass plants in Great Harbor, Wood Hole, MA, USA (Dennison and Alberte, 1985). While the effects of shortened H_{sat} on biomass were detrimental at deep sites, plants at shallow sites recovered within 2 weeks after shading was stopped (Dennison and Alberte, 1985).

Leaf length also shows a positive relationship with light. Eelgrass growing at depth (Dennison and Alberte, 1986), in turbid areas (Vermaat et al., 1997; Cabello-Pasini et al., 2003), and in experimentally reduced light (Olesen and Sand-Jensen, 1993; Short et al., 1995; Bintz and Nixon, 2001), exhibited greater leaf length, sustaining leaf growth even in severe shading, presumably to decrease canopy depths under reduced light intensity. An increase of leaf length represents a mechanism for increasing light interception in low light environments. This was also shown by Olesen and Sand-Jensen (1993), who found that at light intensities too low to support a net gain in plant biomass, eelgrass maintained leaf elongation.

Shading has also been found to result in reduced specific leaf weight (g dw m^{-2}) and increased specific leaf area (cm^2 g dw^{-1}) (Dennison and Alberte, 1985; Olesen and Sand-Jensen, 1993; Bintz and Nixon, 2001), although some other studies did not find any significant change in specific leaf area with depth (Dennison and Alberte, 1986) and with field manipulation of light levels (Dennison and Alberte 1982). Relative leaf growth rate (g g^{-1} dw day^{-1}) (Dennison and Alberte, 1986), leaf turnover time and leaf formation rate (in days) (Olesen and Sand-Jensen, 1994) have all been shown to decrease with decreasing light. In extreme shading, growth observations addressed in previous paragraphs, e.g., longer leaves with decreasing light become confounded. Under such conditions negative growth (representing a reduction in leaf weight per leaf area) has been shown perhaps because leaves become thinner with reduced light (Olesen and Sand-Jensen, 1993; Peralta et al., 2002), and shoot size (by weight and area) remains very small (Bintz and Nixon, 2001). Olesen and Sand-

Jensen (1993) found that under reduced light, leaves continued to elongate, to reduce canopy depth for light capture, and that such elongation occurred at the expense of leaf size and weight per leaf area. Specific growth however, does not seem to be affected by reduced light intensity (Short et al., 2005; Bintz and Nixon, 2001).

The first stage in the flowering process, the induction of flowering shoots, is primarily correlated with the irradiance the plants receive such that reduced irradiance inhibits flowering (Backman and Barilotti, 1976). The authors also showed that the course of the sexual reproductive process in *Z. marina* is affected by day length. De Cock (1981) also found that the stylar exsertion from the spathe was influenced by photoperiod.

The possession of an extensive root/rhizome system distinguishes seagrasses from all other submerged marine plants. It has been argued that the root/rhizome system which offers a competitive advantage in nutrient-poor waters, can make eelgrass plants more susceptible to reduced light conditions (Hemminga 1998). Zimmerman et al. (1996) showed proliferation of new roots in eelgrass occurring only when whole-plant carbon balance was positive, confirming that root production is suppressed in extreme light limitation (Alcoverro et al., 1999). Quantitative data on respiratory demands of eelgrass roots and rhizomes in comparison to net carbon fixation by photosynthetic tissues in the field are scarce. In a laboratory experiment by Olesen and Sand-Jensen (1993), eelgrass responded to light reduction by allocating biomass to leaves at the expense of rhizomes and roots (i.e., reducing the relative proportion of below-ground biomass). The weight of rhizomes and roots decreased faster than the respiration rates of these below-ground parts (Olesen and Sand-Jensen, 1993).

It has been suggested that the reduction in leaf weight while leaf area remains constant is a mechanism adopted by the plants to reduce respiration as increased leaf chlorophyll maximizes light absorption. Decreases in leaf weight per leaf area, leaf number, growth and production of eelgrass with decreasing light are often more dramatic in deeper-growing than shallower-growing eelgrass, i.e. deep growing plants are often more responsive to light reduction than shallow-growing plants, suggesting that light limitation of eelgrass growth increases non-linearly with depth (Dennison and Alberte, 1982; Dennison and Alberte, 1985; Dennison and Alberte, 1986; Dennison, 1987; Boström et al., 2004; Colarusso, 2007).

Community structure responses

In the field, light availability regulates eelgrass shoot density (Backman and Barilotti, 1976; Krause-Jensen et al., 2000). The relationship between shoot density and light is logarithmic as shown in mesocosm experiments (Short et al., 1995). However, in eutrophic estuaries in the Waquoit Bay system (USA) correlative modeling revealed that shoot density was uncoupled from water clarity. The decoupling was attributed to enhanced mortality of established shoots by severe light limitation caused by macroalgal canopies (Hauxwell et al., 2006). Smothering by macroalgae can cause an exponential decrease in shoot densities and bed areas resulting in substantial loss and total disappearance of eelgrass in areas with high nitrogen loading (Hauxwell et al. (2003). The authors attributed the observed decline to a lack of recruitment or enhanced mortality. Thus, another mechanism by which eutrophication affects eelgrass is through light deprivation by macroalgae and not necessarily via water column light attenuation.

In another study, the number of leaves per shoot was found to decrease with decreasing light (Bintz and Nixon, 2001), but other studies have not confirmed this observation (Short et al., 1995).

In Great Harbor Woods Hole, MA, USA, leaf biomass was found to decrease with increasing depth (Dennison and Alberte, 1986). Reduced shoot density with depth is attributed to the plants' response to regulate self-shading and reduce the respiratory demand through limited rhizome development at depth. In shallow water vegetations, where eelgrass shoot density and biomass reach high values, self-shading is less of an issue due to the abundance of light. However, self-shading and possibly space limitation lead to reduced variability in mean values of shoot density (Middelboe et al., 2003). In deeper areas where light is limiting, the risk of self-shading is reduced because of lower biomass and shoot densities (Middelboe et al., 2003). Modelling and field studies in Denmark have also shown that eelgrass biomass-shoot density relations change markedly with depth (Krause-Jensen et al., 2000; Middelboe et al., 2003). The mechanism by which a reduction in the number of eelgrass shoots occurs in response to reduced light has not been clearly demonstrated.

Duration of light reduction and its effect on eelgrass response

Most previous research has focussed on the impact of more modest light reductions on eelgrass (e.g. Backman and Barilotti, 1976; Dennison and Alberte, 1982). The degree of tolerance to and the duration of severe light reduction will determine how long eelgrass can survive below its minimum light requirements, or even under total darkness. Thus, the duration and intensity of the light reduction, and possibly its interaction with other environmental factors, determine the type of plant response (physiological or morphological), the intensity of the response (acclimation or mortality), and the time it takes for the onset of the response (Longstaff and Dennison, 1999).

Seasonal light reductions (to 10% SI or less than 3 mol photons m^{-2} d^{-1}) of 30 days in duration limited the survival of eelgrass transplants (Moore et al., 1997). Transplant mortality at previously vegetated areas in Lower Chesapeake was attributed to seasonally high levels of turbidity (Moore et al., 1996). In another experiment, *in situ* reduction of H_{sat} to 6 h for 30 days resulted in mortality of transplant survival. In an eutrophic coastal area in Japan, survival of eelgrass transplants was inhibited due to sediment deposition on eelgrass leaves that reduced light to only 36% of that without deposition (Tamaki et al., 2002). A study on the effects of light deprivation caused by an *in situ* pulsed turbidity event in the Gulf of Carpentaria, Australia on *Halodule pinifolia* and *Halophila ovalis* showed that only long duration (>38 days) of light reduced to 0.1 mol photons m^{-2} d^{-1} or 0.35% SI would cause total seagrass die-off (Longstaff and Dennison, 1999). Such studies are lacking on eelgrass. Similarly, hardly anything is known about the effect of repetitive turbidity stresses on eelgrass, especially with regard to the time required by plants (or meadows) to recover from a previous turbidity event before they can effectively cope with the next. Integrated whole-plant responses that incorporate the duration and frequency of exposure to light stress is ecologically more relevant to eelgrass management than responses to instantaneous irradiance flux (Biber et al., 2004). The findings of these studies imply that the minimum light requirement concept should incorporate a temporal dimension of light availability.

Storage and mobilization of carbohydrate reserves

Eelgrass plants have the ability to store photosynthetic products in form of non-structural carbohydrates (NSC). Carbohydrate storage varies with latitude as shown by increasing NSC concentrations in leaves and rhizomes and the relative proportion of sugar in the total NSC pool with increasing latitude (Colarusso, 2007). Rhizomes represent the major storage organ in eelgrass for non-structural carbohydrates with sucrose as the main contributor and starch forming less than 15% (Alcoverro et al. (1999); Cabello-Pasini et al., 2002; Colarusso, 2007; Vichkovitten et al., 2007). Eelgrass plants respond to low light by mobilizing these labile carbon compounds as shown by a rapid decline in soluble carbohydrates with short-term light reduction in laboratory experiments (Kraemer and Alberte, 1995; Cabello-Pasini et al., 2002), in 3-week experimental shading in the field (Burke et al., 1996), and from summer to winter (Zimmerman et al., 1995a). These studies suggest that accumulated carbohydrate reserves built up during periods of abundant light are remobilized to meet carbon deficits during periods of light reduction. Carbon depletion can also be caused by rapid early spring growth, grazing (Zimmerman et al., 1996) and high summer temperatures (Burke et al., 1996). Extreme light limitation, however, can prevent full mobilization of carbon reserves stored in below-ground tissues (Alcoverro et al., 1999). In conclusion, carbohydrate reserves can allow eelgrass to survive for certain periods below its minimum light requirements, implying that minimum light requirement models for eelgrass survival need to incorporate the role of carbohydrate reserves.

Timing of light reduction

Given the different phenological status of eelgrass plants at different times of the year, their responses to reduced light is expected to vary temporally. Eelgrass may be most vulnerable to light attenuation during the early growing season, when plants are growing rapidly. Under reduced light conditions, eelgrass invests energy in longer and wider leaves rather than in rhizome growth, preventing accumulation of carbohydrate reserves in below-ground parts. If overwintering plant parts are small (high specific leaf area) or are few in number (low shoot density and number of leaves per shoot) due to spring/summer turbidity, long-term plant survival can be seriously affected (Moore et al., 1997). In this way, the timing of a turbidity event could be critical to continued survival (Moore et al., 1997).

Significant decreases in short-term photosynthetic rates with decreasing light intensity (Bintz and Nixon, 2001) and decreases in photosynthetic rates, growth and biomass with shortened H_{sat} (Dennison and Alberte, 1985) were observed in June but not in August, suggesting that seasonal features of the eelgrass habitat (e.g. phenology) may influence photosynthetic adjustment to light environment. Even relatively short periods of extreme light attenuation can affect eelgrass survival and depth distribution (Zimmerman et al., 1991), and depending on the timing of such stressful conditions, can have major consequences for the long-term survival of eelgrass. For example, a late spring, month long turbidity event caused substantial attenuation of both light intensity and quality (wavelengths useful to eelgrass) resulting in the loss of eelgrass transplants (Moore et al. (1997). The authors of this study speculated that the regular occurrence of such short-term extreme stress events could be the cause of a lack of successful recruitment and colonization of bare areas in parts of Chesapeake Bay. In another study, exposure to high levels of turbidity in spring caused growth reduction and mortality resulting in limited transplant survival in summer (Moore et al., 1996).

In Maryland and Virginia, USA, near the southern edge of eelgrass' distribution range, springtime is the most important growth period and provides the plants with a window of opportunity for storing carbohydrate reserves (Burke et al., 1996). Shading in spring could therefore potentially reduce survival because it does not enable the plants to store adequate carbohydrate reserves to maintain a positive carbon balance throughout the remainder of the year (Burke et al., 1996). However, Colarusso (2007) showed that the timing of the accumulation of carbohydrate reserves, at least in shallow-growing plants, varies with latitude. At lower latitudes, a higher percentage of non-structural carbohydrates was stored as starch in below-ground tissues, with peak concentrations during winter and spring. At higher latitudes, this peak was during summer and fall. With light reduction during the peak carbohydrate build-up having greater negative consequences for eelgrass, the season most sensitive to light reduction will vary accordingly with latitude.

Spring plants may also be more sensitive to shading due to a low above- to below-ground ratio, which limits the plant-mediated oxidation of the sediments and thus the reoxidation of sulfides (Holmer and Laursen, 2002). As a consequence, shaded plants are more exposed to anoxic and sulfidic conditions in the sediment, affecting their growth and survival.

Although the above examples show spring to be an important period for eelgrass growth, additional light reduction during winter when day length is shortest and turbidity may be high could also be detrimental to plant survival because the daily

H_{sat} requirements may increase in winter compared to other seasons (Zimmerman et al., 1995a).

These examples underscore the importance of the timing of human activities that reduce light availability and should draw the attention of managers seeking to reduce the potential impact of,such activities.

Factors that complicate the effects of light reduction

In laboratory experiments that aim to study the effects of light reduction on eelgrass, factors other than light can usually be controlled and kept constant, which is useful to derive at direct impact-effect relationships. In the field, however, other environmental factors (e.g. temperature, nutrient concentrations) are rarely constant, which complicates the effects of light reduction on eelgrass. Furthermore, the eelgrass plants themselves go through seasonal patterns of emergence, growth, flowering and senescence, and such phenological phases of plant development further complicate the actual effects of light reduction observed in the field. Also, the effects of light reduction in the plant are not limited to the process of photosynthesis alone, but also involve complicated cascades of other secondary effects through respiration, anoxia, sulfide toxicity and related processes in the rhizosphere. There have been several studies of these aspects, some of which will be dealt with in this section.

Leaf photosynthesis is the major source of oxygen for eelgrass roots and rhizomes. Reduced light results in proportionally increased respiratory demand of these below-ground parts, negatively influencing the photosynthesis-respiration ratio and consequently the carbon balance of the whole plant. Hypoxic/anoxic and elevated sediment sulfide conditions can reduce the plants' potential for utilization of available light, or prevent them from attaining the depth limit that light levels would allow (Greve and Krause-Jensen 2005b). Both photosynthesis and respiration rates increase with increasing temperature, but respiration can increase more than photosynthesis at progressively higher temperatures (above 30°C) leading to a reduction in net photosynthesis, and resulting in anoxia in eelgrass meristems (Greve et al., 2003).

Sulfide has been shown to intrude rhizomes and meristematic tissues (Pedersen et al., 2004), and its concentrations can sometimes reach levels toxic to eelgrass (Zimmerman et al, 1989) and causing meristem rotting and mortality (Holmer et al., 2005). In high sulfide sediments, increased anoxic stress may require the plants growing in such sediments to increase their oxygen production rates, which is necessary to enhance the plant-mediated oxidation of the sediments and thus the re-oxidation of sulfides. The negative effects of sulfide on eelgrass are greater in low light than in high light. These effects include reductions in P_{max}, increases in I_c, decreases in the initial slope of the PI curve (Goodman et al., 1995), and reductions in growth and survival (Holmer and Laursen, 2002; Holmer et al., 2005). Effects of reduced light and increased sediment sulfide on P_{max} were found to be additive (Holmer and Laursen, 2002).

Prolonged exposure to reduced light can lead to carbon limitation in roots, and even though eelgrass plants have been found to adapt by increasing rates of carbohydrate transport to the roots (Zimmerman et al., 1995b; Cummings and Zimmerman 2003), carbohydrate depletion of the roots still occurs. Thus, besides whole-plant carbon balance, light availability may regulate the depth distribution of eelgrass by controlling carbohydrate transport to roots.

Internally, the physiological status of plant tissues can differ markedly with the phenological phase of the plants. During periods of rapid growth, the rate of leaf formation can be twice as high as at the end of growth season when the leaf canopy is fully developed and senescence begins, while photosynthetic capacity may be lost during flowering and senescence (sensu Dennison and Alberte, 1985).

The response of eelgrass to light reduction may be further complicated by the effects of nutrient enrichment and elevated temperature. Nutrient enrichment has been shown to lead to substantial light reduction in eelgrass areas (see eutrophication section above). Coastal waters may become increasingly sensitive to nitrogen loading as water temperature rises, suggesting that warming trends of climate may be expected to interact with eutrophication to elevate eelgrass decline in warmer areas of its growth range (Bintz et al., 2003; Burkholder et al., 2007). Water-column nutrient enrichment has an inhibitory effect on eelgrass root growth at elevated temperatures. As shown by Bintz et al. (2003), the effects of nutrient enrichment and elevated temperature on the health and survival of eelgrass are additive.

Documented decline and loss of eelgrass due to light reduction

Although a variety of mechanisms can cause eelgrass loss, researchers have increasingly linked decreased water clarity with anthropogenic disturbance, which reduces light availability to eelgrass, with the consensus that the unprecedented decline of eelgrass in recent decades is largely due to light reduction (Short and Wyllie-Echeverria, 1996; Duarte, 2002; Hauxwell et al., 2003). Despite the ability of eelgrass to acclimate to temporary reductions in light availability, decline and loss of eelgrass may still occur when light reduction is severe either in magnitude or duration.

Decline and loss due to suspended sediments

Both natural and human-induced events that cause increased sediment loading in the water column reduce light availability causing eelgrass decline. Storms reduced irradiance to nearly zero for >3 weeks in the eastern Pacific Ocean, leading to mortality of eelgrass shoots after a near depletion of sugar and starch content in the leaves (Cabello-Pasini et al., 2002). Seedlings re-appeared after the storms when water column turbidity decreased. Interpretation of satellite imagery revealed a 34% (457 ha) loss of submerged eelgrass in Bahía San Quintín, Baja California, Mexico, but a 13% (136 ha) gain in intertidal eelgrass. Losses were attributed to sediment loading and turbidity caused by a single flooding event in winter of 1992–1993, possibly exacerbated by subsequent large-scale agricultural development of adjacent uplands (Ward et al., 2003). At the coast of Iwakuni in Japan, aerial photographs showed significant losses of the vegetative cover of eelgrass meadows following a typhoon in September 1999 (Hiraoka, et al., 2001). The authors suggested that shading by fine sediments supplied from flooding rivers and deposited on eelgrass leaves reduced light intensity to less than 50%, causing the deterioration of the eelgrass meadows. A study by Onuf and Quammen (1983) revealed a 10-fold increase in silt and clay content in the eastern arm of the Mugu lagoon, California, USA, during a storm period that lasted a week, leading to complete disappearance of shallow subtidal eelgrass, which remained absent for four years. A flood that followed two years after further reduced eelgrass cover, but these recovered within 2 years.

The construction of a 32 km causeway ("Afsluitdijk") caused significant changes in the tidal range, tidal curve, and current velocities resulting in increased sedimentation and erosion in the Dutch Wadden Sea. In addition to a subsequent increase in demersal fishing frequency, resulting increases in turbidity have been blamed for the decline of eelgrass in the Dutch Wadden Sea (de Jonge and de Jong, 1992). There has been virtually no subsequent recovery of eelgrass in the Dutch Wadden Sea (Giesen et al., 1990), apparently because the effects of the construction created a new turbidity threshold in which water clarity has failed to return to the original state (van der Heide et al., 2007). Dredging operations were directly linked to varying areas of eelgrass loss in the United States (between 1.8 and 8.3 ha) (Erftemeijer and Lewis, 2006). Sedimentation on eelgrass leaves caused by "coastal development" reduced photosynthetic photon flux density to less than 36% resulting in a significant decline in eelgrass transplant survival in a eutrophic bay in Japan (Tamaki et al., 2002).

The above examples show that both natural events and human activities can reduce water clarity through increased suspended solids in the water column, eventually resulting in eelgrass decline. It is however difficult to tease apart the exact cause of loss as both natural and human factors typically co-occur. Eelgrass decline related to natural events, as opposed to human activities, may have a greater potential to recover owing to the temporary nature of such events.

Decline and loss due to eutrophication

In recent years, an explosion of eutrophication-related research has made it unequivocally clear that excess amounts of nitrogen and phosphorus entering the sea, especially those stemming from human activities, have an indirect negative effect on light availability in eelgrass, resulting in mass decline and losses of eelgrass vegetation (Short et. al., 1995; Short and Wyllie-Echeverria, 1996; Moore and Wetzel, 2000). For example, anthropogenic inputs of nutrients from groundwater (from inland farms and septic tanks) favour microalgae and macro-algal mats whose increasing biomass causes reduction of light available to eelgrass (Giesen et al., 1990; Valiela et al., 1997; Thybo-Christesen et al., 1993). Light reduction by algal blooms through it effects on vegetative shoot density, seedling density, shoot height, and growth rate have been implicated for eelgrass decline in Chincoteague Bay, Maryland (Short et al., 2006) and in the coastal waters of Japan (Sugimoto et al., 2007). In segments of Chesapeake Bay, a bloom of the red tide dinoflagellate, *Prorocentrum minimum*, in spring caused a decline in submerged aquatic vegetation (SAV), and interrupted the recovery of SAV in other parts of the bay (Gallegos and Bergstrom, 2005).

Coastal eutrophication, in addition to intensive fishing activities and altered water exchange due to construction of road banks and leisure boat harbours have been suggested as plausible causes of a 60% decrease (over two decades) in the areal extent of *Zostera marina* in the archipelago of the Swedish Skagerrak (Baden et al., 2003). In Danish coastal waters, impoverished light conditions hampered recovery after the wasting disease of the 1930s, resulting in a 75% reduction in areal coverage of eelgrass between 1900 and 1990 (Boström et al., 2003). Maximum depth of eelgrass distribution decreased from 5-6 to 2-3 m in estuaries and from 7-8 to 4-5 m in open waters. Rapid declines (up to 60% in 6 years) especially of deep water eelgrass populations were attributed to eutrophication (Frederiksen et al., 2004a, b) and now recovery is hampered by unfavourable environmental conditions (Frederiksen et al., 2004a).

Eutrophication due to increased nitrogen loading impacted an estimated 10 – 100 km^2 of eelgrass in Rhode Island and 1000 km^2 in Massachusetts (Hauxwell et al., 2001; Short and Burdick, 1996; Short et al., 1996), reducing shoot density, areal cover, biomass and productivity. Brown tides in Chesapeake Bay shaded eelgrass causing an estimated loss of about 10 km^2 (Dennison, 1989). Correlations of housing densities in the watersheds of Waquoit Bay, MA with nitrogen load, macroalgal biomass and loss of eelgrass (Short and Burdick, 1996) were experimentally confirmed with results showing macroalgal blooms as the cause of eelgrass decline (Hauxwell et. al., 2001; Hauxwell et al., 2003). The examples presented here clearly demonstrate that light reduction caused by eutrophication plays a large role in eelgrass decline worldwide.

CONCLUDING REMARKS

Eelgrass growth and distribution is controlled primarily by light availability. Increased light attenuation deriving from both natural and anthropogenic sources can lead to eelgrass decline. The concept of minimum light requirements has improved our understanding of when light availability becomes insufficient to sustain eelgrass plants. While several MLR models have been developed, examples of the practical application of these models for management purposes are few. That there are at least three different models to assess MLR makes a clear understanding of light needs difficult and might be the cause of the limited application by managers. The highly variable underwater light environment, which is complex and difficult to measure with precision as well as the specific physiological characteristics upon which the minimum light requirement estimations rely both contribute to the complexity of defining and accurately estimating the minimum light required by the plants for long-term survival. In future discussions about minimum light levels that should reach eelgrass plants, there is a clear need for greater consistency in the interpretation of *where* that light should reach (*i.e.* top of canopy, middle part of canopy, or bottom?) and *for what* this minimum is actually required (*i.e.* physiological compensation, positive carbon balance, survival of an individual eelgrass shoot or year-round persistence of an eelgrass meadow), as both of these interpretations may influence the outcome of MLR calculations.

Anthropogenic factors, in particular sediment loading and eutrophication, are the most widespread causes of light reduction leading to eelgrass decline. The water column light regime can be highly variable, the variability resulting from numerous causes that can be hard to identify or differentiate with certainty. There is a natural variability in space and time and at a variety of scales. While the natural background turbidity could determine the initial limit of acceptable turbidity increase, its high variability makes predicting or detecting what constitutes a significant "increase" in light reduction a challenge. Research on seasonal and year-to-year changes in eelgrass and the relationship of such changes to natural variation in light availability, however, is limited. Hence there is dearth of knowledge that clearly delineates changes caused by natural from those caused by human impacts. An understanding of the natural dynamics in eelgrass systems and environmental variability is required to fine-tune management of human impacts.

Eelgrass plants have adapted to a low and variable light environment by displaying plasticity in physiological and morphological responses, which allows them to continue to exist under these sub-optimal conditions. Under extreme light reduction (either as trends or as pulse events), widespread eelgrass loss occurs. A more thorough knowledge of these processes leading to eelgrass loss as well as of the factors determining success and time scales of eelgrass recovery (e.g. see Van der Heide et al., 2007) is necessary in order to fully understand the factors that regulate eelgrass distribution and may help prevent further losses.

Despite extensive work on the responses of eelgrass to light, little is known about the duration of tolerance to light reduction, and whether or not the effects of light reduction are reversible. For example, after how long and at what light levels are the first symptoms expressed? Will 10 weeks of 50% increase in turbidity have the same effect as 5 weeks 100% increase? Thus, to better understand man's role in the decline of eelgrass, studies of the frequency, duration, and intensity of changes in the light regime are needed. Deeper-growing plants are more sensitive to light reduction than shallower-growing plants, but plant dynamics at the maximum depth limit, where plants live near the minimum light for survival, has received little attention. It is at the maximum depth where plant performance and survival are sensitive to small changes in water clarity. Also, the sensitivity of eelgrass plants to changes in light quality is less understood.

Eelgrass plants presumably respond to light reduction by mobilizing carbohydrate reserves built up during periods of abundant light to meet the carbon deficit during extended periods of low light. While the major premise for monitoring carbohydrate reserves is to provide an index of potential for re-growth following events of light reduction, no studies have quantified re-growth from carbohydrate reserves. Further carbohydrate storage patterns in reproductive shoots and in eelgrass seeds is unknown.

Finally, periods of high turbidity are difficult to predict, but have the potential to limit eelgrass survival. Setting critical thresholds for water quality improvements based on the responses of eelgrass plants to light availability and minimum light levels requires that research examines further not only the cascade effects of light reduction and the other factors (e.g. temperature) that interrelate with light, but also the timescale for eelgrass response to changing light conditions (quality and quantity). This complexity implies that site-specific information is required as a first step towards a full understanding of the relationship between light availability and eelgrass light requirements (and hence depth limits).

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Table 2.1. Minimum light requirements of eelgrass (*Zostera marina* L.), expressed as % of surface irradiance.

Location	%surface irradiance	Reference
Aarhus Bight, Denmark	11	Olesen and Sand-Jensen, 1993
New Hampshire, USA	11	Short et al., 1995
Eastern Long Island Sound, USA	12	Koch and Beer (1996)
Western Long Island Sound	13	Koch and Beer (1996)
Japan	18.2	Duarte (1991)*
Woods Hole, USA	18.6	Dennison (1987)*
Roskilde, Denmark	19.4	Borum (1983)*
Chesapeake Bay, USA	20	Dennison et al., 1993
Kattegat, Denmark	20.1	Ostenfeld (1908)*
Denmark	20.6	Duarte (1991)*
York River, VA, USA	20 - 30	Moore (1991)**
The Netherlands	29.4	Duarte, 1991*

* Taken from calculations made by Dennison et al. (1993) using maximum depth limit (m) and K_d; light attenuation from respective papers.

** Based on light levels monitored at the deepest limits of seagrass growth, with variations depending on season.

Table 2.2. Overview of literature values for the minimum saturating irradiance (I_k) in μmol photons m^{-2} s^{-1} of eelgrass (*Zostera marina* L.).

Location	I_k	**Source**
Woodshole, MA, USA	9 - 88	Dennison (1987)
Zandkreek, Netherlands	80	Vermaat et al. (1997)
Woodshole, MA, USA	100	Dennison and Alberte (1982)
Great Bay Estuary, NH, USA	47 - 137	This thesis (Chapter III)
Catalonia, Spain	140	Vermaat et al. (1997)
Chesapeake Bay, USA	35 - 265	Ralph and Gademan (2005)
Sylt, Wadden Sea, Germany	863	Leuschner and Rees (1993)

Table 2.3. Summary of the daily period of photosynthesis saturating irradiance (I_k) in hours (H_{sat}) reported for eelgrass, *Zostera marina* L., where photosynthesis was measured as oxygen production.

Experimental design	H_{sat} availability	H_{sat} requirement	Study area	Method	Temperature °C	Plant part	Reference
Ungrazed plants	7 - 10	5.5 - 6	Monterey Bay, California	mesocosm	-	WP	Zimmerman et al., 1996
Grazed plants	7 - 10	13.5			-	WP	Zimmerman et al., 1996
Seasonality							
variable turbidity	4 - 12	-	Baja California, Mexico	field	15 - 26	-	Cabello-Pasini et al., 2003
Depth							
0.8m	5.8	-	Woods Hole, MA	field	-	-	Dennison and Alberte, 1986
7m	12.7	-					
Seasonality							
summer	12	3	San Fransisco Bay	field	-	WP	Zimmerman et al., 1995
winter	10	5					
depth							
0.5	10.3 - 10.8		San Fransisco Bay	field	-	-	Zimmerman et al., 1995
1.5	6.8 - 9.1						
Temperature		3 - 12 *		laboratory	10 - 20	WP	Zimmerman et al., 1989
H_{sat} varied							
2, 4, 6, 12		> 6	California, USA	laboratory	14 ± 2	-	Kraemer and Alberte, 1995
(14 days)							
H_{sat} varied							
2 and 7 hrs		7.4	California	150 l tanks	12	-	Alcoverro et al., 1999
(45 days)							
Seasonality	6 - 12	-	Woods Hole, MA	field	-	-	Dennison, 1987
Hsat, Hcomp							
H_{sat}, depth varied							
shallow	12.5	approx. 6	Woods Hole, MA	field	-	leaf	Dennison and Alberte, 1985
deep	8.9						
H_{sat}, depth varied							
shallow	9		Woods Hole, MA	field	-	-	Dennison and Alberte, 1982
deep	6.4						
CO_2							
enriched		2.7		laboratory		leaf	Zimmerman et al., 1997
non-enriched	12	7					

WP = whole plant
*depending on S:R and P_{net}:R ratios

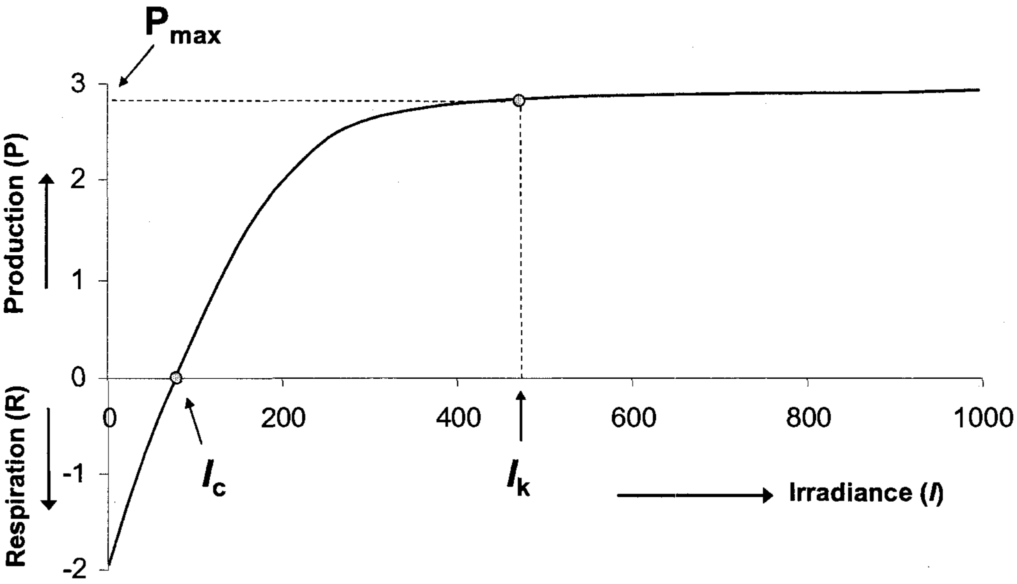

Figure 2.1. A hypothetical (oxygen) production-irradiance (*P*-*I*) curve, illustrating maximum production (P_{max}), compensation irradiance (I_c) and minimum saturating irradiance (I_k). Values shown for irradiance (µmol photons m^{-2} s^{-1}) and production (mg O_2 dm^{-2} min^{-1}) on the graph are examples only.

CHAPTER III

PHOTOSYNTHETIC AND MORPHOLOGICAL RESPONSES OF EELGRASS (*ZOSTERA MARINA* L.) TO A GRADIENT OF LIGHT CONDITIONS

ABSTRACT

Zostera marina L. (eelgrass) seedlings (thirteen weeks old) collected from drifting wrack in Great Bay Estuary, New Hampshire, and mature plants collected from Fishing Island, Maine (USA), were transplanted in outdoor mesocosms and – after an acclimation period of 22 days - were subjected to four light treatments, 100, 58, 34 and 11% surface irradiance (SI), between May and September 2003 to investigate the relationship between light availability and the growth and survival of eelgrass. Differences in photosynthetic activity measured between seedlings and mature eelgrass plants disappeared within the acclimation period. During at least the first 19 days of shading, maximum electron transport rate of seedlings did not differ significantly between light treatments. A significant reduction in maximum electron transport rate (ETR_{max}) and minimum saturating light (I_k) was observed in plants growing at 34% SI and below at 40 days. Plants shaded to 34% SI exhibited drastic reductions (to less than 25% of control) in rhizome growth, shoot density, shoot production, number of nodes per plant and plant weight at the end of the experiment. Shoot to root ratio (S:R) at this light level increased by >50%. Plants shaded to 58%

SI showed no significant difference from the control in plant parameters except the rate of rhizome elongation. Morphological responses exhibited a linear increasing trend with greater light. The results link lower shoot densities with shading to slow growth rate of horizontal rhizomes and to a total lack of lateral expansion at 11% SI. Low and declining maximum electron transport rate over time in plants at 11% SI resulted in 81% mortality, no lateral branching and no morphological development, indicating that the minimum light required for long-term eelgrass growth and survival is greater than the previously suggested 11% SI. The results demonstrate that eelgrass plants at these latitudes can persist at light levels of 58% SI and above, and are light-limited at 34% SI and below.

INTRODUCTION

Light reduction due to anthropogenic impacts is the most widespread cause of seagrass decline worldwide (Walker and McComb, 1992; Short and Willey-Echeverria, 1996; Green and Short, 2003), although other, non-light-related impacts such as disease (Muehlstein et al., 1991), herbivory (Tubbs and Tubbs, 1983; Heck and Valentine, 2007; Rivers and Short, 2007) and boat anchoring and propeller scarring (Walker et al., 1989; Creed and Amado Filho, 1999) contribute to seagrass losses. The seagrass *Zostera marina* L. (eelgrass) is found in temperate coastal estuarine environments, often influenced by anthropogenic nitrogen loading from watersheds in highly developed areas (Short and Burdick, 1996). Chronic nutrient loadings can cause excessive growth of macroalgae, phytoplankton or algal epiphytes which attenuate light in the water column. Algal growth as well as chronic (Giesen et al., 1990) or temporary (Moore et al., 1997; Longstaff and Dennison, 1999) increases in suspended sediments reduce water clarity, leading to a reduction in the amount of light available to eelgrass (Short et al, 1995; Hauxwell et al., 2003). Changes in the light regime of eelgrass habitats have caused extensive losses (den Hartog and Polderman, 1975; Giesen et al., 1990; Green and Short, 2003).

Light availability is the most important factor regulating eelgrass depth distribution limits (Duarte, 1991; Nielsen et al., 2002; Greve and Krause-Jensen, 2005). Most empirical models relating Secchi depths to maximum depth limits for eelgrass growth estimate minimum light requirements for eelgrass to be equivalent to 11% of *in situ* surface irradiance (SI), which closely agrees with minimum light demands for eelgrass survival estimated in laboratory experiments (Zimmerman and Alberte, 1991; Olesen and Sand-Jensen, 1993). Responses of eelgrass to light manipulations, both in the field and laboratory, have shown that light availability imposes an ultimate limit on eelgrass biomass and production (Backman and Barilotti, 1976; Dennison and Alberte, 1982; Short et al., 1995; Bintz and Nixon, 2001). Few have studied the effects of light reduction on lateral expansion and branching patterns of individual plants (Bintz and Nixon, 2001), and the extent to which plants respond to reduced light availability through acclimation in biomass partitioning between shoots and rhizomes (Olesen and Sand-Jensen, 1993; Hemminga, 1998).

Responses of eelgrass plants to light reduction in the field (Dennison and Alberte, 1985; Dennison, 1987) have not been compared between seedlings and mature plants, while experiments using seedlings are rare (Bintz and Nixon, 2001) or have not incorporated photosynthetic parameters (van Lent and Verschuure, 1995). Established eelgrass meadows are maintained primarily by vegetative production of lateral shoots (Olesen and Sand-Jensen, 1994; Olesen, 1999), while the colonization of new areas depends largely on seed dispersal and subsequent seedling establishment (Harwell and Orth, 2002; Greve et al., 2005; Erftemeijer et al., 2008). In order to better understand the relationship between light availability and the growth and survival of eelgrass, we examined the photosynthetic responses (photosynthetic rates and maximum quantum yield of photosystem II) of eelgrass seedlings in comparison to mature plants under different light conditions over time. In addition, eelgrass seedlings grown for 103 days in mesocosms were examined for their morphological (lateral branching, rhizome growth, shoot production and biomass allocation) responses to light reduction. The study demonstrates that eelgrass growing well above 11% SI can be light limited. A parallel study (Walker et al., in prep.) examined morphological and reproductive changes in the same plants to understand the seedling development and dependence of the timing of plant resource allocation on light availability.

MATERIALS AND METHODS

Experimental design

Three month old *Z. marina* seedlings, their age identified by the presence of a seed coat still attached to the cotyledon and by the number of leaves, were collected from drifting wrack in Great Bay, New Hampshire, USA (43°05'N, 70°50'W) in May, 2003. On May 22nd, 2003 at the Jackson Estuarine Laboratory, a total of 360 pre-weighed seedlings were transplanted into 10 cm deep seawater, 30 plants in one half of each of twelve 1 m^3 outdoor mesocosms equipped with flow-through seawater from the adjacent estuary and with pumps providing water circulation (Short et al., 1995). The mesocosms had been filled with 15 cm-deep sandy/muddy sediment from the bay. Mature eelgrass shoots (leaves, roots and 10 cm of rhizome), collected from a shallow subtidal eelgrass bed at Fishing Island, Maine, USA (43° 04'N, 070° 42'W), were transplanted in the other half of each mesocosm at 30 shoots per half tank on June 2nd, 2003. After 13 days, the water level in all 12 mesocosms was raised an additional 30 cm. Epiphytes and macroalgae were removed regularly by hand while mud snails (*Ilyanassa obsoleta*) were added to each tank to control diatoms and green algae.

After allowing 22 days' recovery from transplant stress, light treatments were randomly applied by shading the mesocosms using neutral density screening, so that mean surface irradiance under the screens was 11%, 34% and 58% of solar photosynthetically active radiation (PAR) at the surface measured with a Licor 2π sensor (Short et al., 1995). Three unshaded (100% SI) replicate mesocosms were used as controls. Each irradiance level (treatment) was replicated three times, with each mesocosm representing an experimental unit.

Incoming irradiance was monitored by the Jackson Estuarine Laboratory weather station, where measurements were recorded at 15-min intervals with a Licor 2π sensor and datalogger. Seawater temperature in each mesocosm was monitored every 30 minutes using Onset Stowaway TidbiT® temperature loggers suspended in the water column during the study. Temperature data were averaged by month.

Fluorescence measurements

Photosynthetic characteristics of both seedlings and mature eelgrass plants under different light treatments were quantified once before shading, and again after 6, 19, 40, 47, and 63 days of shading. Two measurements were made per tank and then averaged. Using the Diving-PAM® (Pulse Amplitude Modulated) fluorometer (Walz, Germany), we determined (i) the rate of electron transport (ETR) between photosystem II and photosystem I, which measures the ability of the plants' photosystems to use incident light, and (ii) the maximum quantum yield of PSII (F_v/F_m).

Electron transport rate (ETR)

ETR was determined using Rapid Light Curves (RLCs) (Ralph and Gademann, 2005). The middle section of the third leaf (Durako and Kunzelman, 2002) of both seedlings and mature eelgrass shoots was enclosed in a leaf clip and exposed to eight incremental steps of artificial irradiance pre-programmed in the PAM. Each step lasted 10 s to allow relaxation after each saturating pulse while the effective quantum yield was being measured. The irradiances used ranged from 0 to 1740 μmol photons m^{-2} s^{-1}.

Electron transport rate (ETR) was estimated according to Beer et al. (2001) using the effective quantum yield (Y) as follows:

$$\mathrm{ETR} = \mathrm{Y} * \mathrm{PAR} * 0.5 * 0.84$$

where, Y is effective quantum yield calculated as: $(F_m' - F)/F_m'$; PAR is the corresponding actinic light step generated by the internal halogen lamp of the PAM; 0.5 is the assumed equal distribution of photons absorbed by the two photosystems; and 0.84 is the PAM's default value for the proportion of incident photons absorbed by the photosynthetic pigments, a value comparable to a mean absorption factor of

0.846 ± 0.004 ($n = 209$) measured for eelgrass plants from Great Bay Estuary in June and September, 2004 using the quantum sensor of the PAM and reading without leaf (A), then with leaf (B) and calculating the relative light absorption by using the formula: AF = 1-B/A according to Beer et al. (2001). AF values were found to be comparable to absorptance of eelgrass leaves measured in the laboratory using an integrating sphere and a light source from a high intensity illuminator (Dolan Jenner Industries Inc.) set at high. All measurements were standardized to the middle section of the third leaf devoid of epiphytes.

Maximum quantum yield (F_v/F_m)

To estimate the maximum quantum yield (= potential photosynthetic efficiency) of photosystem II (PSII) (*sensu* Beer et al., 2001), leaves were dark adapted for 10 minutes using "dark leaf clips" to allow the reaction centers of PSII to be oxidized or "open", and thus the minimum fluorescence (F_o) to be recorded. The leaves were subsequently exposed to a 0.5 – 1.0 s period of saturating light (> 2000 μmol photons m^{-2} s^{-1}), which reduced the PSII reaction centers, consequently raising the fluorescence yield to a maximum value (F_m). Maximum quantum yield was calculated by deriving F_v/F_m as follows:

$$F_v/F_m = (F_m - F_o)/ F_m$$

where F_v is the variable fluorescence.

ETR versus irradiance curves

ETR_{max} was derived by fitting the RLCs of each replicate to the Jassby and Platt (1976) equation to establish the relationship between ETR and absorbed irradiance, where absorbed irradiance is: (PAM generated PAR)*0.5*0.84 (Saroussi and Beer, 2007). An iterative process (using Solver in Excel, Microsoft©) was applied using the least squares method (Zar, 1984). The minimum saturating irradiance (I_k) was calculated by dividing the maximum electron transport rate by the initial slope.

Plant measurements

Eelgrass Production

After 45 days of shaded growth in the mesocosms, the rhizomes of 5 shoots per tank were tagged to measure shoot production and rhizome growth (Short and Duarte, 2001); the sediment was carefully fanned to expose the rhizome meristem for tagging. The rhizome plastochrone interval (P_R) (days) was determined by dividing the number of new nodes produced by the time interval between tagging and harvesting (35 – 42 days).

Eelgrass Morphometric Parameters

At the end of the study (81 days of shading; 103 days of growth in mesocosms), all surviving seedlings were individually harvested and brought to the laboratory, keeping all the lateral shoots intact and retaining the structure of each individual plant (one terminal shoot (originally one seedling) and associated lateral shoots). Morphological features of the means of 5 plants per mesocosm were analyzed by measuring total number of rhizome nodes per plant, number and order of laterals per plant, number of leaves per shoot, sheath length, and length and width of the third leaf of each sample shoot. Specific leaf area (SLA) was calculated as leaf area divided by leaf weight. Weight per plant was determined after drying each plant part at 60 °C to constant dry weight.

Statistics

Means and standard errors for all measured variables were calculated for each light treatment for seedlings and mature eelgrass plants. Data were tested for normality and proportions arcsine-transformed before performing parametric analyses. A 2 x 4 factorial ANOVA was used to test the response of eelgrass seedlings vs. mature plants to light reduction. For seedlings, a one-way ANOVA followed by a post hoc analysis (Tukey's test) of significant effects set at $\alpha = 0.05$, and a least squares regression analysis, were used to test the effects of light treatment on photosynthetic characteristics, growth and morphology. The effects of shading on photosynthetic parameters were tested on combined data of measurements made after 6 through 63 days of shading treatment on seedlings only. In addition, the effects of shading level on photosynthetic parameters were tested using an analysis of covariance (ANCOVA) with days of shading (time) as the covariate, followed by a regression analysis for each light treatment. All statistical tests were performed using Systat Software Inc. version 11, California, USA.

RESULTS

Mean monthly PAR during the study period varied between 289 (in August), and 387 (in July) µmol photons m^{-2} s^{-1} (Table 3.1), providing calculated values of 168 – 223 µmol photons m^{-2} s^{-1} in the shade screens of mesocosms receiving 58% SI, 98 – 131 µmol photons m^{-2} s^{-1} in the mesocosms receiving 34% SI, and 32 – 42 µmol photons m^{-2} s^{-1} in the mesocosms receiving 11% SI. Monthly mean temperature in the mesocosms increased with month (Table 3.1), with no significant temperature differences between light treatments (greatest difference 0.6 °C).

Photosynthetic characteristics

Seedlings vs. mature eelgrass

With the exception of the first sampling (20 days after transplanting and before shading), there were no significant differences (ANOVA; $p > 0.05$) between seedlings and mature eelgrass for the photosynthetic parameters ETR$_{max}$ (Figure 3.1) and I_k. Thus, photosynthetic data described in the subsequent paragraphs below are those from seedlings only, and represent combined measurements from day 6 through day 63 of shading for each treatment. At 20 days after transplanting, ETR$_{max}$ of seedlings was 30.5 ± 1.3 µmol electrons m^{-2} s^{-1}, 1.5 times lower (ANOVA; $n = 10$; $p = 0.0001$) than that of mature eelgrass plants (48.1 ± 0.7 µmol electrons m^{-2} s^{-1}). I_k was 46.9 ± 2.2 µmol photons m^{-2} s^{-1} in seedlings, 1.6 times lower (ANOVA; $n = 10$; $p = 0.002$) than in mature plants (72.9 ± 2.9 µmol photons m^{-2} s^{-1}). Maximum quantum yield of PSII (F_v/F_m) ranged between 0.69 ± 0.02 and 0.75 ± 0.004, and was not significantly different (ANOVA; $n = 15$; $p = 0.583$) between seedlings and mature eelgrass plants throughout the study. Neither seedlings nor mature plants showed evidence of down-regulation of photosynthesis even at elevated irradiances (Figure 3.1a & b).

Effects of shading (seedlings only)

ETR_{max} of eelgrass shoots showed a logarithmic relationship with light after 63 days of shading (Figure 3.2a), with the effect of shading becoming apparent at 34% SI for ETR_{max} (Table 3.2). I_k showed similar results (Table 3.2). In contrast, maximum quantum yield of PSII (F_v/F_m) was significantly higher ($p < 0.0001$) in the most shaded eelgrass (receiving 11% SI), and decreased with increasing light (Figure 3.2b).

Over time, however, photosynthetic activity did not differ significantly between light treatments until day 40 (Figure 3.3), when plants at 34% and 11% SI exhibited significantly lower ETR_{max} and I_k than plants at 100% SI ($p < 0.05$). At 63 days, even the difference between the two lower light levels was significant ($p = 0.02$; Tukey's HSD).

While the ETR_{max} and I_k of the most shaded plants significantly decreased over 63 days of shading, ETR_{max} and I_k of unshaded plants significantly increased (Figure 3.3a and b). The trends seen in the two most extreme light treatments were corroborated by a significant interaction between time and light treatment for ETR_{max} (ANCOVA; $n = 60$; $p = 0.001$) and I_k (ANCOVA; $n = 60$; $p = 0.022$). The ETR_{max} of eelgrass plants receiving 11% SI decreased by 14% ($r^2 = 0.3918$; $p = 0.029$) between the 6th and 63rd days of shading, while that of plants receiving 100% SI increased by 40% ($r^2 = 0.700$; $p = 0.0001$) (Figure 3.3a and b). Similarly, I_k of plants receiving 11% SI decreased by 20% ($r^2 = 0.39$; $p = 0.013$), while that of plants receiving 100% SI increased by 37% ($r^2 = 0.36$; $p = 0.018$ (Figure 3.3b). There were no significant changes ($p > 0.05$) in ETR_{max} and I_k at intermediate (58% SI and 34% SI) light treatments. Maximum quantum yield (F_v/F_m) of plants at all light treatments, except at 34% SI, increased significantly over time (ANCOVA; $n = 16$; $p = 0.0001$) (Figure 3.3c).

Plant characteristics and growth

All plant morphological and plant growth parameters showed an increasing trend with greater light ($p < 0.05$) (Table 3.2; Figure 3.4). Eelgrass seedlings receiving 100% SI exhibited the highest rate of growth and lateral expansion, while those receiving 11% SI remained small. Relative to control (100% SI), plants growing in the 34% SI mesocosms exhibited relatively low survival (46% compared to 76% in controls), 82% reduction in rhizome elongation, 77% reduction in shoot production, 74% reduction in weight per plant, significant reduction in below ground storage (> 50% increase in S:R), and significant reduction in lateral expansion (only first order lateral branches compared to up to 6th order in 100% SI) (Table 3.2; Figures 3.4 & 3.5).

At the end of the experiment, none of the surviving plants in the 11% SI treatment showed lateral branching: only the original terminal shoot of the seedlings remained. At 34% SI, only first order lateral shoots had been produced, while at 100% SI more than 50% of the shoots were higher order laterals (laterals off the original branching) (Figure 3.5a). Up to 6th order laterals were observed in some plants at 100% SI. Both above- and below-ground plant weight was greater with increasing light (Figure 3.5b); the S:R was lower with increasing light (ANOVA; $n = 3$; $p = 0.0001$). A decreasing trend was evident in S:R at light levels between 34 and 100%

SI, with values ranging from 5.3 ± 0.5 in plants receiving 34% SI to 2.8 ± 0.1 in plants receiving 100% SI. At 11% SI, the mean S:R was 3.8 ± 0.8, and was not significantly different from the higher light treatments (Table 3.2). Mean total plant weight was significantly lower at lower light levels (11 and 34% SI) than higher light levels (58% and 100% SI) (Figure 3.5b; Table 3.2).

The mean leaf width of both terminal and lateral shoots measured at the end of the experiment was significantly lower in plants receiving 34% and 11% SI (p = 0.0001) than the other light treatments (Table 3.2). Leaf length, on the other hand, was significantly lower (p = 0.0001) only in terminal shoots receiving 11% SI (Table 3.2). Leaf length of lateral shoots did not respond significantly to shading (Table 3.2). SLA averaged 612 ± 62 $cm^2 g^{-1}$ for lateral, and 417 ± 21 $cm^2 g^{-1}$ for terminal shoots and was not affected by shading (ANOVA; $n = 3$; $p = 0.86$). The number of leaves per shoot averaged 3.0 ± 0.6 for lateral and 5.0 ± 0.8 for terminal shoots, and was also not affected by shading (ANOVA; $n = 3$; $p = 0.384$). Seedling survival in the most shaded mesocosms was significantly lower (81% mortality; ($p = 0.041$) than in the mesocosms receiving 34%, 58% and 100% SI (Table 3.2).

DISCUSSION

The present mesocosm study demonstrates that 11% SI is inadequate for long-term eelgrass survival. The study further shows that eelgrass at these latitudes (with temperatures between 18°C and 23°C) can persist when light availability is reduced to 58% SI, and that eelgrass is light-limited at 34% SI and below. In the present experiment, it took 28 days for the maximum electron transport rate of 13-week old eelgrass shoots transplanted at the monocotyledon stage to equal that of mature eelgrass. Although significant growth occurred at 34% SI, with surviving seedlings reaching maturity and even flowering, plant vigor at this light treatment was compromised. The work of Beer et al. (1998) demonstrated that PAM fluorometry can efficiently yield photosynthetic rates of *Zostera marina* L. in the laboratory as well as *in situ* under natural conditions. Based on Beer et al. (1998), we assume, in the current study, that the maximum electron transport rate (ETR_{max}) represents photosynthetic capacity, i.e., the rate of photosynthesis measured at saturating light intensity.

Increasing photosynthetic rate with greater light is a typical response of temperate seagrasses adapted to low light and low temperature regimes (Dennison and Alberte, 1982). In agreement, the 63-day shading experiment showed a logarithmic relationship of photosynthetic capacity (denoted by ETR_{max}) with light (Figure 3.2). Eelgrass plants receiving 100% SI adapted to full sunlight and were tolerant of PAM-generated irradiances as high as 1740 μmol photons $m^{-2}s^{-1}$, showing no evidence of photosynthetic down-regulation (Figure 3.1). A high photosynthetic capacity (ETR_{max}) represents the ability to transfer more electrons at high light and therefore to process more solar energy. Because a high photosynthetic capacity is often matched with a greater investment in the plant's biochemical machinery for carbon dioxide fixation (Lawlor, 2001), high-light adapted plants display a higher minimum saturating light (higher I_k) (Figure 3.3b). At low light, a limited number of photons reaches the leaf surface, such that there is no advantage for plants at low light to have a large capacity electron chain (Loomis and Amthor, 1999). Per leaf surface area, shade-adapted plants have fewer functional reaction centers (Chow et al., 1990), leading to a lower saturation point (i.e., lower I_k at low light).

ETR_{max} measured in mature plants after 20 days of growth in full sunlight in this study (48 μmol electrons m^{-2} s^{-1}) was lower than that measured by Ralph and Gademan (2005) (63 μmol electrons m^{-2} s^{-1} when an absorption factor of 0.84 is assumed) in mature eelgrass maintained at high light for two weeks. The discrepancy may be attributed to using different irradiances (absorbed irradiance vs. incident irradiance) in estimating ETR. Consistent with previous reports (Dawson and Dennison, 1996; Major and Dunton, 2002; Durako et al., 2003), low light plants (34% and 11% SI) in the present study exhibited a higher maximum quantum yield of PSII (F_v/F_m) than high-light adapted plants (58% and 100% SI), demonstrating a greater efficiency at capturing photons under low light conditions as well as a greater efficiency at transducing light energy than high-light adapted plants (Bulchov et al., 1995). At 34% SI, after 63 days of shading, photosynthetic capacity of eelgrass (Table 3.2) was significantly less than measured at 100% SI, but significantly greater than the 11% SI treatment, implying that these plants would survive but not persist. At 11% SI, a high (Figure 3.2c) and increasing F_v/F_m over time (Figure 3.3c), coupled with a low and decreasing ETR_{max} and I_k (Figure 3.3a and b) confirm trends of photoacclimation to low light, serving to increase the effectiveness of light utilization in an attempt to reach sufficient photochemical activity to achieve daily carbon gain under light-limitation. Despite such effective adjustment, the 11% SI light level was insufficient for the plants to sustain growth at the prevailing temperatures (18 - 23°C): the decreasing photosynthetic capacity with time (Figure 3.3a) implies that these plants were actually dying.

The present mesocosm experiment was done at the prevailing seasonal water temperature, with average water temperature in the mesocosms increasing from 18°C

(June) to 23°C (July) (Table 3.1) and not different between light treatments. The water temperature in the mesocosms was likely to be slightly higher than that in Great Bay Estuary, which ranges between 0°C in winter and 17 or 22°C in summer depending on the position in the estuary, with maximum values in August. The seasonal trend in water temperature in the bay suggests that the mesocosm temperatures would also be higher in August. Over time, photosynthetic capacity (ETR_{max}) and saturation point (I_k) increased significantly in plants at 100% SI, did not show a significant trend in plants at 58 and 34% SI, and decreased significantly in plants at 11% SI (Figure 3.3). The increases at 100% SI, indicate that plants growing in full sunlight were acclimating to the seasonal increase in water temperature, which reaches its peak in September in Great Bay (Lee et al., in prep). There was no significant difference in plant photosynthetic capacity between 58% SI and the control at any time during the experiment, but at 34% SI, drastic reductions were observed in both photosynthetic and morphological attributes at the end of the experiment (Figure 3.3; Table 3.2). Stable and comparable photosynthetic activity over time at 58% and 34% SI (as opposed to increasing photosynthetic activity at 100% SI) suggests that the potential positive effect of increasing temperature on photosynthesis of plants (Marsh et al., 1986; Zimmerman et al., 1989) growing at these light levels was reduced by light availability.

Plant morphology results of this study (Figure 3.4; Table 3.2) confirmed the linear increase in eelgrass morphological and growth parameters with increasing light reported earlier by Short et al. (1995), and confirm light availability to be a limiting factor for eelgrass growth (Dennison et al., 1993). Here we show morphological analysis along with photosynthetic activity over time, which together suggest that for survival, the minimum light requirement for eelgrass must be higher than the previously reported 11% SI (Duarte 1991; Zimmerman and Alberte, 1991; Olesen and Sand-Jensen, 1993). We show that although eelgrass growth can be supported at 34% SI during the summer growing season, significantly lower photosynthetic capacity, shoot weight, and shoot production relative to control, coupled with significantly greater maximum quantum yield of PSII and S:R (Table 3.2) (which are photo- and structural acclimations to increase the effectiveness of light use and reduce respiratory costs of rhizomatous tissue) demonstrate less than optimal capacity of plants at 34% SI to sustain eelgrass meadows in the long-term.

Vegetative reproduction through rhizome branching in seagrasses generally makes it difficult to delineate what constitutes an individual plant. The present experiment made the first attempt to quantify rhizome growth and lateral branching of individual eelgrass seedlings. By tracking individual ramets (genetically identical shoots formed by rhizome branching) within experimental treatments, we were able to show that the observed linear increase in the number of shoots per plant (which determined shoot density) was a direct consequence of increased lateral branching with greater light (0 branches per plant at 11% SI vs. up to 29 shoots per plant in 100% SI) (Walker et al., in prep). Rhizome elongation was the only morphological parameter which showed significant differences between 58% and 100% SI (Table 3.2), demonstrating that even at 58% SI, eelgrass is growing at sub-optimal light conditions.

Low shoot density at low light is a well-known response adopted by eelgrass to reduce self-shading (Bulthuis, 1983; Gordon et al., 1994; Short et al., 1995; Krause-Jensen et al., 2000). Results of the present study of low shoot density with less light (Figure 3.3a; Table 3.2) provide evidence that links lower shoot densities with moderate shading to the slow growth rate of horizontal rhizomes and the total lack of lateral expansion in extreme shading. Inhibited lateral branching at low light provides a mechanism to explain the lower shoot densities reported from eelgrass growing in more turbid environments (Moore et al., 1997; Hauxwell et al., 2006) or at greater depths (Krause-Jensen et al., 2000; Middelboe et al., 2003), and confirms that eelgrass bed structure, defined by shoot density and shoot length, may be controlled by light availability in estuarine waters (Dennison and Alberte, 1985).

The contribution of roots and rhizomes to total eelgrass respiration depends, at least in part, on the biomass of such organs relative to that of photosynthetic organs, represented by the shoot-to-root ratio (S:R) (Zimmerman et al, 1989; Olesen and Sand-Jensen, 1994; Hemminga, 1998). By initially planting individual eelgrass seedlings and subsequently monitoring their development, This study showed that plants responded to reduced light conditions by an increasing S:R as a structural acclimation that reduces the respiratory demand of non-photosynthetic tissues. At 34% SI, S:R ratio increased by > 50% of the control, suggesting that with less light, individual plants allocated greater amounts of photosynthate to leaf growth rather than to storage in the rhizomes. However, at 11% SI, plants had insufficient light to produce substantial above- or below-ground tissue such that the S:R was not different from control (Figure 3.5b; Table 3.2).

Counts of the number of rhizome nodes allowed reconstruction of the number of leaves produced during eelgrass growth in the mesocosms (Jacobs, 1979; Duarte et al., 1994). After 81 days of shading, an average of only 7 leaves had been produced by a surviving seedling at 11% SI, but as many as 63 leaves had been produced per seedling at 100% SI (Table 3.2). The linear increase in leaf production with greater light confirms the strong effect of light availability on new leaf production (Dennison and Alberte, 1986; Short et al., 1995; Bintz and Nixon, 2001). Leaf width of eelgrass plants in this study demonstrated a significant logarithmic increase with increasing light whereas leaf length remained constant at light levels above 34% SI. Plants hardly grew at 11% SI. In the mesocosms, maximum leaf length for all plants at 34% SI and higher was controlled by the water depth in the tanks, a limitation of the mesocosm set-up in investigating this plant parameter (Burdick-Whitney and Short, in prep).

The lack of build-up of below-ground carbon reserves suggests that seedlings exposed to light levels less than 34% SI during the growing season are unlikely to survive winter light and temperature stress (Burke et al., 1996). In winter, shorter periods of daylight, reduced light levels and low temperatures limit carbon assimilation and growth, and accumulated carbohydrates in eelgrass rhizomes may play a critical role in the survival and re-growth of the plants in spring (Alcoverro et al., 1999; Ochieng and Short, in prep).

CONCLUSION

For the first time, we related temporal measurements of photosynthesis with measurements of plant morphology to explain eelgrass plant response to a gradient of light conditions. Plant weight, rhizome elongation, leaf production, lateral branching and shoot density increased linearly with greater light, confirming light as a limiting factor for eelgrass growth. At 58% SI and above, eelgrass plants would persist , but rhizome elongation was significantly lower than at full light. At 34% SI, plants were light limited, but photosynthetic activity did not change over time. Plants responded to light reduction at 34% SI level with a significant increase in S:R as a structural acclimation to reduce the respiratory costs of non-photosynthetic tissues. Although growth and reproduction could be maintained at 34% SI, this light level was less than optimal for plant morphological development and produced plants that quite likely would not survive the winter due to their limited underground resources. At 11% SI, low survival and decreasing photosynthetic capacity and low biomass production in surviving plants suggested that 11% SI is below the minimum required for long-term growth and survival of eelgrass at the prevailing temperatures. The present study suggests that when eelgrass is subjected to severe light reduction, the symptoms of physiological stress may not appear until at least 19 days. The study provides evidence that links low shoot density caused by sahding to the slow growth rate of horizontal rhizomes and a lack of lateral expansion in extreme shading; and confirming that eelgrass bed structure is also controlled by light availability.

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Table 3.1. Integrated daily PAR, daily average and daily maximum at Jackson Estuarine Laboratory weather station, and the mean, maximum and minimum seawater temperature (°C) measured in 12 mesocosms. Numbers are means (± SE).

	June	July	August
Incident irradiance:			
Integrated Daily PAR (mol photons m^{-2} d^{-1})	28 (2)	33 (2)	25 (2)
Daily Average PAR (μmol phtons m^{-2} s^{-1})	326 (26)	387 (23)	289 (18)
Daily Max. PAR (μmol photons $m^{-2}s^{-1}$)	1039 (69)	1194 (60)	1019 (50)
Water temperature (°C):			
Mean	18.4 (0.5)	22.6 (0.3)	
Maximum	27.8 (0.8)	28.4 (0.5)	
Minimum	11.2 (0.2)	17.8 (0.2)	

Table 3.2. Analysis of variance of eelgrass photosynthetic responses to light manipulation (measured after 63 days of shading) and morphological, below-ground growth and flowering responses to light manipulation (measured after 81 days of shading). Water temperature in mesocosms varied between 18°C and 23°C. Mean values followed by the same letters are not significantly different at $p < 0.05$.

Variable	Treatment				*df*	MS	ANOVA *F*-value	*p*
	11%	34%	*58%*	100%				
ETR$_{max}$ (μmol electrons m^{-2} s^{-1})	47.79^{a}	67.85^{b}	74.89^{bc}	90.39^{c}	3	937.226	23.024	< 0.0001
I_k (μmol photons m^{-2} s^{-1})	64.57^{a}	98.34^{ab}	114.67^{bc}	137.19^{c}	3	2801.676	15.363	< 0.0001
F$_v$/F$_m$	0.811^{a}	0.775^{b}	0.751^{b}	0.762^{b}	3	0.010	11.426	< 0.0001
Number of shoots (shoots plant^{-1})	1^{a}	5^{b}	11^{bc}	17^{c}	3	143.525	166.629	0.006
Number of nodes (nodes plant^{-1})	7^{a}	18^{ab}	45^{c}	63^{c}	3	1903.940	23.414	< 0.0001
Rhizome growth (g dw plant^{-1} d^{-1})	0^{a}	0.017^{ab}	0.068^{bc}	0.112^{c}	3	0.008	15.174	0.001
Rhizome elongation (cm plant^{-1} d^{-1})	0.05^{a}	3.17^{ab}	8.07^{c}	18.00^{d}	3	184.585	54.538	< 0.0001
Shoot production (new shoot d^{-1})	0.01^{a}	0.05^{a}	0.10^{ab}	0.21^{b}	3	0.023	10.425	0.004
Weight (g dw plant^{-1})	0.081^{a}	0.578^{a}	1.787^{b}	2.241^{b}	3	13.158	23.256	< 0.0001
shoot to root ratio (S:R)	3.832^{a}	5.32^{b}	3.906^{a}	2.763^{ac}	3	0.548	8.688	< 0.0001
Leaf length (cm)								
Terminal shoots	25.9^{a}	71^{b}	69.1^{b}	67.1^{b}	3	849.563	26.537	< 0.0001
Lateral shoots		29.6	36.5	32.5	2	35.779	1.084	0.396
Leaf width (mm)								
Terminal shoots	1.8^{a}	3.7^{b}	4.7^{bc}	5.3^{c}	3	7.055	40.627	< 0.0001
Lateral shoots		2.6^{a}	3.1^{ab}	3.4^{b}	2	0.512	8.954	< 0.0001
Survival (% plants)	19^{a}	46^{b}	72^{b}	76^{b}	3	0.129	4.411	0.041
Flowering frequency (% generative plants)	0^{a}	24^{b}	26^{b}	33^{b}	3	0.201	33.127	< 0.0001

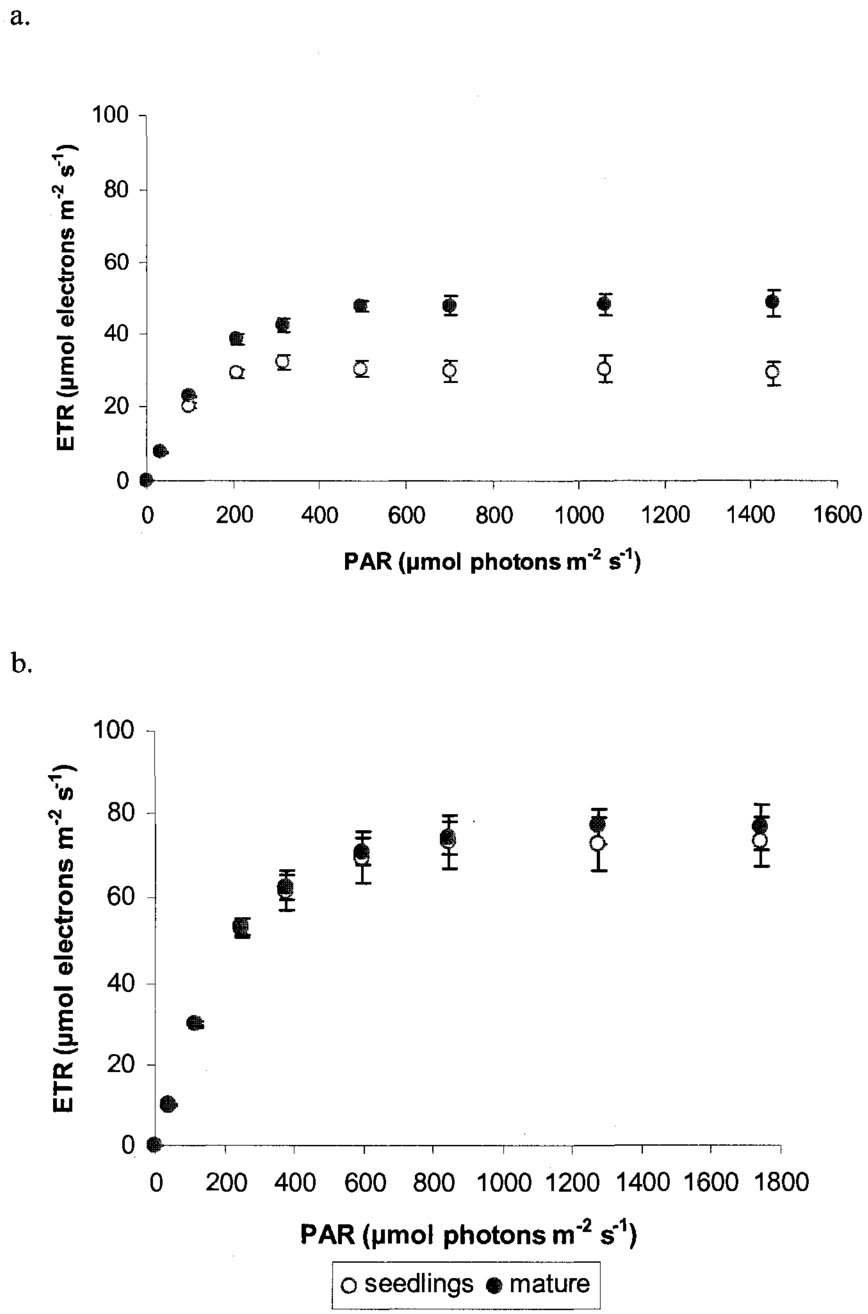

Figure 3.1. Photosynthetically active radiation (PAR) using a diving-PAM versus electron transport rate (ETR) plotted from rapid light curves for seedlings and mature eelgrass plants (a) 20 days after transplanting and (b) 28 days after transplanting. Values are means ± SE, *n* = 6.

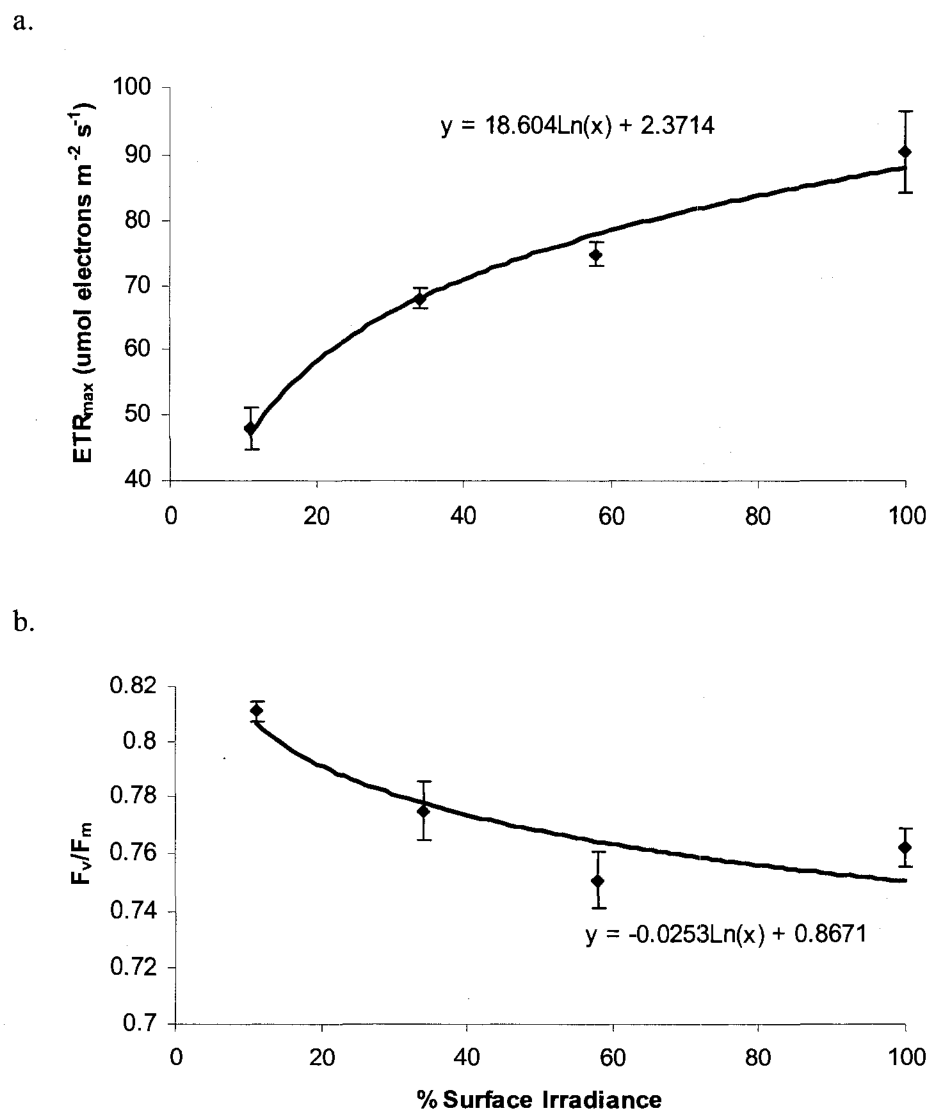

Figure 3.2. Effect of shading on (a) maximum electron transport rate (ETR$_{max}$) and (b) maximum quantum yield of photosystem II (F$_v$/F$_m$) of eelgrass seedlings. Values are means ± SE of measurements taken on day 63 of shading treatment, *n* = 3.

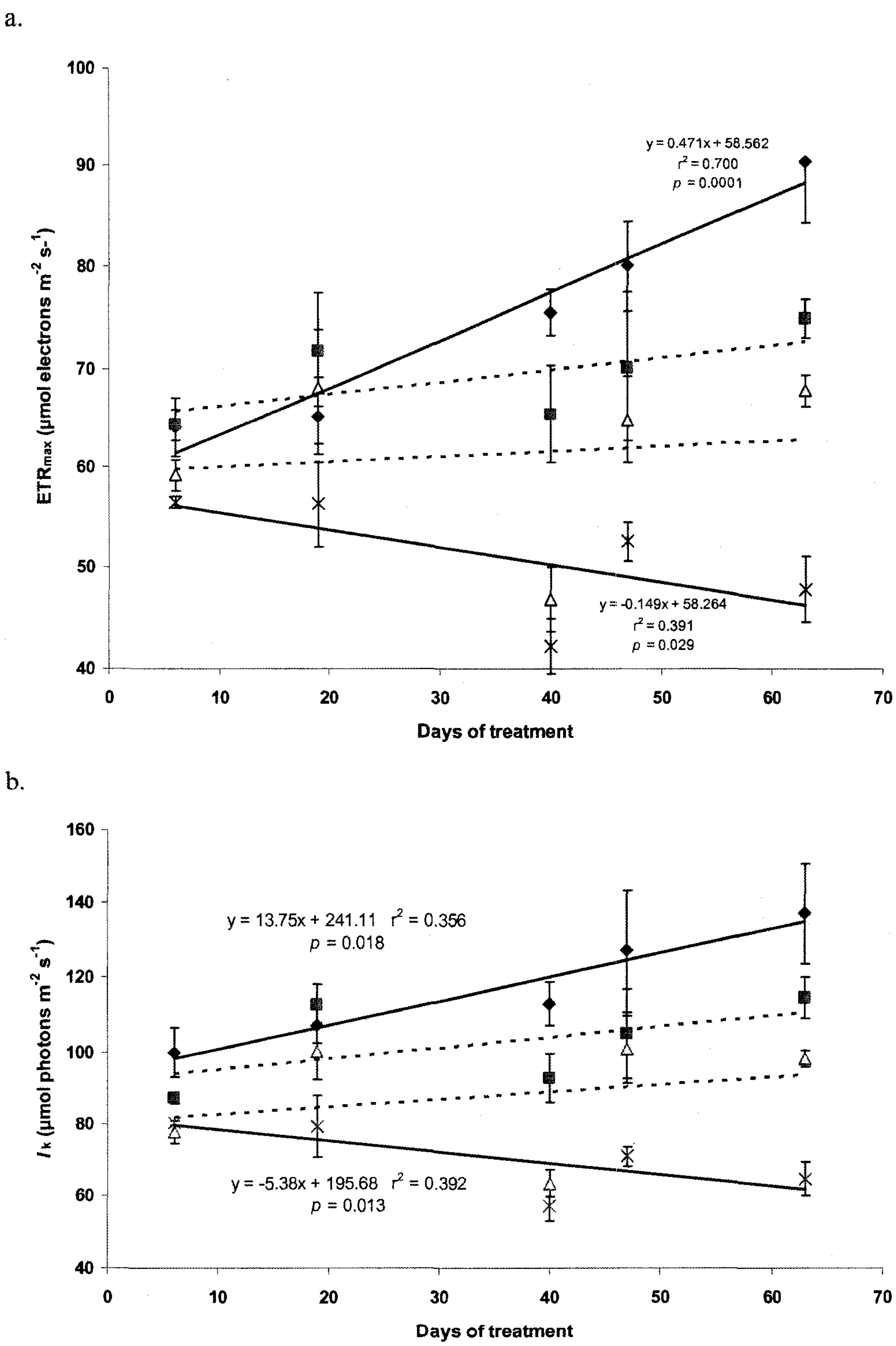

Figure 3.3. (see next page for figure caption)

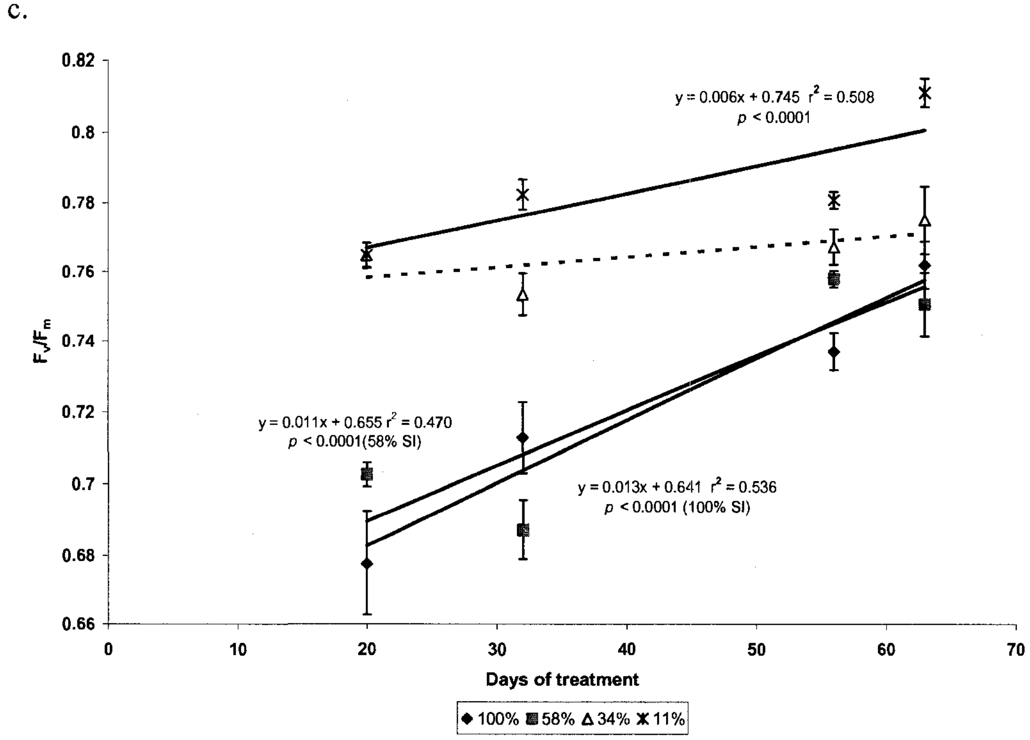

Figure 3.3. Response of eelgrass seedlings to reduced light from shading over time (days of treatment) on the (a) mean maximum electron transport rate (ETR_{max}), (b) minimum saturation irradiance (I_k), and (c) maximum quantum yield of photosystem II (F_v/F_m) of eelgrass seedlings growing in mesocosms. Light treatments are represented by diamond, (◆) (100% SI); square (■) (58% SI); triangle (▲) (34% SI) and x (x) (11% SI). Significant ($p < 0.05$) trends are shown by bold regression lines. Non-significant slopes are shown by dashed lines. Values are means ± SE, $n = 3$.

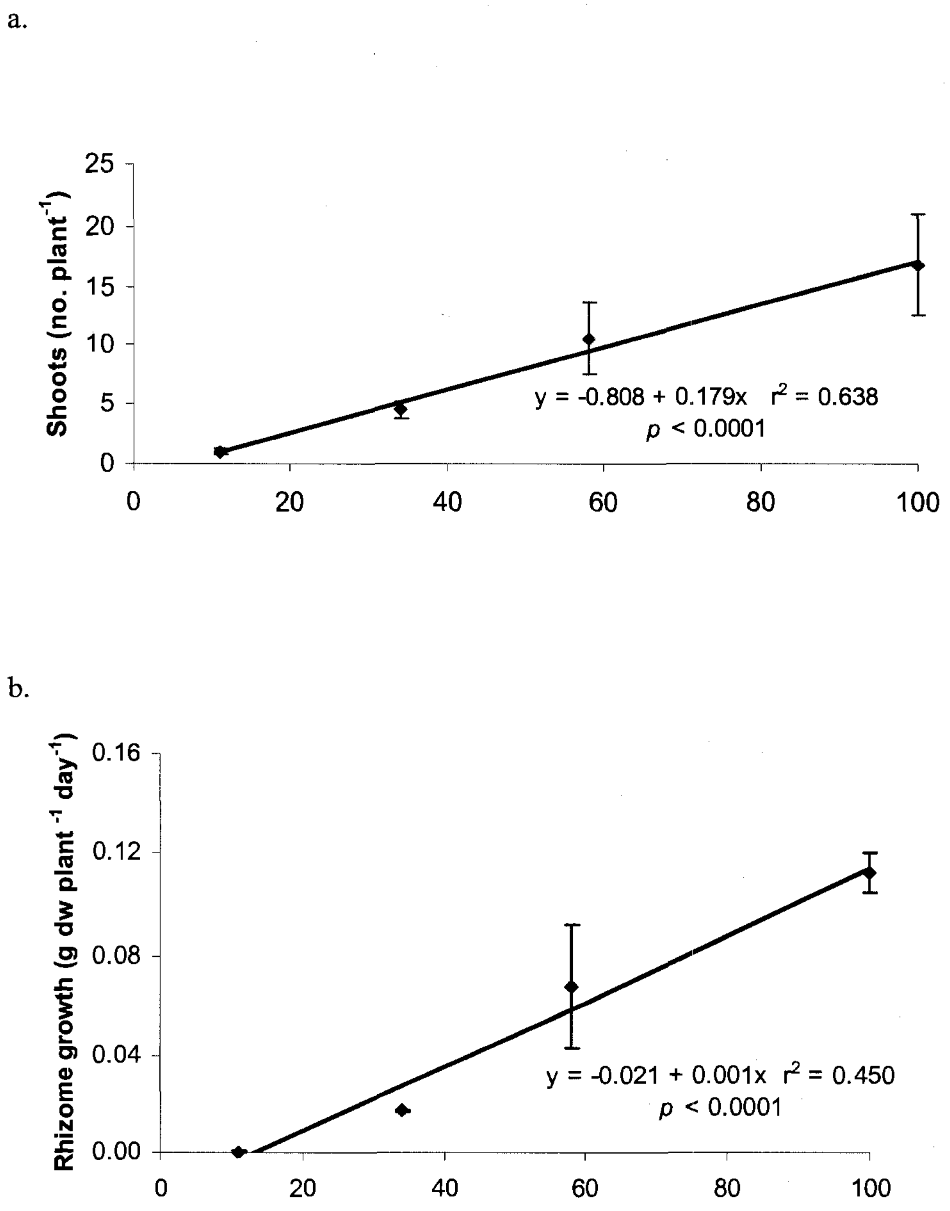

Figure 3.4. (see next page for figure caption)

c.

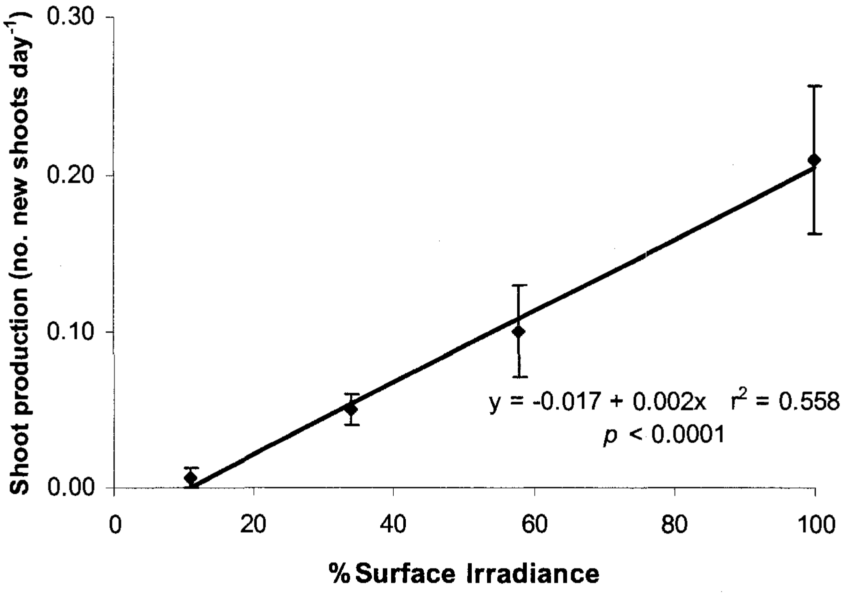

Figure 3.4. Effect of reduced light from shading on (a) the number of shoots per plant, (b) rhizome growth, and (c) shoot production of eelgrass seedlings after 81 days of shading. Means ± SE, *n* = 3.

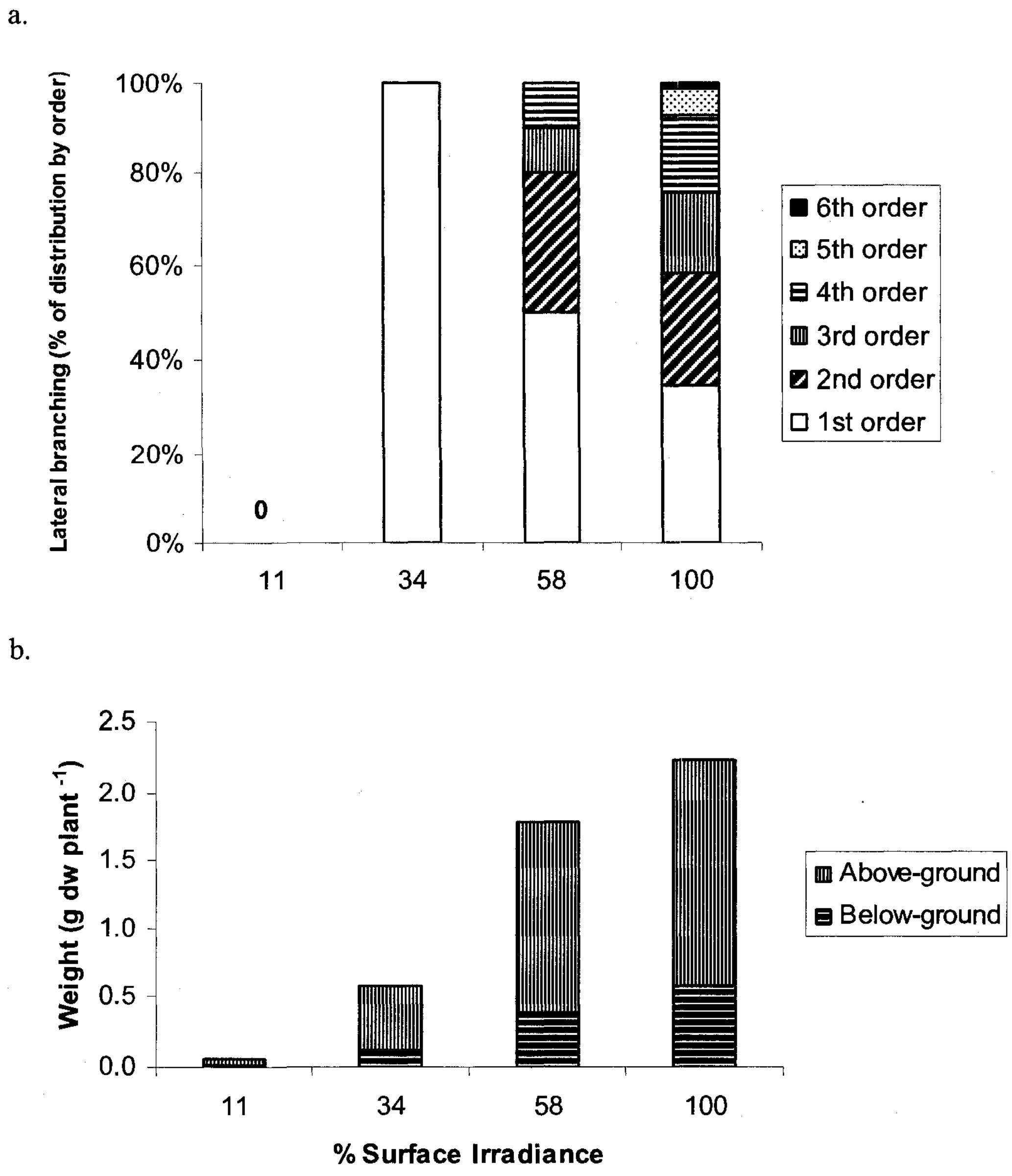

Figure 3.5. Effect of reduced light from shading on (a) lateral branching and (b) above- and below-ground weight of eelgrass seedlings after 81 days of shading.

CHAPTER IV

SURVIVAL OF *ZOSTERA MARINA* L. AT THE MAXIMUM DEPTH LIMIT AND THE ROLE OF RECOVERABLE PLANT RESERVES

ABSTRACT

Photosynthesis, carbohydrate storage, morphology and biomass of eelgrass plants were assessed quarterly during one year at the maximum depth limit of plant growth ("deep edge") at 5 sites along an estuarine gradient in Great Bay Estuary (GBE), NH and along a depth gradient at the site with the clearest water, to understand mechanisms of plant adaptation to low light. Mean daily PAR available to deep edge plants in winter (December) was 81% less than peak values in summer (June), while photosynthetic capacity (ETR_{max}) was 75% less. However, because of photosynthetic adjustments (reduced minimum saturating irradiance, I_k), the daily period of light for saturating photosynthesis (H_{sat}) in December (5.5 hrs) was high. Periods of lowest H_{sat} (April: 2.5 hrs; September: 3 hrs) were typically followed by a drop in rhizome soluble sugar concentrations (lowest levels in June); mobilization of stored reserves occurred during transition periods (spring and autumn) rather than in winter. Plants at the deep edge (1.4 – 4.3 m in GBE), while growing under light-limited conditions (approximately 13% SI), built up biomass and sugar reserves comparable to intertidal and shallow subtidal plants. Deep edge plants, however, exhibited much greater acclimation in response to changes in light regime than plants at shallower depths. Acclimations to light reduction from September to December at all depths included increases in chlorophyll *a/b* and maximum quantum yield of PSII (F_v/F_m) as well as significant declines in ETR_{max}, I_k, leaf size, above-ground shoot weight and total biomass. Despite increasing mobilization of carbon from rhizomes with depth (measured as leaf re-growth in g dw (g rhizome sugar)$^{-1}$ from incubated rhizome fragments, and inferred from a decrease in sugar concentrations along the depth gradient), the relative contribution of rhizome sugar reserves to winter eelgrass growth at the deep edge was small (6%). The study demonstrates that eelgrass plants at their maximum depth limit exhibit considerable photosynthetic and morphological acclimation; their survival of winter light stress primarily depends on wintertime photosynthesis, with remobilization of stored carbohydrate reserves playing only a minor role. However, stored carbohydrates played an important role in spring and fall as plants adapted to increasing and decreasing light levels.

INTRODUCTION

Water quality deterioration, and in particular, loss of water column light availability, has been implicated in the decline and/or loss of seagrass beds worldwide (Short and Wyllie-Echeverria, 1996; Orth et al., 2006). Understanding the responses of seagrass plants to light reduction has therefore received significant research interest over the last 30 years (Backman and Barilotti, 1976; Dennison and Alberte, 1982, 1985; Pirc, 1986; Dennison, 1987; Gordon et al., 1993; Short et al., 1995; Zimmerman et al., 1995a; Moore et al., 1997; Vermaat et al., 1997; Moore and Wetzel, 2000; Alcoverro et al., 2001; Bintz and Nixon, 2001; Peralta et al., 2002). Light available for primary production in a water body depends on incident light, light attenuation in the water column and water depth. Underwater irradiance shows a negative exponential decrease with depth (Kirk, 1994). Light availability, and hence, water clarity, primarily controls the lower depth distribution limit of seagrasses (Dennison, 1987).

Assuming negligible epiphyte cover, the relationship between water column light attenuation and seagrass colonization depth (Duarte, 1991) implies that the light level reaching the seagrass at its maximum depth in a given location (the "deep edge"), expressed as incident light in % of surface irradiance (*SI*), is the minimum required for long-term eelgrass growth and survival (Kenworthy and Fonseca, 1996). Values reported for the minimum light requirements of eelgrass vary widely (11 – 30% SI) depending on location and methodologies used (Dennison et al., 1993; Olesen and Sand-Jensen, 1993; Erftemeijer and Lewis, 2006).

The location of the deep edge has been found to relate to light availability (Dennison and Alberte, 1985; Zimmerman et al., 1991), leaf growth rates, and soluble carbohydrate concentrations (Kraemer and Alberte, 1995). That reduced light availability can result in reduced growth and transplant survival has been shown (Zimmerman et al., 1995a), but no studies have made actual measurements of light availability at the maximum depth limit, or combined seasonal changes of such light with seasonal eelgrass photosynthetic acclimations to aid understanding of eelgrass adaptations under light stress in winter. Because eelgrass plants at the deep edge live near or at the minimum light required for survival and growth, these plants are likely to be more sensitive to small changes in water clarity than plants at shallower depths. Furthermore, the effects of increased water column light attenuation and the effects of high turbidity pulses are likely to be seen first on deep edge plants.

Past studies have suggested, based on changes in carbohydrate concentrations, that carbohydrate reserves in rhizome tissues support continued eelgrass growth during winter when light is limiting (Zimmerman et al., 1995a; Cabello-Pasini et al., 2002), ensure the sustenance of below-ground structures during dormant periods (Burke et al., 1996) and replenish carbon loss from herbivory (Zimmerman et al., 1996). However, quantifying plant re-growth from recoverable stored reserves in seagrasses has never been attempted. Experimental manipulation of light availability to eelgrass revealed that substantial carbohydrate stores and a low rate of carbon consumption enable eelgrass plants to sustain metabolism during brief episodes of reduced light availability allowing for enough metabolic activity to prevent below-ground tissues from suffering the negative effects of anoxia (Kraemer and Alberte, 1995).

It has been hypothesized that eelgrass plants at the light limited edge of their depth distribution may not have abundant carbohydrate reserves in roots or rhizomes due to chronic light limitation (Kraemer and Alberte, 1995). Assessing eelgrass tissue carbohydrate reserves, along with measurements of light availability and photosynthetic performance at the maximum depth, may contribute to a better understanding of how eelgrass plants survive at locations where light availability reaches minimal levels during the winter season, and may provide insight into potential consequences of increased light attenuation on deep water eelgrass plants growing at or close to their minimum light requirements.

In the present study, the effect of changing light availability over time on photosynthetic and morphological parameters of eelgrass (*Zostera marina* L.) at the deep edge was studied in Great Bay Estuary, to answer the following questions: How do deep edge eelgrass plants respond to winter light stress? To what extent do carbohydrates stored in eelgrass rhizomes buffer the plants against periods of presumed limited photosynthate production due to light reduction? How does carbohydrate remobilization compare with wintertime photosynthesis of the deep edge plants? The following hypotheses were tested: (1) changing light availability with season has no effect on rhizome sugar concentrations, photosynthetic capacity or morphology of eelgrass at the deep edge, and (2) rhizome sugar reserves do not contribute to eelgrass winter growth.

MATERIALS AND METHODS

Site description

The study site was in the Great Bay Estuary, situated at the southern New Hampshire-Maine border (43°05'N, 70°50'W), USA (Figure 4.1). Great Bay Estuary is a tidally dominated embayment, extending inland to a distance of 25 km from the mouth of the Piscataqua River to the Great Bay proper. Tidal waters from the Gulf of Maine enter the estuary at Portsmouth Harbor, flooding the three major portions of the estuary: the Piscataqua River, Little Bay and Great Bay. Mean tidal amplitude ranges from 2.7 m at the mouth of the estuary to 2.0m at Dover Point. Among a variety of habitats in the estuary are extensive subtidal eelgrass meadows covering approximately 2023.4 hectares (Short, 1992; Jones, 2000). The intertidal measurements for this study were made at a 10 ha intertidal eelgrass meadow near Fishing Island in Portsmouth Harbor, with a subtidal area located adjacent to the main channel of Portsmouth Harbor not far from Fishing Island.

Depth gradient

The effect of depth on eelgrass photosynthesis, morphology, biomass and carbon reserves was tested by sampling eelgrass along a depth gradient running from the intertidal bed to the deep edge of the meadow at Fishing Island (FI), situated at the mouth of the estuary. Three depth strata along the gradient were identified: deep edge (-4.3 m MLW), shallow subtidal (-2.4 m MLW) and intertidal. At each depth, three random replicate sediment core samples containing eelgrass (each replicate at least 100 m apart) were taken in April, September and December, 2004.

Deep edge

Additionally, eelgrass was sampled in April, June, September and December, 2004 at the deepest edge of each of five beds in the estuary: Fishing Island (FI; -4.3 m MLW) at the mouth of the estuary, Outer Cutts Cove (OCC; -2.0 m MLW) in Portsmouth Harbor, Great Bay Fish Pier (GBF; -2.6 m MLW) in the Piscataqua River, Dover Point (DP; -1.6 m MLW) in Little Bay, and Red Nun (RN; -1.4 m MLW) in Great Bay proper. All five sites are characterized by strong tidal currents (between 1.5 to 2.3 m/s) and vertical mixing. The depth of maximum eelgrass distribution decreases up-estuary where frequent re-suspension of sediments by tidal currents and waves result in higher turbidity, particularly in the upper portion of the Great Bay (Figure 4.1). Salinity also decreases from the mouth of the estuary to the upper portions of Great Bay.

Light measurements

With the help of SCUBA, wide spectrum light sensors (HOBO, Onset Computer Corp, Bourne, MA) in submersible cases were deployed at each deep edge site at the top of the canopy of the eelgrass bed, firmly secured to a pole. These light sensors were launched simultaneously and set to log data every 30 minutes. Data were down-loaded after every four weeks, after which the sensors were cleaned and redeployed. Only readings from the first eight days of each quarter were used in order to avoid potential influences of biofouling on the cases. In addition, a HOBO light sensor and a Li-Cor sensor were mounted at the rooftop of the Jackson Estuarine Laboratory (JEL), located along the shore of Great Bay, to simultaneously measure luminosity and PAR respectively during June (23 days), July (22 days), September (6 days) and December (7 days). Comparisons of the flux range reaching the two instruments were made from data collected between 0800 and 1700 hrs. The resulting data readings were used to establish a linear regression, allowing conversion of luminosity data logged continuously by HOBOs at the deep edge sites (in lumens per square foot) into PAR (μ mol photons m^{-2} s^{-1}), assuming similar performance under water as in air. Surface irradiance (SI) was estimated from continuous PAR readings taken at the JEL roof top, assuming an average 5.2% water surface reflection (calculated from readings taken by a 4 *pi* PAR sensor held at the surface and at 10 cm below the water surface in April, June, September and December ($n = 18$)). Percent light available to deep edge plants was calculated as: Estimated PAR at the deep edge divided by PAR measured at JEL rooftop (minus 5.2% reflection).

Temperature and salinity

Water temperature at each site was monitored hourly throughout the study period using Onset Stowaway TidbiT temperature loggers. Monthly mean temperature was calculated from daily averages. Seawater salinity at each site was measured using a refractometer during each field visit.

Plant material

At each of the five deep edge sites (Figure 4.1), 24 eelgrass shoots (including several internodes of root/rhizome), at least a meter apart, were harvested randomly by hand in April, June, September and December. Half of the shoots were brought up into the boat in a mesh bag and kept shaded.

The other half of the eelgrass shoots were kept in a cooler for further processing. At the laboratory, this second set of shoots was rinsed in freshwater and the number of leaves per shoot, the sheath length and the length and width of the third leaf of each sample shoot recorded. The first 4 to 5 rhizome internodes were separated and their lengths measured. The third leaf, remaining leaves, the first 4 to 5 rhizome internodes and associated roots were dried separately at 50 – 60 °C to constant dry weight. Rhizome material was ground to fine powder using a Wig-L-Bug® Dentsply. The powdered rhizome samples were placed in capped vials and stored in a desiccator for further analysis (see recoverable reserves).

At each of three of the five deep edge sites (i.e., FI, GBF, RN), three random replicate cores were taken to a depth of 20 cm using a 15 cm diameter corer with each replicate at least 100 m apart (9 cores in total) in order to assess eelgrass biomass and shoot density. Core samples were cleaned of sediment by washing with seawater, and then transported in a cooler to the laboratory. After rinsing with freshwater, samples were separated into leaves, roots, and rhizome. The number of reproductive shoots (when present) was noted and dead plant material discarded. Plants parts were dried at 50 – 60 °C to constant dry weight.

Fluorescence measurements

Fluorescence measurements were performed on the first set of 12 shoots immersed in seawater in a shaded bucket using a diving-PAM (Walz, Germany), within 30 - 60 minutes of harvesting. The electron transport rate (ETR) of eelgrass plants was determined using Rapid Light Curves (RLCs): the middle section of each third leaf was enclosed in a leaf clip and exposed to eight incremental steps of artificial irradiance pre-programmed in the PAM, each light step lasting 10 s (Ralph and Gademan, 2005; Ochieng et al., in prep). Irradiances used ranged between 0 and 1500 μ mol photons m^{-2} s^{-1}. Electron transport rate (ETR) was estimated using the effective quantum yield (Y) and measured corresponding absorption factors (AF) according (Beer et al., 2001) as follows:

$$ETR = Y*PAR*0.5*AF.$$

where, Φ_{PSII} is effective quantum yield calculated as: $(F_m'–F)/ F_m'$; PAR is the corresponding actinic light step generated by the internal halogen lamp of the PAM; the result is multiplied by 0.5 under the assumption of equal distribution of photons absorbed by the two photosystems.

The maximum quantum yield (= potential photochemical efficiency) of photosystem II (F_v/F_m) was measured by dark adapting the middle section of the third leaf in a leaf clip for 10 minutes and subsequently exposing the leaf to a 0.5 – 1.0 s period of saturating light (> 2000 μmol photons m^{-2} s^{-1}). F_v/F_m was derived according to Beer et al. (2001) as follows:

$$F_v/F_m = (F_m - F_o)/ F_m$$

where, F_o is the minimum fluorescence value, F_m is the maximum fluorescence value, and F_v is the variable fluorescence. For alternative terminology, see Papageorgiou and Govindjee (2004).

ETR versus irradiance curves

The electron transport rates were plotted against absorbed irradiance (I_a), where I_a = Irradiance*AF*0.5 (Saroussi and Beer, 2007), and then fitted according to the Jassby and Platt (1976) model using Solver in Excel, Microsoft© following the least squares method (Zar, 1984) to derive the photosynthetic parameters: light saturated maximum rate of electron transport (ETR_{max}, umol electrons m^{-2} s^{-1}), minimum saturating light (I_k, umol photons m^{-2} s^{-1}) and initial slope (α, mol electrons photons mol^{-1}). The daily period of irradiance-saturated photosynthesis, or hours of saturating light (H_{sat}), defined as $I > I_k$, where I is the estimated PAR at the deep edge, was calculated from irradiance estimations and photosynthetic characteristics from eelgrass from the 5 deep edge sites in Great Bay Estuary measured in April, June, September and December. Underwater light curves were plotted from irradiance estimated over 8 days at each of the 5 deep edge sites in Great Bay Estuary in April, June, September and December. The length of the day that irradiance was greater than the light saturation point for photosynthesis, in hours, was determined.

Chlorophyll content

Chlorophyll measurements were standardized to the middle portion of a healthy third leaf taken from the same set of 12 eelgrass shoots. Epiphytes (when present) were removed carefully by scraping. Approximately 1 cm^2 leaf material was pre-soaked in 100% acetone in the dark. Leaf tissue was macerated using a pre-chilled (in ice) mortar and pestle with the help of small amounts of clean sand. A small amount of $MgCO_3$ was added to a final volume of 10 ml of 90% acetone. The acetone extracts were centrifuged to settle suspended material. Chlorophyll pigment content was determined spectrophotometrically at 725, 664 and 647 nm (Granger and Iizumi, 2001). Care was taken to keep samples away from direct sunlight and acids. Chlorophyll *a* and *b* were expressed as μg Chl cm^{-2} leaf.

Sugar reserves

Dried, ground rhizome material collected in April, June, September and December from each sampling site was analyzed for soluble carbohydrates (sugars) according to Burke et al., (1992). Sugars were extracted from rhizome samples in hot ethanol (80%), and the extract measured using the phenol (5%)-sulfuric acid (96%) colorimetric reaction (Hodge and Hofneiter, 1962) after which the absorbance was read spectrophotometrically at 490 nm using glucose as a standard.

Estimating growth from underground rhizome sugars

Shoots harvested in September and December from each sampling site were rid of photosynthetic tissue by clipping each shoot just above the meristem, leaving approximately a third of the sheath, a known length of rhizome material (4 to 5 rhizome internodes), and undamaged meristematic tissue intact. The rhizome-meristem units were then incubated moist, but not wet, in separate, sealed, spacious plastic bags in a cooler in a dark room maintained at 5°C. The amount of recoverable underground reserves, considered to represent the potential for growth, was quantified by repeatedly cutting, drying and weighing leaf tissue re-growth every 7 – 9 days until no more re-growth occurred. The sum of leaf tissue re-growth for each site was considered to be its recoverable underground reserves. Rhizome material was analysed for any remaining soluble sugars after the re-growth process, the initial measure of quarterly rhizome sugar content being the time zero (T_0) values.

The dry weight of the first 4 to 5 internode rhizome segments (Kraemer and Alberte, 1993) incubated in the dark was estimated using the corresponding weight to length ratio (W:L) (from the site and month in question). The amount of sugar present in the incubated rhizomes was determined by multiplying rhizome dry weight by the average sugar concentration of those rhizomes. Growth (g dw rhizome $(\text{sugar g})^{-1}$) was then estimated by relating the measured dry weight of leaf material (re-growth from incubated rhizome) to the amount of sugar in rhizome tissue.

Predicting winter growth

Winter (December) growth of deep edge plants was predicted in three different ways: (1) growth (g dw m^{-2} d^{-1}) based on measured sheath lengths, a regression equation derived by Gaeckle et al. (2006), and shoot density; (2) growth derived from carbon equivalents (g C m^{-2} d^{-1}) of measured electron transport rates (ETR) (Uku, 2005); and (3) growth (g dw m^{-2} d^{-1}) based on growth measurements of intertidal plants in the same study location (Gaeckle et al., 2006) and deep edge shoot density. Photosynthetic capacity of deep edge plants was 42.2% that of intertidal plants in December in the present study. Assuming that the difference in growth between deep edge and intertidal plants in December was in the same order of magnitude as photosynthetic capacity, the growth measurements by Gaeckle et al. (2006) were upgraded accordingly.

Data analysis

Photosynthetic potential and rates, chlorophyll, soluble sugar content, morphology and biomass data were analyzed as either one-way or two-way analysis of variance using the ANOVA procedure in Systat Software Inc. version 11. When there was a significant difference at $\alpha = 0.05$, Tukey's Post Hoc test of the parameter was done using least squares to compare means. June data were excluded from the tests for interaction between depth and month in order to maintain an equal number of groups. Homogeneity of variance was checked. Proportions were arcsine-transformed before performing parametric tests. Data are presented as means ± standard error.

RESULTS

Light availability

Ambient light conditions at the deep edge were highly variable even within a single day, with daily total irradiance ranging from 0.1 to 11.7 mol photons m^{-2} d^{-1}.

The daily integrated light availability to eelgrass plants at the 5 deep edge sites varied with month, with the highest mean recorded in June (4.2 ± 0.4 mol photons m^{-2} d^{-1}) and the lowest in December (0.8 ± 0.1 mol photons m^{-2} d^{-1}) (Table 4.1). The annual average daily period of light-saturated photosynthesis (H_{sat}) was 4.8 ± 0.3 hours. The monthly average H_{sat} at the deep edge was 2.5 ± 0.8 hrs in April, 7.1 ±0.4 hrs in June, 3.0 ± 0.5 hrs in September and 5.5 ± 0.3 hrs in December and was significantly higher in June and December than in the other months ($n = 5$ sites) (Table 4.1). The average light available for June, September and December to eelgrass plants at the deep edge sites was 13.4 ± 1.7 percent of surface irradiance (% SI), and the percentage did not change significantly with month ($p = 0.458$; $n = 5$ deep edge sites) (Table 4.1).

Water temperature

Mean daily water temperature at the deep edge sites in Great Bay Estuary was 6.9 ± 0.2 °C in April, increasing to 14.5 ± 0.2 °C in June, reaching a peak of 16.9 ± 0.1 °C in September, and dropping to 4.2 ± 0.1 °C in December. Moving upstream into the estuary, mean daily water temperature increased linearly with distance from the ocean during April, June and September, but decreased linearly in December. Fishing Island, the most seaward site, was the warmest in winter and the coldest in spring and summer relative to the other sites, whereas Red Nun, the furthest site from the ocean was the coldest in winter and the warmest in spring and summer relative to the other sites (Figure 4.2).

Salinity

Mean salinity at the deep edge was 22.3 ± 1.0 in June, increasing to a peak of 26.7 ± 0.8 ($n = 5$) in September with no significant differences in salinity between June and December. Salinity decreased linearly moving upstream into the estuary, from 28 at Fishing Island to 14 at Red Nun in December ($r^2 = 0.988$).

Photosynthetic rates

Maximum electron transport rate (ETR_{max}), minimum saturating light (I_k) and the maximum quantum yield of PSII (F_v/F_m) of eelgrass plants from the deep edge varied between seasons. ETR_{max} increased from an average of 19.4 ± 2.4 μmol electrons m^{-2} s^{-1} in April to 45.2 ± 2.3 μmol electrons m^{-2} s^{-1} in September and then dropped to 11.3 ± 0.8 μmol electrons m^{-2} s^{-1} in December (Table 4.2). I_k increased from an average of 29.5 ± 3.0 μmol photons m^{-2} s^{-1} in April to 68.8 ± 3.6 μmol photons m^{-2} s^{-1} in September and dropped significantly to 14.1 ± 1.0 μmol photons m^{-2} s^{-1} in December (Table 4.2). A reverse trend was observed in F_v/F_m, which decreased significantly from an average ratio of 0.760 ± 0.004 in April to the lowest value of 0.749 ± 0.002 in June, after which the F_v/F_m increased significantly through September (0.782 ± 0.002) to a peak in December (0.813 ± 0.001) (Table 4.2).

ETR_{max} of plants sampled along a depth gradient at Fishing Island showed the same pattern as that of deep edge plants. A significant interaction ($p < 0.0001$) between depth and month was apparent (Figure 4.3a). Results of ANOVA statistical analyses of comparisons between different depths along a depth gradient at Fishing Island for each of the four months of sampling are shown in Table 4.4. In April, the ETR_{max} of shallow subtidal plants was significantly higher than that of the intertidal and deep edge plants (Table 4.4). In September, the month when plants exhibited highest ETR_{max}, both the intertidal and shallow subtidal plants had higher ETR_{max} than the deep edge plants. And in December, plants from all three depths had significantly lower ETR_{max} than September, with intertidal plants (18.17 ± 1.05 μmol electrons m^{-2} s^{-1}) having significantly higher ETR_{max} than shallow subtidal (11.56 ± 0.72 μmol electrons m^{-2} s^{-1}) and deep edge plants (7.66 ± 0.29 μmol electrons m^{-2} s^{-1}) (Table 4.4; Figure 4.3a).

Minimum saturating light (I_k) of plants sampled along a depth gradient at Fishing Island showed a significant interaction between depth and month ($p < 0.0001$). I_k of intertidal and deep edge plants increased between April and September then decreased between September and December, while the I_k of shallow subtidal plants did not change significantly between April and September but decreased significantly between September and December (Table 4.5). While the I_k of shallow subtidal plants was higher than those of the deep edge throughout April, June and September, no significant difference in I_k was found between these two depths in December, while the I_k of intertidal plants (30.1 ± 2.1 μmol photons m^{-2} s^{-1}) was significantly higher than that of both the shallow subtidal (12.8 ± 2.7 μmol photons m^{-2} s^{-1}) and deep edge plants (9.5 ± 0.4 μmol photons m^{-2} s^{-1}) in December (Table 4.4; Figure 4.3b).

The maximum quantum yield of PSII (F_v/F_m) of eelgrass plants sampled along a depth gradient demonstrated similar seasonality to that of the deep edge plants, with a significant interaction ($p < 0.0001$) between depth and month (Figure 4.3c). Intertidal plants had a relatively lower maximum quantum yield (F_v/F_m) compared to shallow subtidal and deep edge plants throughout the months for which data is available. The difference was smaller in December, when F_v/F_m was 0.801 ± 0.003 (intertidal), 0.822 ± 0.002 (shallow subtidal) and 0.820 ± 0.003 (deep edge), with no significant difference between the shallow subtidal and deep edge plants (Table 4.4).

Chlorophyll content

Total leaf chlorophyll content (Chl *a+b*) of eelgrass plants growing at the deep edge was 44.4 ± 2.0 μg cm^{-2} in April, decreasing significantly ($p < 0.0001$; Tukey's HSD) to 28.9 ± 0.8 μg cm^{-2} in June, and increasing back to 45.0 ± 1.3 μg cm^{-2} in September and 43.8 ± 0.8 μg cm^{-2} in December, with no significant differences between September and December ($p = 0.883$; Tukey's HSD) (Table 4.2).

Leaf chlorophyll *a/b* of deep edge plants also decreased significantly from 2.46 ± 0.02 in April to 2.11 ± 0.01 in June ($p < 0.0001$; Tukey's HSD), and then increased significantly to 2.16 ± 0.01 in September and further to 2.39 ± 0.01 in December ($p < 0.0001$; Tukey's HSD) (Table 4.2).

Total leaf chlorophyll of eelgrass plants sampled along a depth gradient at Fishing Island demonstrated a significant interaction ($p < 0.0001$) between depth and month. Leaf chlorophyll increased significantly with depth in April and September (Table 4.4). In April, leaf chlorophyll was 23.0 ± 1.0 μg cm^{-2} (intertidal plants), 33.4 ± 2.3 μg cm^{-2} (shallow subtidal plants), and 40.8 ± 2.0 μg cm^{-2} (deep edge plants). The trend was similar and more pronounced in September (Figure 4.4a). However, in December, the difference in chlorophyll between intertidal (44.6 ± 1.5 μg cm^{-2}) and deep edge plants (43.2 ± 1.7 μg cm^{-2}) was not significant (Table 4.4). The chlorophyll of shallow subtidal plants (51.6 ± 2.0 μg cm^{-2}) was significantly higher in December than the intertidal and deep edge plants (Table 4.4; Figure 4.4a). No June chlorophyll data were collected.

On the other hand, there was a significant effect of depth and of month (but no interaction between depth and month) on the chlorophyll *a/b*. The chlorophyll *a/b* of intertidal plants (2.59 ± 0.03) was higher than the chlorophyll *a/b* of shallow subtidal (2.49 ± 0.02) and deep edge plants (2.52 ± 0.03) throughout April, September and December (Table 4.4). Chlorophyll *a/b* increased significantly from September to December for shallow subtidal and deep edge plants (Table 4.5), but there was no significant difference in leaf chlorophyll *a/b* between plants from the two depths (Table 4.4; Figure 4.4b).

Morphology and biomass

Leaf length

The average eelgrass leaf length of deep edge plants for all five sites measured was 24.7 ± 1.2 cm in April, increasing significantly to 78.4 ± 4.9 cm in June ($p < 0.0001$), remaining high in September (88.6 ± 3.0 cm) and dropping significantly to 70.6 ± 2.7 cm in December ($p = 0.00001$) (Table 4.3).

There was a significant interaction ($p < 0.0001$) between depth and month in leaf length sampled along the depth gradient at Fishing Island (Figure 4.5a). In April, leaves from the shallow subtidal (35.1 ± 2.1 cm) were significantly longer than those from the deep edge (27.9 ± 2.2 cm) and intertidal (11.2 ± 0.7 cm) (Table 4.4). By September, leaf length had increased at all depths with the average leaf length of deep edge plants (92.8 ± 4.5 cm) significantly higher than that of both the shallow subtidal (73.4 ± 7.0 cm) and intertidal plants (23.8 ± 1.9 cm). In December, there was no significant difference in leaf length between shallow subtidal and deep edge plants (Table 4.4). Leaf length of plants at the deep edge significantly decreased between September and December, while leaf length of plants at the shallow subtidal did not change (Table 4.5; Figure 4.5a). In December, the intertidal plants remained relatively small.

Leaf width

The average eelgrass leaf width of deep edge plants was 3.0 ± 0.1 mm in April, increasing significantly to 4.8 ± 0.2 mm in June ($p < 0.0001$), and remaining high in September (4.7 ± 0.1 mm) before dropping significantly to 4.1 ± 0.1 mm in December ($p < 0.0001$) (Table 4.3).

There was a significant interaction ($p = 0.003$) between depth and month in leaf width at the Fishing Island depth gradient. In April, average leaf width of plants from the shallow subtidal and the deep edge were not statistically different (3.0 ± 0.2 and 2.9 ± 0.1 mm respectively), but both were significantly wider than leaves of plants from the intertidal (2.1 ± 0.1 mm) (Table 4.4). In June, average leaf width of plants from the shallow subtidal was significantly greater than that of deep edge plants. In September, the trend was reversed with average leaf width of deep edge plants (4.7 ± 0.1 mm) significantly greater than that of shallow subtidal (4.2 ± 0.2 mm) and intertidal plants (2.7 ± 0.2 mm). In December, there was no significant difference in leaf width between the shallow subtidal and deep edge plants (Table 4.4). Leaf width of deep edge plants significantly decreased between September and December, while leaf width of plants at the shallow subtidal did not change (Table 4.5; Figure 4.5b). The maximum leaf width of shallow subtidal plants occurred in

June, while the maximum in deep edge plants occurred in September, with both peaks differing significantly ($p < 0.05$) from adjacent months).

Number of leaves per shoot

The number of leaves per shoot of eelgrass plants varied significantly ($p < 0.05$) with month (all sites). The average number of leaves per shoot of deep edge plants was 4.4 ± 0.1 in April, increasing to a peak in June (5.8 ± 0.2), before decreasing to 5.1 ± 0.1 in September and further decreasing to the lowest value in December (3.9 ± 0.1) (Table 4.3).

A significant interaction ($p = 0.007$) in the number of leaves per shoot was evident between depth and month (September vs. December) at Fishing Island. The main differences in the number of leaves per shoot of eelgrass along a depth gradient occurred between intertidal plants and deep edge plants, while there was no significant difference between shallow subtidal and deep edge plants throughout the year (Table 4.4). In April, the number of leaves per shoot in deep edge plants (4.6 ± 0.2) was significantly higher ($p < 0.0001$) than in intertidal plants (3.1 ± 0.1 leaves per shoot) (Figure 4.5c). However, in September, the number of leaves per shoot in intertidal plants (6.5 ± 0.2 leaves per shoot) was higher than in deep edge plants (5.2 ± 0.2). In December, no significant differences were observed in the number of leaves per shoot between deep edge and intertidal plants (Table 4.4; Figure 4.5c).

Shoot density

Eelgrass shoot density of deep edge plants ranged from 293 ± 33 to 431 ± 29 shoots m^{-2} and did not change significantly with month (Table 4.3).

However, there was a significant effect of depth on shoot density ($p < 0.0001$) (but no effect of month and no interaction between depth and month). The average shoot density at Fishing Island was consistently higher in the intertidal (3 – 7-fold) than at the shallow subtidal and deep edge during all sampling months (Table 4.4; Figure 4.5d).

Above-ground weight per shoot

A significant difference between months in the weight per shoot was notable in eelgrass plants from all deep edge sites. Average above-ground weight per shoot from deep edge plants was 0.12 ± 0.02 g dw in April, increasing significantly to 0.40 ± 0.04 g in June and further to a peak of 0.72 ± 0.04 g dw in September, before decreasing significantly to 0.33 ± 0.03 g dw in December (Table 4.3).

Along the depth gradient at Fishing Island, a significant interaction in shoot weight between depth and month was evident ($p < 0.0001$). Shoot weight of deep edge plants increased three-fold between April and June compared to a six-fold increase of shallow subtidal plants. After June, shoot weight of deep edge plants continued to increase significantly to a peak in September, while those of shallow subtidal plants began to decrease (Table 4.5). After September, the average shoot weight of deep edge plants dropped considerably (55%) from 0.953 ± 0.086 g in September to 0.429 ± 0.028 g in December, while those of shallow subtidal plants dropped only moderately (33%), from 0.727 ± 0.104 g in September to 0.486 ± 0.044 g in December. The drop in the average weight per shoot of intertidal plants from 0.128 ± 0.019 g in September to 0.11 g ± 0.011 g in December was very small (13%) in comparison (Figure 4.6a).

Biomass

Mean eelgrass biomass (above + below-ground) at three deep edge sites in the estuary ranged from 68.1 ± 7.4 g dw m^{-2} (April) to 128.5 ± 14.0 g dw m^{-2} (September). Biomass was significantly higher in September ($p = 0.001$), while there was no significant difference between April and December (Table 4.3). No biomass cores were taken in June.

A significant interaction in biomass between depth and month was apparent along the depth gradient at Fishing Island ($p = 0.009$). In April, eelgrass biomass was significantly higher at the shallow subtidal than at the intertidal and deep edge (Table 4.4). In September, there were no significant differences in total biomass between the three depths. In December, biomass of intertidal plants was significantly higher ($p = 0.015$) than that of shallow subtidal and deep edge plants (Table 4.4; Figure 4.6b). Mean plant biomass (all months pooled together) at the deep edge (120.7 ± 9.6 g dw m^{-2}) and intertidal (186.9 ± 19.3 g dw m^{-2}) was significantly lower ($p < 0.001$) than at the shallow subtidal (237.9 ± 29.0 g dw m^{-2}).

Shoot to root ratio

The distribution of biomass between photosynthetic and non-photosynthetic tissues of eelgrass plants showed significant differences between months. The shoot to root ratio (S:R) of deep edge plants was 1.2 ± 0.2 in April, increasing significantly to 3.5 ± 0.3 in September ($p < 0.0001$; $n = 3$ sites; FI deep edge, GBF and Red Nun) and to 3.7 ± 0.5 in December (Table 4.3). The difference between September and December was not significant.

A significant interaction in S:R ($p = 0.006$) between depth and month along a depth gradient at Fishing Island was evident. S:R of plants from all depths tripled between April and September, although S:R of intertidal plants was significantly lower than that of plants from other depths in all months (Table 4.4). After September, S:R of deep edge plants increased by 17%, from 3.3 ± 0.4 in September to 3.9 ± 0.3 in December, but S:R of intertidal plants increased only by 7%, from 0.7 ± 0.1 in September to 0.8 ± 0.3, and S:R of shallow subtidal plants remained largely unchanged (Figure 4.6c). As a result of these changes, the differences in S:R between the intertidal, shallow subtidal and deep edge plants were greater in December than in other months. In April, S:R was only significantly different between deep edge and intertidal plants; in September not only was the difference in S:R between deep edge and intertidal plants significant, but between shallow subtidal and intertidal plants as well. In December, S:R at all three depths was significantly different (Table 4.4; Figure 4.6c).

Weight to length ratio of rhizomes

Weight to length ratio (W:L) of the first five rhizome internodes from deep edge plants varied with month. W:L was 0.015 ± 0.001 in April, increasing significantly to 0.019 ± 0.001 in September before dropping significantly to 0.011 ± 0.000 in December (Table 4.3).

There was a significant interaction between depth and month in W:L ($p < 0.0001$). Whereas the W:L of deep edge rhizomes at Fishing Island decreased sharply (60%) from 0.028 ± 0.002 in September to 0.011 ± 0.001 in December, the W:L of shallow subtidal plants decreased only moderately (36%) from 0.022 ± 0.002 to 0.014

± 0.001, while that of intertidal plants remained constant at 0.010 ± 0.001 (Figure 4.6d).

Root biomass

The percentage of root biomass to the total below-ground biomass of deep edge plants decreased from 35 ± 3% in April to 9 ± 1% in December ($n = 5$ deep edge sites). There was a significant interaction ($p < 0.01$) in percentage of roots to the total below-ground biomass between depth and month along a depth gradient at Fishing Island. In April, the proportion of roots was highest in deep edge plants (35 ± 3%) followed by shallow subtidal plants (25 ± 2%) and lowest in intertidal plants (19 ± 4%). After a significant drop from April to September for both shallow subtidal and deep edge plants (Table 4.5), the proportion of roots in September was larger in intertidal plants compared to that of plants at other depths (Table 4.4). A further decline at all depths to lowest values in December left intertidal plants with a higher contribution of roots of total below-ground biomass (13 ± 1%) than deep edge (9 ± 1%) and shallow subtidal plants (7 ± 1%) in December (Figure 4.6e).

Soluble sugars

There was clear seasonality in concentrations of soluble sugars in rhizome material collected from deep edge sites. In April, the average soluble sugar concentration was 189.5 ± 33.7 mg g^{-1} dw, decreasing significantly to the lowest level measured, 83.4 ± 6.6 mg g^{-1} dw in June ($p = 0.004$), then increasing significantly to a peak of 252.4 ± 19.2 mg g^{-1} dw in September ($p < 0.0001$), and decreasing significantly to 169.1 ± 18.7 mg g^{-1} dw in December ($p = 0.01$; ANOVA, Tukey's

HSD) (Table 4.2). The mean soluble sugar concentrations in June (the low) and September (the high) represent 9.3 ± 0.7 and 25.2 ± 1.9 % of rhizome dry weight, respectively.

A significant interaction between month and depth was evident in sugar levels measured along the depth gradient at Fishing Island ($p < 0.0001$). In April, rhizome sugar content of intertidal plants (21.1 ± 2.1 mg g^{-1} dw) was significantly lower than that of both shallow subtidal (211.2 ± 16.0 mg g^{-1} dw) and deep edge plants (282.7 ± 31.7) (Table 4.4). In September, soluble sugar content ranged between 240.0 ± 19.1 mg g^{-1} dw (shallow subtidal) and 306.8 ± 31.4 mg g^{-1} dw (deep edge) with no significant difference between the three depths. In December, however, sugar content at the deep edge (128.8 ± 17.6 mg g^{-1} dw) was significantly lower than at both shallow subtidal (236.8 ± 35.5 mg g^{-1} dw) and intertidal sites (225.5 ± 9.8 mg g^{-1} dw) (Table 4.4). Decline in sugar levels was greatest in rhizomes from deep edge plants compared to plants from other depths both between April and June (69% vs. 30% (shallow subtidal plants)) and between September and December (58.2% vs. 1% (shallow subtidal plants) and 17% (intertidal plants)) (Figure 4.7a).

Recoverable rhizome sugars

Leaf re-growth from rhizomes

Rhizome sections collected from all five deep edge sites yielded an average of 0.086 ± 0.007 g dw of leaf material per g dw of rhizome material after dark incubation of rhizome sections collected in September (Table 4.2). After a period of 48 days, no more re-growth occurred. The average re-growth from rhizomes was significantly lower in December when growth was 0.064 ± 0.004 g dw of leaf material per g dw of rhizome material ($p = 0.010$) (Table 4.2). Most re-growth (74%) from deep edge rhizomes occurred within the first 24 days.

There was a significant interaction between depth and month in leaf re-growth sampled along the depth gradient at Fishing Island ($p < 0.0001$). In September, the average leaf re-growth of intertidal plants (0.116 ± 0.016 g g^{-1} dw) was significantly higher than that of shallow subtidal (0.069 ± 0.005 g g^{-1} dw) and deep edge plants (0.073 ± 0.010 g g^{-1} dw) (Table 4.4). In December, however, a reverse trend was evident, where leaf re-growth of intertidal plants (0.031 ± 0.006 g g^{-1} dw) was significantly lower than re-growth of both shallow subtidal (0.059 ± 0.008 g g^{-1} dw) and deep edge plants (0.061 ± 0.008 g g^{-1} dw) (Table 4.4; Figure 4.7b).

Sugar concentrations before and after leaf re-growth

Soluble sugars stored in rhizomes collected from all sites in September, the month with the highest sugar concentrations, decreased ($p < 0.00001$) from an average of 253 ± 15 mg g^{-1} (before re-growth) to 82 ± 11 mg g^{-1} (after re-growth), demonstrating that eelgrass plants consumed up to 67.5% of the sugars stored in the rhizomes to generate new leaf tissue in the absence of light. Rhizomes sampled along the depth gradient at Fishing Island showed no significant difference between depth in the decreases in sugar concentrations after re-growth ($p > 0.05$) (Figure 4.7c). Rhizome sugars were reduced by 83% (intertidal plants), 45% (shallow subtidal plants and 54% (deep edge plants) after re-growth (Figure 4.7c).

Estimated leaf re-growth from rhizome sugars

In September, the estimated average leaf re-growth from rhizome sugars from plants collected from five deep edge sites in the estuary was 0.366 ± 0.032 g dw (g sugar)$^{-1}$. In December, leaf re-growth was 0.393 ± 0.029 g dw (g sugar)$^{-1}$ and was not significantly different from September ($p = 0.641$) (Table 4.2).

There was a significant interaction between depth and month ($p < 0.0001$) in re-growth sampled along the depth gradient at Fishing Island. Leaf re-growth decreased with depth in September, but increased with depth in December (Table 4.4). In September, leaf re-growth of intertidal plants was (0.462 ± 0.048 g dw (g sugar)$^{-1}$) significantly higher than that of shallow subtidal (0.285 ± 0.023 g dw (g sugar)$^{-1}$) and deep edge plants (0.236 ± 0.032 g dw (g sugar)$^{-1}$) (Table 4.4). Shallow and deep edge re-growth were not significantly different. In December, however, leaf re-growth from rhizomes of deep edge plants (0.472 ± 0.058 g dw (g sugar)$^{-1}$) was significantly higher than that of shallow subtidal (0.250 ± 0.035 g dw (g sugar)$^{-1}$) and intertidal plants (0.135 ± 0.025 g dw (g sugar)$^{-1}$), with re-growth in the latter two not significantly different from each other (Table 4.4; Figure 4.7d).

DISCUSSION

The quarterly assessment of eelgrass plants growing along a depth gradient in an eelgrass bed near the mouth of the Great Bay Estuary, as well as eelgrass plants growing at the deep edge along a gradient up the Great Bay Estuary, demonstrated that in response to winter light reduction, deep edge plants exhibit a greater degree of photosynthetic and morphological acclimation compared to shallow subtidal and intertidal plants. I interpreted such acclimation, along with remobilization of stored carbohydrate reserves, to be an adaptation that allows the deep edge plants to survive low winter light. The relatively low contribution of remobilized reserves to winter growth underscores the importance of wintertime photosynthesis for winter survival of eelgrass.

In agricultural crops, the majority of photosynthesis has been shown to occur at light levels below that required to saturate photosynthesis, due to the fact that only a small amount of the total radiation is intercepted by the plants (Ort and Baker, 1988). On average, about 70% of the canopy photosynthesis of terrestrial crops is contributed by the upper 40% of the leaves. Photosynthetic efficiency under limited light, thus, should be of greater importance in determining the overall photosynthetic performance of terrestrial crops (Ort and Baker, 1988), and the same seems to be the case for deep edge eelgrass. Generally, eelgrass is considered to be a shade-adapted plant because of the generally low I_c and I_k, and a high photosynthetic efficiency, α (Dennison and Alberte, 1985; high levels of light harvesting proteins per photosystem (Cummings and Zimmerman, 2003) and an increment in photosystem unit size at low light (Dennison and Alberte, 1985). In the present study, plants displayed a very efficient use of low light (see high F_v/F_m at depth (Figure 4.3c) and falling F_v/F_m as light decreased with season (Table 4.2)) and were sensitive to high light (see depressed in June (Table 4.2), indicating that eelgrass plants are low-light adapted.

Photosynthetic and morphological acclimations to reduced light (Tables 4.2 and 4.3), further demonstrated by significant statistical interaction between depth and month in all the measured parameters (except shoot density) (Figures 4.1 through 4.6), a reduction in the concentration of light-capturing pigments (Figure 4.4a), and a nearly year-round utilization of rhizome soluble sugars with late summer replenishment (Table 4.2), indicate that deep edge plants (depths from 1.4 – 4.3 m) throughout Great Bay Estuary are chronically light-limited. Yet eelgrass plants persisted year-round under these light-limited conditions at the deep edge, attaining high biomass, especially during the month of September. In agreement with other work (Dennison, 1987; Dennison and Alberte, 1986), plants in the present study exhibited clear photoacclimation in which the efficiency of the light reaction of photosynthesis was adjusted in response to falling light intensity and light period with season and in response to depth. Photosynthetic capacity (ETR_{max}) and the minimum saturating light (I_k) of eelgrass plants decreased 4-fold (Figure 4.3a, 4.3b; Table 4.2) as the plant maximum quantum yield of PSII (F_v/F_m) increased between September and December (Figure 4.3c). Highly reduced photosynthetic capacity limits carbon assimilation and growth and may indicate a danger of plants approaching a carbon deficit and/or root anaerobiosis (Dennison and Alberte, 1985; Kraemer and Alberte, 1995). However, in winter increasing F_v/F_m with decreasing light may have prevented metabolic carbon deficit, while low temperatures reduced respiration rates.

The lowest quantum yields were measured in intertidal plants relative to deeper growing plants (Figure 4.3c), while a depression in the maximum quantum yield (F_v/F_m) (and chlorophyll *a+b*) was observed in June in all plants, coinciding with the period of highest underwater irradiance (Table 4.1). Reduction of F_v/F_m and chlorophyll at high light (in intertidal plants and in all plants in the month of June) may be an adaptation strategy to prevent absorption of excess light energy to avoid photodamage. These trends are consistent with photoacclimatory responses to low light that serve to increase the efficiency of capturing photons and transducing light energy so as to increase the effectiveness of light utilization (Bulchov et al., 1995).

Depth, seasonal fluctuations in incident irradiance, photosynthetic performance and turbidity, all influenced the daily photoperiod during which deep edge eelgrass shoots could maintain maximal photosynthetic rates in hours (defined as H_{sat}) (Zimmerman et al., 1995a). Measurements of H_{sat} and/or the H_{sat} requirement at the deep edge, however, are lacking, and studies on seasonal dynamics of deep edge eelgrass are rare (but see Rivers, 2006). H_{sat} directly affects carbon transport and anoxic stress in root tissues by influencing the daily period of seagrass root aerobiosis (Dennison and Alberte, 1985; Zimmerman et al., 1994). In deep edge plants, the effect of winters, with shorter periods of daylight resulting in extreme light limitation and significantly reduced plant metabolism, is amplified (Figure 4.3), suggesting that to maintain a positive carbon balance, longer hours of saturating light may be necessary in winter than in other seasons.

In the present study, H_{sat} fluctuated greatly (annual average H_{sat} = 4.8 hrs), not only because of seasonal fluctuations in incident irradiance, but also due to seasonal plant acclimation of photosynthetic performance (Dennison 1987), in particular, the minimum saturating light (I_k), which when low, results in longer H_{sat} and vice versa. I_k decreases with decreasing temperature; since $I_k = P_{max}/\alpha$, and then since the slope α is unaffected by temperature, I_k must decrease with P_{max} (Kirk, 1994). In eelgrass, I_k was found to increase from 7 μmol photons m^{-2} s^{-1} at 0°C to 90 μmol photons m^{-2} s^{-1} at 15°C (Marsh et al. 1986). Thus, seasonal increase in I_k is part of the increase in photosynthetic capacity with increasing temperature. H_{sat} was shortest in April (2.5 hours) and in September (3.0 hours) (Table 4.1). These periods of shortest H_{sat} were typically followed by a drop in soluble rhizome sugar concentrations (April-June and September-December, Table 4.2, Figure 4.7a), suggesting that stored reserves were being mobilized, and implying that very short H_{sat} periods may indeed coincide with a negative carbon balance in the plant. Such changes in rhizome sugar content support the proposition that seasonal sugar accumulation in rhizomes during favorable periods provides a supplemental source of energy for growth and respiration during periods of negative carbon balance (Zimmerman et al., 1995b; Burke et al., 1996; Alcoverro et al., 1999; Alcoverro et al., 2001; Cabello-Pasini et al, 2002) and after leaf losses (Vermaat and Verhagen, 1996; Zimmerman et al., 1996).

In spring (April), the daily period of saturating photosynthesis was low because incident light was still relatively low, yet I_k was rising as a result of rising temperatures. Although light was increasing, biomass ratios (see high root biomass, low S:R and small shoot size (i.e. leaf length, leaf width and above-ground shoot weight)) were unfavorable as there was little photosynthetic tissue to process available light fast enough to meet the rapidly increasing respiratory requirements (due to rising temperatures) of large non-photosynthetic tissue. Consequently, the plants needed to use reserves, since the need for growth and respiration exceeded photosynthate availability.

The length of H_{sat} was greatest (7 hours) in late spring-early summer (June) because of high incident irradiance and long days in that month and a less than maximum I_k (Table 4.1). Stored sugars continued to be depleted, reaching their lowest levels in June, coinciding with the period of maximum leaf growth. In the present study, the relative drop in soluble sugar concentrations was greater in deep edge plants (69% between April and June and 58% between September and December) compared to shallow subtidal plants (30% April - June and 0% September - December) (Table 4.5), suggesting a greater need for deep edge plants to compensate for low photosynthate availability by mobilizing more carbohydrate reserves to maintain a positive carbon balance. Relatively more sugars were mobilized between April and June than between September and December in deep edge plants (Table 4.2).

In autumn (September), the daily period of saturating photosynthesis was low because incident light had dropped significantly, yet I_k was at its peak due to high water temperatures. High temperatures in September (Figure 4.2) favored maximum photosynthesis and, at the same time, respiration rates of both photosynthetic and non-photosynthetic tissue increased, possibly resulting in a negative carbon balance and thereby necessitating mobilization of reserves.

In winter (December), however, H_{sat} deviated from integrated daily PAR because of seasonal changes in the relationship between photosynthesis and irradiance as explained below. I_k was very low due to low water temperatures and low light availability in December. With low ambient light, a very low I_k resulted in a relatively long H_{sat} (5.5 hours). An 80% reduction in I_k of deep edge plants from 69 μmol photons m^{-2} s^{-1} in September to 14 μmol photons m^{-2} s^{-1} in December effectively lengthened H_{sat} from 3 hours in September to 5.5 hrs in December. Lowering of I_k, therefore, is an adaptation by the plants to lengthen H_{sat}, extending the period of root aerobiosis and allowing for sustained photosynthesis under conditions of very low light availability and low plant respiration.

Thus, during both the periods of highest underwater irradiance (June) and lowest underwater irradiance (December), deep edge plants received the longest hours of saturating light owing to adjustments in the minimum saturating light (I_k). Increasing H_{sat} in December has been shown previously (Dennison, 1987;

Zimmerman et al., 1995a). The results of the present study show that an H_{sat} of 3 hours may represent a negative carbon balance, necessitating the use of stored carbohydrate reserves during spring and autumn. Mobilization of below-ground reserves and photoacclimation, therefore, work jointly to maximize carbon gain and maintain growth during periods of low light.

The present study found that the daily period of saturating photosynthesis (H_{sat}) is not lowest in winter. Instead, the times of the year when light may not be sufficient are the transition periods (spring and autumn) when light, temperature, plant morphology and biomass ratios are out of balance. Although plants adjust their saturation point to lower values under light stress conditions, photosynthetic rates (Table 4.2) and carbon consumption rates (Kraemer and Alberte, 1995) remain low in winter.

H_{sat} at the deep edge (average 4.8 hrs) was comparable to an experimental shortening of the light period by 3-5 hrs in June (Dennison and Alberte, 1985), and to a 2-hr H_{sat} treatment (Alcoverro et al. 1999), both of which resulted in eelgrass mortality within 30 days. In the same way, shading eelgrass plants to 11% SI for 3 months in a mesocosm experiment (at temperatures between 18 °C and 23°C) during the peak growth period led to > 80% plant mortality, with the few surviving plants succumbing and not expected to survive the winter (Chapter III). In the present study, there was no loss of vegetation with a H_{sat} of 2.5 hours in April (average temperature, 7 °C). According to the literature, healthy eelgrass generally requires 4 – 6 hours of saturating light to meet daily carbon demand (Dennison and Alberte, 1986; Kraemer and Alberte, 1995; Zimmerman et al., 1989, Zimmerman et al., 1991, 1995a). Since no plant carbon balance was determined in the present study, it can only be speculated that a H_{sat} of 2.5 (April) - 3.0 (September) hours was insufficient to meet the plants' respiratory requirements and, for that reason, plants began using stored reserves. The difference in responses between field and manipulative studies might be explained by the fact that in the field, light conditions change gradually with season (as opposed to the sudden change imposed on plants in manipulative experiments), which allows plants in the field to acclimate to falling light intensity and period, enabling effective use of ambient light paramount to plant survival under light stress. Secondly, the timing of light reduction in the field ensures plants have built up large below-ground reserves. Thirdly, along with falling light, temperature decreases dramatically, reducing plant respiratory demand and thereby reducing the oxygen requirement to maintain aerobiosis. However, temperature and light interactions are rarely captured in experiments.

A common ontogenetic response of aquatic plants to variations in light regime is to regulate the quantity of light-capturing pigments (Kirk, 1994). Higher pigment content at low light enables plants to absorb a greater percentage of incident irradiance (Falkowski and Raven, 1997). In agreement, the data of the present study showed increasing leaf chlorophyll $a+b$ content with falling light levels between June and September (Table 4.2) as well as with depth (Figure 4.4a). The extreme light stress at the deep edge (at Fishing Island), however, resulted in a significant reduction ($p = 0.03$) in pigments of plants there between September and December (Figure 4.4a). Such a chlorotic response to extreme light stress has been demonstrated in algae (Falkowski and Owens, 1980).

These adjustments in pigments and photosynthetic activity with falling light imply that plants reallocate energy and resources from the carbon assimilation process to that of light harvesting as light becomes limiting, i.e. plants increase the overall photon yield of photosynthesis by slowing the maximum rate of carbon assimilation (Major and Dunton, 2002).

Morphological acclimation to low light stress displayed by eelgrass plants serves to reduce carbon requirements and ensure survival (Dennison and Alberte, 1986). In the present study, leaf length and width (Figures 4.5a and b), the number of leaves per shoot (Figure 4.5c), and the weight per shoot (Figure 4.6a) of deep edge plants decreased significantly between September and December when underwater light was at its lowest (Table 4.1), but the change in shoot size of shallow subtidal and intertidal plants was negligible (Figure 4.5). Over the same period, rhizome weight (represented by W:L of the 1st 5 internodes) decreased by 60% in deep edge plants, and by 36% in shallow subtidal plants, but no change occurred in intertidal plants (Figure 4.6d). These data further suggest that for deep edge plants to survive winter low light conditions, they must exhibit considerable acclimation compared to shallow subtidal and intertidal plants.

Reduced shoot density with depth (Figure 4.5d), also observed by Dalla Via et al.(2003), Hauxwell et al. (2003) and Middelboe et al. (2003), confirms findings from earlier studies that attributed this phenomenon to the need of plants to reduce self-shading and increase the light received by individual plants (Krause-Jensen et al., 2000; Olesen et al., 2002). No changes in shoot density with season, however, were observed at the deep site as light levels dropped substantially during winter. In spite of a relatively low photosynthetic capacity, deep edge plants did not differ significantly from shallow subtidal plants in shoot weight (Figure 4.6a) and carbohydrate storage (Figure 4.7a), and in September they had significantly higher leaf length (Figure 4.5a) in comparison to shallow subtidal and intertidal plants. There was no significant difference in September in the total biomass (Figure 4.6b) between deep edge and shallow subtidal plants despite a significantly lower shoot density (Figure 4.5 d). Apparently, biomass build-up of deep edge plants occurred primarily through increasing shoot size rather than through increased shoot density.

The difference in biomass between deep edge plants and shallower plants, however, was portrayed in allocation between photosynthetic and non-photosynthetic tissues (S:R), a ratio that has been shown to affect the plant's carbon balance, and consequently the period of light saturating photosynthesis (Zimmerman et al., 1989). An increasing S:R with decreasing light availability indicates a phenotypic adjustment in the allocation of photosynthate in an attempt to reduce the respiratory burden of below-ground biomass so as to maintain a positive carbon balance (Zimmerman et al., 1989; Kraemer and Alberte, 1993; Olesen and Sand-Jensen 1994; Zimmerman et al., 1995b; Olesen et al., 2002). Thus, deep edge plants in the present study displayed a greater adaptation to light reduction compared to shallow-growing plants (Figure 4.6c).

Carbohydrates in eelgrass appear to be stored primarily as sugars, mainly sucrose, and can represent between 80 and 96% of total plant carbohydrates; maximum concentration of sugars appears to occur in rhizome tissues (Zimmerman et al., 1989; Burke et al., 1996; Alcoverro et al., 1999; Touchette and Burkholder, 2000; Colarusso, 2007), followed closely by leaves (Zimmerman et al., 1989; Zimmerman et al., 1995a, b; Alcoverro et al. 1999; Touchette, 1999; Touchette and Burkholder, 2000; Cabello-Pasini et al., 2002). Unlike *Posidonia oceanica*, where starch constitutes almost 50% of the carbohydrate in lignified rhizomes (Pirc, 1985), starch concentrations are generally low in *Z. marina* except in roots, where starch may account for more than 65% (up to 140 mg g^{-1}dry weight) of the total non-structural carbohydrate content (Burke et al., 1996). Carbon accumulation occurs when photosynthate availability exceeds the need for growth and/or respiration. In the present study, the seasonal peak in storage of below-ground sugar reserves by the eelgrass plants at the deep edge in Great Bay Estuary occurred in the late summer (Table 4.2) - early autumn period, and did not parallel the growth pattern found by Orth and Moore (1986) and Burke et al. (1996). Instead, the period of great carbon build-up in storage tissues occurred after the peak growth season, consistent with reports on *Zostera noltii* by Vermaat and Verhagen (1996) and on *Ruppia maritima* by Lazar and Dawes (1991), and coincided with the peak in estuarine water temperature.

Previous studies, in which carbohydrates in eelgrass were measured in leaves only, showed that leaf carbohydrates declined with decreasing light, and suggested that accumulated carbohydrates were likely to be critical for supporting metabolic activity and growth during the winter and early spring (Zimmerman et al., 1995a; Cabello-Pasini et al., 2002). In a carbohydrate-light manipulative experiment, Colarusso (2007) demonstrated that, in particular, the depletion of the sugar pool (compared to starch) in rhizome tissues (compared to leaf) was most significant. In his study, 41% of sugars in rhizomes were depleted in 4 days in the dark, compared with 13% in leaves. Furthermore, after 12 weeks in the dark, plants showed a marked decline in the ratio of leaf: rhizome carbohydrates, suggesting that in low light (that the plants have not adapted to), rhizome carbohydrates, particularly sugars, are utilized prior to starch (Colarusso, 2007). During this period the change in starch content was small.

Other than inference of sucrose mobilization based on changes in concentrations and the activity of sucrose synthase (Zimmerman et al., 1995b; Alcoverro et al., 1999; Touchette and Burkholder, 2007), however, no previous studies have quantified recoverability of stored reserves for growth, or the relative contribution of wintertime photosynthesis to stored carbon for winter growth. As the first attempt to quantify leaf re-growth from rhizomes, the present study demonstrated that eelgrass plants could potentially remobilize up to 68% of stored rhizome sugars for leaf re-growth in the absence of light, with no more re-growth occurring after 48 days. However, the total pool of rhizome sugars at the deep edge in December was relatively small, barely more than 2 g sugars m^{-2}. The sugar pool was small possibly due to the fact that these plants had far less below-ground biomass than above-ground biomass (see high and increasing S:R at the deep edge after September, Figure 4.6c).

Using growth predicted in three different ways, in addition to winter eelgrass growth values from the literature at depths ranging between -2.3 and -5.5 m, the average contribution of sugar reserves to winter (December) eelgrass growth at the deep edge was estimated to be in the order of 6% (range 1 to 13%). In comparison, the contribution to September growth was only 0.1%. Since experiments have shown that leaf sugars can also contribute to winter growth (Zimmerman et al., 1995a; Cabello-Pasini et al., 2002), and assuming equal biomass of plant parts and an equal contribution of stored starch, reserves stored in leaves and roots could potentially increase the contribution of reserves to winter growth. Based on literature values of the percentage of rhizome sugars of the total pool of reserves (i.e., sugars and starch in leaves, rhizomes and roots; see Zimmerman et al., 1989; Burke et al., 1996; Alcoverro et al., 1999; Colarusso, 2007), values calculated in the present study could be scaled up to 13% (ranging from 1 to 15%). The surprisingly small contribution of rhizome reserves to winter plant growth at the deep edge underscores the importance of photosynthetic acclimation for winter survival of eelgrass at the deep edge.

Substantial carbohydrate stores, low rates of carbon consumption and the absence of the pasteur effect (i.e., oxygen does not have an inhibiting effect on the fermentation process) in below-ground tissues, as well as sustained protein synthesis by eelgrass roots, all help to maintain metabolism during very low light (Kraemer and Alberte, 1995). Kraemer and Alberte (1995) suggested that at the deep edge, such adaptations would be restricted, as small reserves due to chronic light limitation would lead to lethal stress of root tissues with prolonged reduction in H_{sat}. The present study demonstrates that deep edge plants accumulate comparable reserves to shallow-growing plants, and persist with H_{sat} as low as 2.5 hours. Deep edge plants survive wintertime light stress owing to substantial acclimation in photosynthetic and morphological parameters and greater mobilization of stored reserves compared to shallow plants. Despite greater mobilization of carbon from rhizomes (inferred from the sharp decrease in sugar concentration (Figure 4.7a) and measured as leaf re-growth in g dw (g sugar)$^{-1}$ (Figure 4.7d)) by deep edge plants than plants at other depths, the relative contribution of rhizome sugar reserves to winter eelgrass growth was small.

The results of the present study demonstrate that light-limited deep edge eelgrass plants exhibit a greater degree of photosynthetic and morphological acclimations to further light reduction in winter than plants at other depths. The amount of carbon mobilized from rhizome reserves increases with depth, but the relatively small contribution of these reserves to winter eelgrass growth at the deep edge underscores the importance of wintertime photosynthesis in plant survival.

The hypotheses that seasonal light availability has no effect on rhizome sugar concentrations, photosynthetic capacity or morphology of eelgrass at the deep edge, and that rhizome sugar reserves do not contribute to eelgrass winter growth are rejected.

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Table 4.1. The light characteristics at the maximum depth limit of eelgrass distribution in Great Bay Estuary. Mean daily PAR values are in mol photons m^{-2} d^{-1}; I_k values are in μmol photons m^{-2} s^{-1}. The daily period of light-saturated photosynthesis in hours (H_{sat}) was calculated using corresponding minimum saturating light (I_k) for each site in each month. I_k was derived from rapid light curves (RLCs) and light logged at 5 deep edge sites. Values are mean (SE), *n* = 5 deep edge sites. Different letters, read by column, indicate significant differences set at $\alpha = 0.05$ using 1-way ANOVA and Tukey's comparison of means.

	Mean **Daily PAR**	Mean **% SI**	Mean I_k	Mean H_{sat}
April	**1.6 (0.7)^{a}**	**15.7 (2.5)^{a*}**	**29.5 (3)^{a}**	**2.5 (0.8)^{a}**
June	**4.2 (0.4)^{b}**	**10.2 (1.3)^{a}**	**42.9 (9.8)^{b}**	**7.1 (0.4)^{b}**
September	**2.4 (0.3)^{a}**	**10.1 (3.3)^{a}**	**68.8 (3.6)^{c}**	**3.0 (0.5)^{a}**
December	**0.8 (0.1)^{c}**	**16.5 (6.0)^{a}**	**14.1 (1.0)^{d}**	**5.5 (0.3)^{c}**

*** March 2005**

Table 4.2. Photosynthetic capacity (ETR_{max}), maximum quantum yield of PSII (F_v/F_m), leaf chlorophyll content, rhizome soluble sugar concentration, and leaf re-growth of eelgrass plants sampled quarterly during 2004 at the maximum depth limit in Great Bay Estuary. Values are means (SE), $n = 5$ deep edge sites. Different letters, read by row, indicate significant differences set at $\alpha = 0.05$ using 1-way ANOVA and Tukey's comparison of means.

	April	**June**	**September**	**December**
ETR_{max} (µmol electrons $m^{-2}s^{-1}$)	$19.4(2.4)^a$	$28.9\ (1.7)^b$	$45.2\ (2.3)^c$	$11.3\ (0.8)^d$
F_v/F_m	$0.760\ (0.004)^a$	$0.749\ (0.002)^b$	$0.782\ (0.002)^c$	$0.813\ (0.001)^d$
Chl *a+b* (µg cm^{-2})	$44.4\ (1.9)^a$	$28.9\ (0.9)^b$	$45.0\ (1.3)^a$	$43.8\ (0.8)^a$
Chl *a/b*	$2.46\ (0.02)^a$	$2.11\ (0.01)^b$	$2.16\ (0.01)^c$	$2.39\ (0.01)^d$
Soluble sugars (mg g^{-1})	$189.5\ (33.7)^{ab}$	$83.4\ (6.6)^c$	$252.4\ (19.2)^b$	$169.1(18.7)^a$
Leaf re-growth (g dw (g rhizome)$^{-1}$)	no data	no data	$0.086\ (0.007)^a$	$0.064\ (0.005)^b$
Leaf re-growth (g dw (g sugar)$^{-1}$)	no data	no data	$0.366\ (0.032)^a$	$0.393\ (0.029)^a$

Table 4.3. Variation with month of morphological and biomass parameters of eelgrass plants sampled quarterly during 2004 at the maximum depth limit in Great Bay Estuary. Values are means (SE), *n* = 5 deep edge sites. Different letters read by row indicate significant differences set at $\alpha = 0.05$ using 1-way ANOVA and Tukey's comparison of means.

	April	**June**	**September**	**December**
Leaf length (cm)	24.7 (1.2)^{a}	78.4 (4.9)^{bc}	88.6 (3.0)^{c}	70.6 (2.7)^{b}
Leaf width (mm)	3.01 (0.08)^{a}	4.79(0.16)^{b}	4.65 (0.11)^{b}	4.05 (0.11)^{c}
Leaves shoot^{-1} (#)	4.4 (0.1)^{a}	5.8 (0.2)^{b}	5.1 (0.1)^{c}	3.9 (0.1)^{d}
Above ground weight shoot^{-1} (g)	0.12 (0.02)^{a}	0.40 (0.04)^{b}	0.72 (0.04)^{c}	0.33 (0.03)^{b}
Total biomass (g m^{-2})	68.12 (7.39)^{a}	no data	128.49 (13.98)^{b}	85.57 (12.49)^{ac}
Shoot to root ratio (S:R)	1.155 (0.165)^{a}	no data	3.455 (0.311)^{b}	3.718 (0.517)^{b}
Rhizome Weight to Length ratio (g cm^{-1})	0.0145 (0.0009)^{a}	no data	0.0194 (0.0010)^{b}	0.0111 (0.0007)^{c}
Shoot density (shoots m^{-2})	330 (42)	no data	293 (33)	431 (29)

Table 4.4. Analysis of variance of depth-by-month responses of eelgrass measured at Fishing Island: pair-wise comparison by depth.

		April		**June**		**September**		**December**	
Parameter	**Depths compared**	**ANOVA *F* value**	***p***	**ANOVA *F* value**	***p***	**ANOVA *F* value**	***p***	**ANOVA *F* value**	***p***
ETR_{max}	intertidal vs. shallow subtidal	83.494	0.00080	-	-	0.657	0.46300	26.549	0.00660
	intertidal vs. deep edge	0.197	0.68030	-	-	63.767	0.00130	92.621	0.00060
	shallow subtidal vs. deep edge	30.459	0.00530	13.366	0.02160	56.974	0.00160	25.594	0.00720
I_k	intertidal vs. shallow subtidal	28.780	0.00700	-	-	0.769	0.43000	25.952	0.00700
	intertidal vs. deep edge	2.701	0.17800	-	-	9.925	0.03400	93.718	0.00060
	shallow subtidal vs. deep edge	29.574	0.00600	1.535	0.28300	47.859	0.00200	1.535	0.28300
F_v/F_m	intertidal vs. shallow subtidal	14.409	0.00220	-	-	51.991	0.00001	31.346	0.00002
	intertidal vs. deep edge	32.016	0.00008	-	-	50.891	0.00001	23.297	0.00014
	shallow subtidal vs. deep edge	13.364	0.00440	1.486	0.25100	0.829	0.37250	0.857	0.36416
Chl *a+b*	intertidal vs. shallow subtidal	17.856	0.00035	-	-	17.867	0.00035	7.660	0.01120
	intertidal vs. deep edge	63.665	0.00001	-	-	42.224	0.00001	0.352	0.55900
	shallow subtidal vs. deep edge	5.478	0.02920	-	-	12.013	0.00231	9.291	0.00810
Chl *a/b*	intertidal vs. shallow subtidal	8.795	0.01890	-	-	28.252	0.00005	5.464	0.02990
	intertidal vs. deep edge	3.923	0.06120	-	-	30.909	0.00001	5.690	0.02700
	shallow subtidal vs. deep edge	0.339	0.56880	-	-	0.006	0.94000	0.042	0.83900
Leaf length	intertidal vs. shallow subtidal	87.181	0.00001	-	-	338.758	0.00001	141.548	0.00001
	intertidal vs. deep edge	19.234	0.00008	-	-	238.833	0.00001	241.350	0.00001
	shallow subtidal vs. deep edge	4.852	0.03230	6.089	0.02500	4.855	0.03820	2.788	0.10980
Leaf width	intertidal vs. shallow subtidal	42.984	0.00001	-	-	35.640	0.00001	33.925	0.00001
	intertidal vs. deep edge	27.298	0.00001	-	-	72.000	0.00001	40.753	0.00001
	shallow subtidal vs. deep edge	0.043	0.83700	12.374	0.00280	5.211	0.03250	1.865	0.18650
# leaves sht^{-1}	intertidal vs. shallow subtidal	-	-	-	-	8.250	0.00880	5.604	0.02700
	intertidal vs. deep edge	43.464	0.00001	-	-	22.000	0.00011	11.355	0.00280
	shallow subtidal vs. deep edge	-	-	0.341	0.56870	1.158	0.29400	28.714	0.00003
shoot density	intertidal vs. shallow subtidal	4.619	0.04300	-	-	21.447	0.00090	28.047	0.00035
	intertidal vs. deep edge	20.759	0.00015	-	-	37.647	0.00010	32.569	0.00019
	shallow subtidal vs. deep edge	40.258	0.00001	-	-	2.886	0.13220	2.049	0.18280
weight shoot^{-1}	intertidal vs. shallow subtidal	59.568	0.00001	-	-	26.858	0.00004	88.732	0.00001
	intertidal vs. deep edge	14.197	0.00080	-	-	81.013	0.00001	115.675	0.00001
	shallow subtidal vs. deep edge	0.185	0.67090	13.406	0.00200	0.919	0.34800	1.192	0.28670
Total biomass	intertidal vs. shallow subtidal	8.174	0.00939	-	-	0.294	0.59970	6.302	0.03090
	intertidal vs. deep edge	2.004	0.17092	-	-	4.173	0.08830	6.041	0.03380
	shallow subtidal vs. deep edge	19.005	0.00028	-	-	2.745	0.12860	0.075	0.78920
S:R	intertidal vs. shallow subtidal	11.807	0.00250	-	-	11.833	0.00880	9.445	0.01180
	intertidal vs. deep edge	9.219	0.00750	-	-	41.148	0.00068	116.398	0.00010
	shallow subtidal vs. deep edge	0.778	0.38940	-	-	1.561	0.24670	7.026	0.02430
Rhizome W:L	intertidal vs. shallow subtidal	-	-	-	-	39.734	0.00001	12.265	0.00210
	intertidal vs. deep edge	-	-	-	-	84.089	0.00001	2.050	0.16690
	shallow subtidal vs. deep edge	-	-	-	-	4.486	0.04570	5.006	0.03570
Root biomass	intertidal vs. shallow subtidal	7.356	0.01270	-	-	62.394	0.00001	22.108	0.00010
	intertidal vs. deep edge	45.331	0.00010	-	-	71.502	0.00001	7.903	0.01080
	shallow subtidal vs. deep edge	10.183	0.00440	7.475	0.01360	8.327	0.02010	1.753	0.19980
Rhizome soluble sugars	intertidal vs. shallow subtidal	130.233	0.00029	-	-	0.416	0.55420	0.093	0.77520
	intertidal vs. deep edge	67.920	0.00118	-	-	0.357	0.58240	23.190	0.00880
	shallow subtidal vs. deep edge	4.055	0.11430	10.374	0.03220	3.301	0.14340	7.432	0.05270
Leaf regrowth (g g^{-1} dry weight)	intertidal vs. shallow subtidal	-	-	-	-	7.583	0.01160	5.718	0.02940
	intertidal vs. deep edge	-	-	-	-	5.152	0.03340	7.233	0.01550
	shallow subtidal vs. deep edge	-	-	-	-	0.124	0.72760	0.024	0.87850
Leaf regrowth (g dry weight (g sugar)$^{-1}$	intertidal vs. shallow subtidal	-	-	-	-	9.849	0.00520	6.349	0.02200
	intertidal vs. deep edge	-	-	-	-	13.007	0.00180	23.658	0.00012
	shallow subtidal vs. deep edge	-	-	-	-	1.111	0.30440	10.180	0.00480

Table 4.5. Analysis of variance of depth-by-month responses of eelgrass measured at Fishing Island: pair-wise comparison by month (as referred to in the text).

		Intertidal		**Shallow subtidal**		**Deep edge**	
Parameter	**Months compared**	**ANOVA *F* value**	***p***	**ANOVA *F* value**	***p***	**ANOVA *F* value**	***p***
ETR_{max}	September - December	644.205	0.00001	619.260	0.00002	565.633	0.00002
I_k	April - September	90.539	0.00070	0.419	0.55240	-	-
	September - December	95.224	0.00060	639.074	0.00001	325.403	0.00005
Chl *a/b*	September - December	3.182	0.08960	27.045	0.00004	27.368	0.00004
Leaf length	September- December	5.985	0.02300	0.184	0.67200	11.970	0.00200
Leaf width	September- December	0.220	0.64400	0.005	0.94560	22.000	0.00010
Shoot weight	June - September	-	-	0.033	0.85810	28.080	0.00004
Root biomass	April - September	6.365	0.01940	34.708	0.00001	111.463	0.00001
Rhizome soluble sugars	April - June	-	-	7.971	0.04770	33.520	0.00440
	September - December	0.966	0.38130	0.007	0.93890	24.551	0.00770

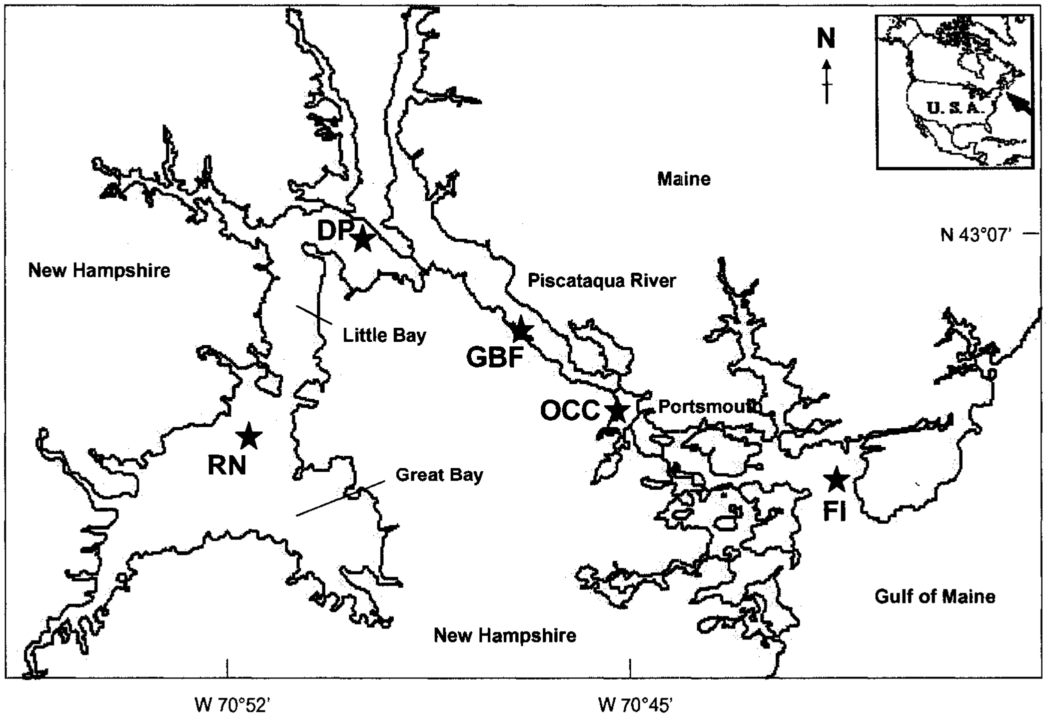


Figure 4.1. Map of the study area (Great Bay Estuary), showing the location of names mentioned in the text (research sites marked with asterix: FI = Fishing Island; OCC = Outer Cuts Cove; GBF = Great Bay Fishing Pier; DP = Dover Point; RN = Red Nun).

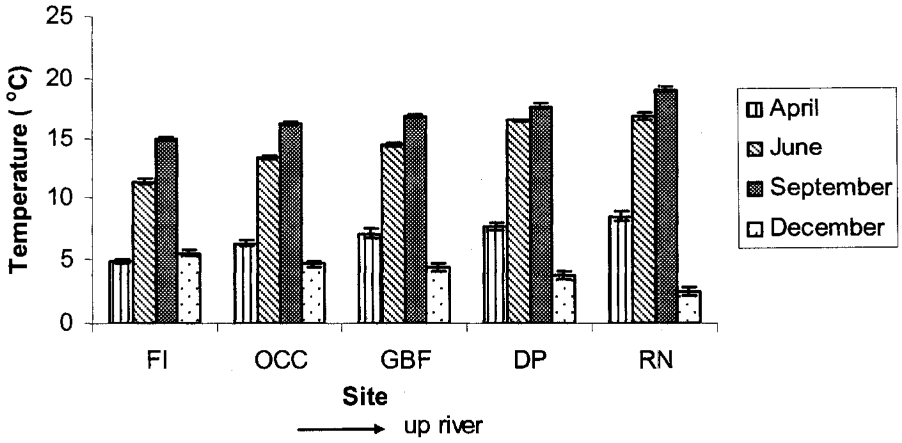
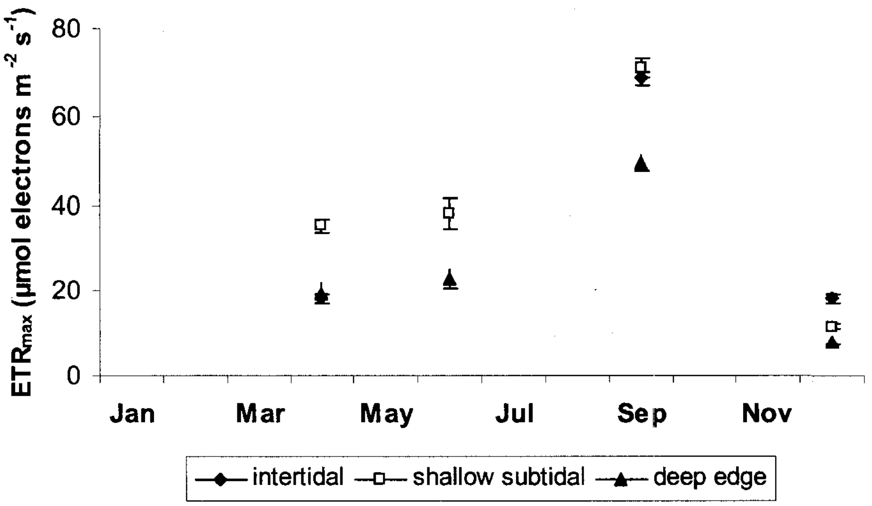


Figure 4.2. Mean monthly water temperature (°C) monitored at five deep edge sites in the Great Bay Estuary during 2004.

a.



b.

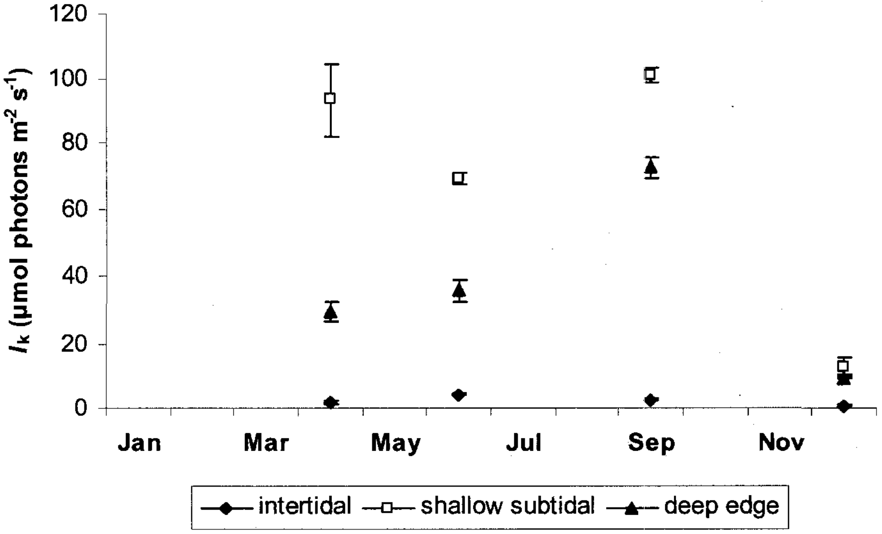


Figure 4.3. (see next page for figure caption)

c.

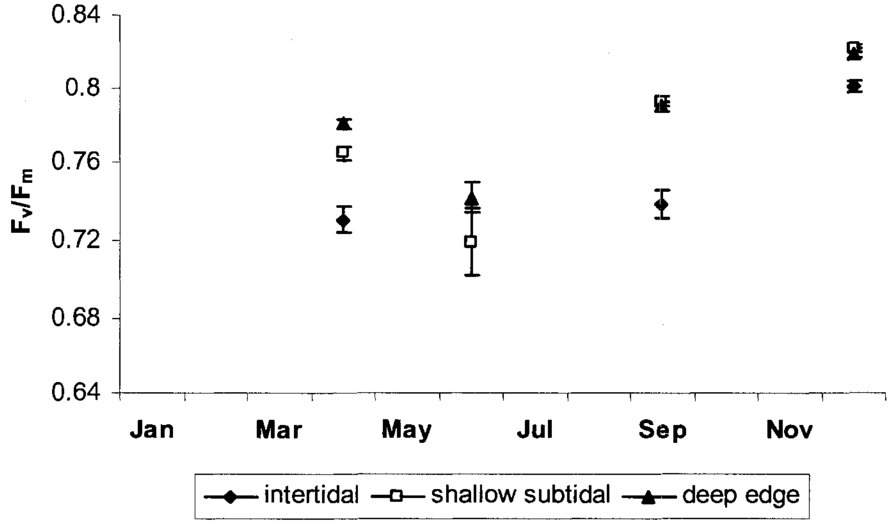
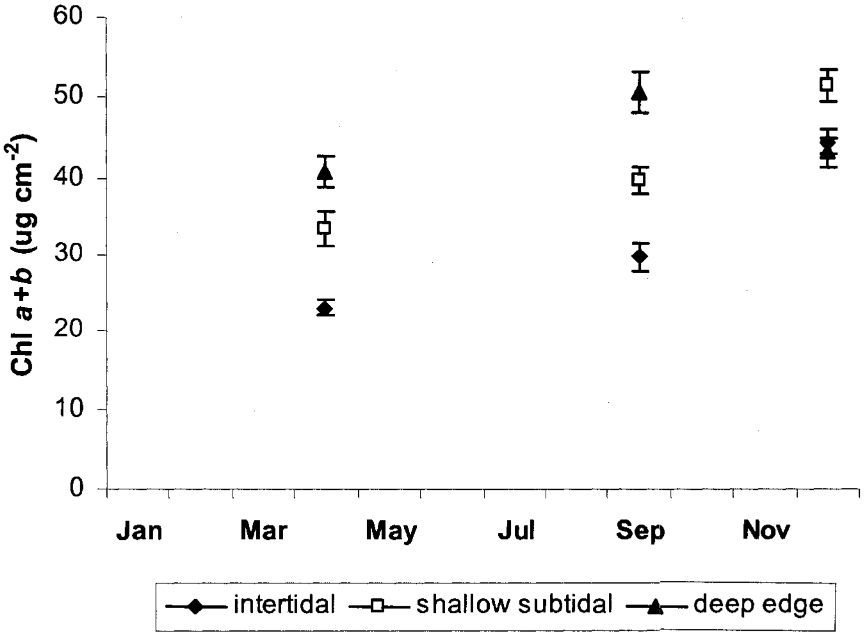


Figure 4.3. Variation with month of (a) maximum electron transport rate (ETR_{max}), (b) minimum saturation irradiance (I_k), and (c) maximum quantum yield of photosystem II (F_v/F_m) of eelgrass plants along a depth gradient at Fishing Island. Values are means ± SE, $n = 3$.

a.



b.

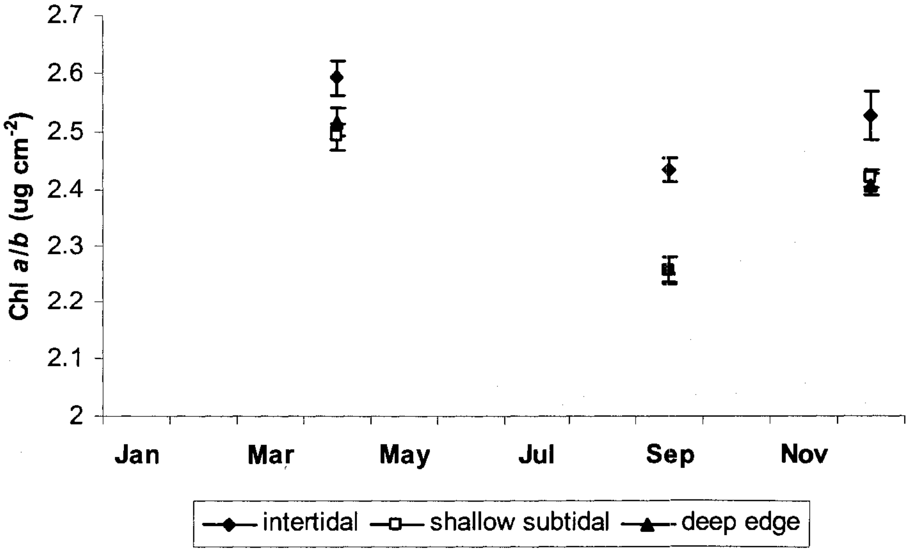


Figure 4.4. Variation with month of (a) chlorophyll a+b content, (b) chlorophyll *a/b* of eelgrass plants along a depth gradient at Fishing Island. Values are means ± SE, *n* = 3.

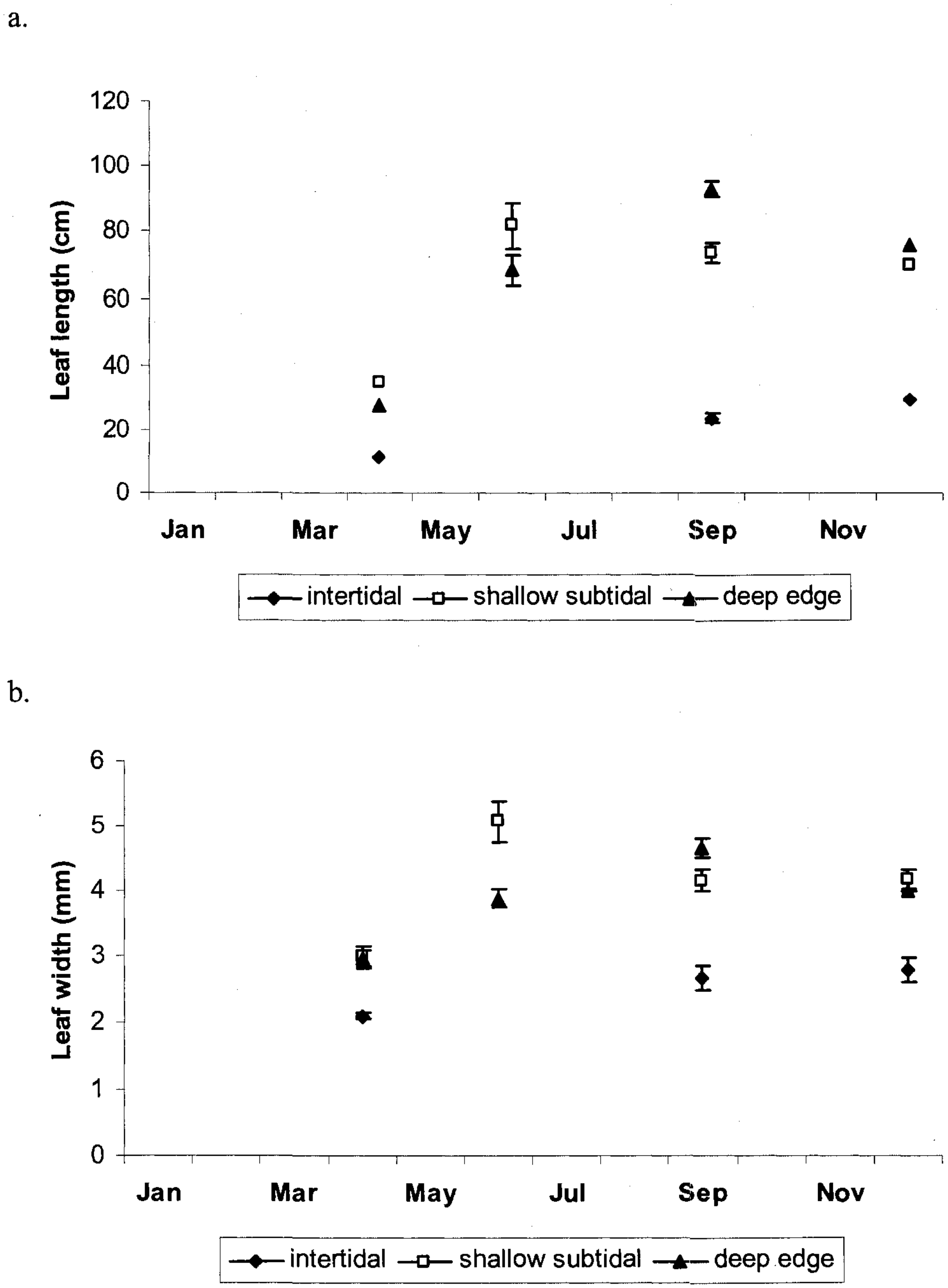


Figure 4.5. (see next page for figure caption)

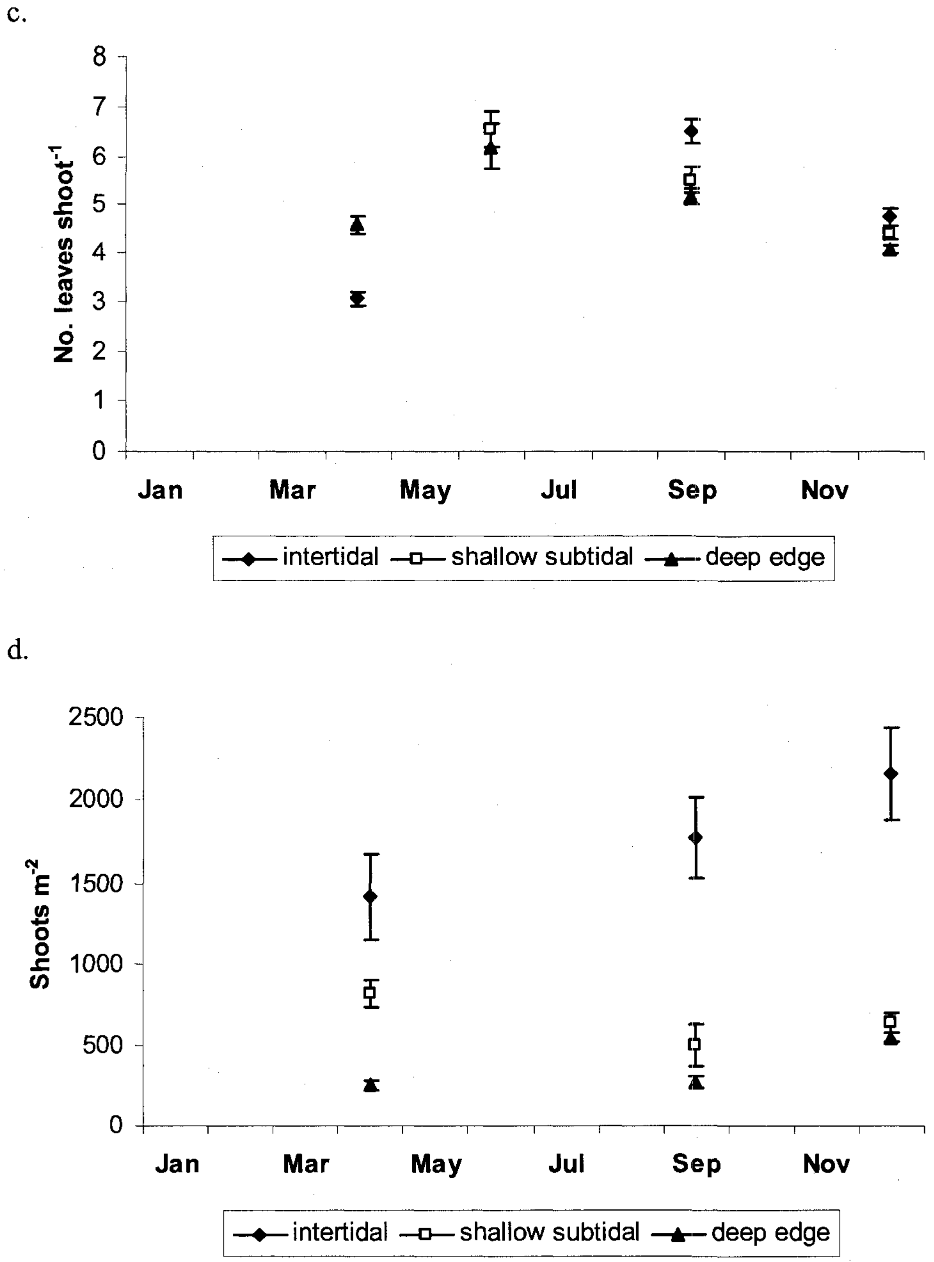


Figure 4.5. Variation with month of (a) leaf length, (b) leaf width, (c) the number of leaves per shoot, and (d) shoot density of eelgrass plants along a depth gradient at Fishing Island. Values are means ± SE, *n* = 3.

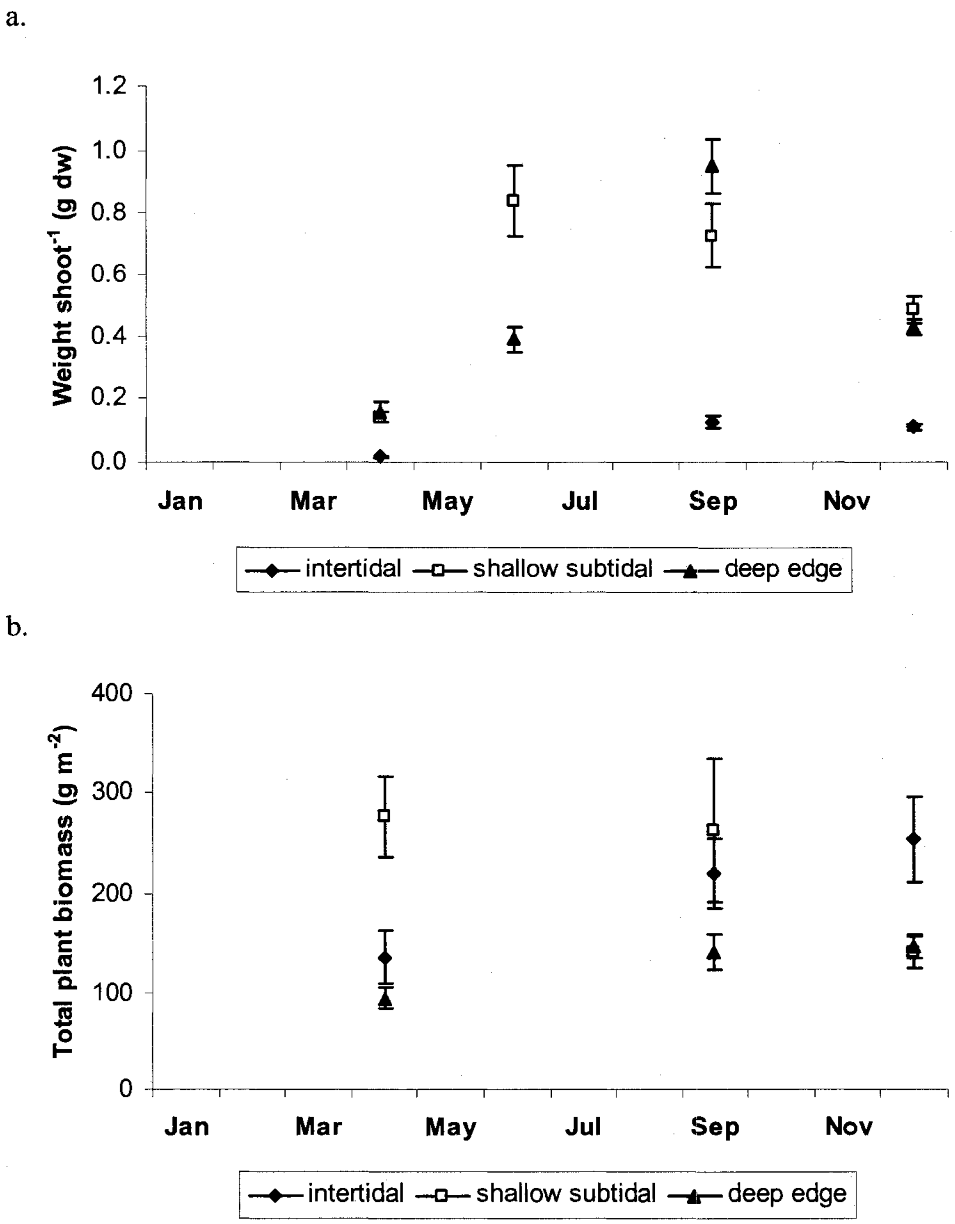
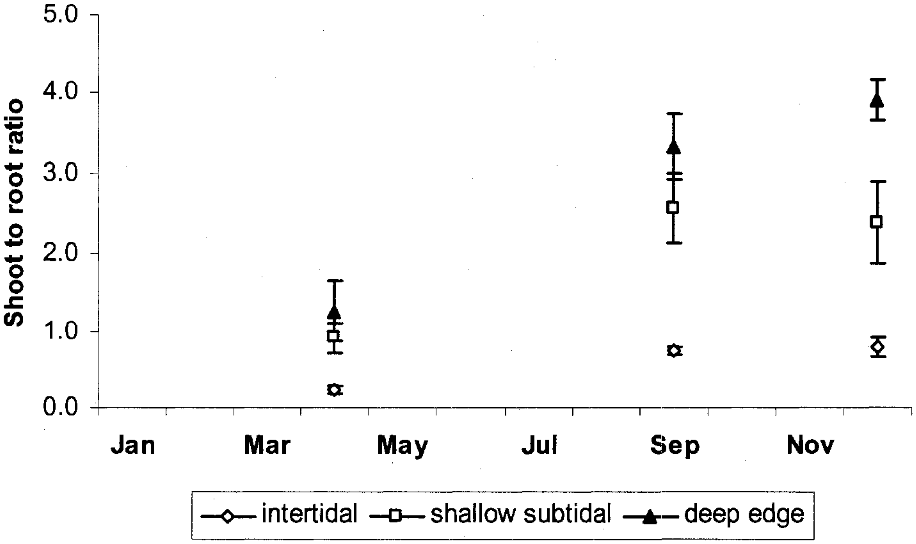


Figure 4.6. (see next page for figure caption)

c.



d.

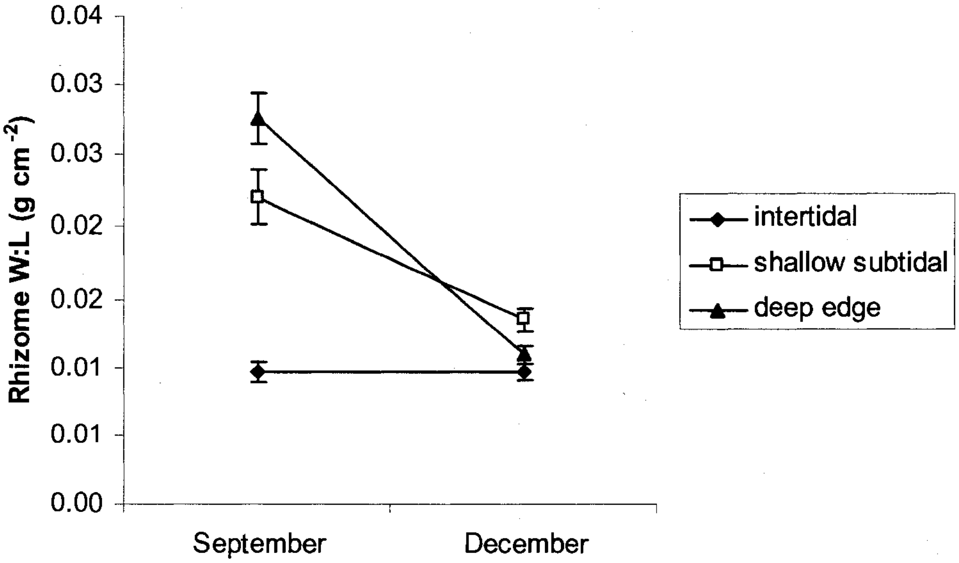


Figure 4.6. (see next page for figure caption)

e.

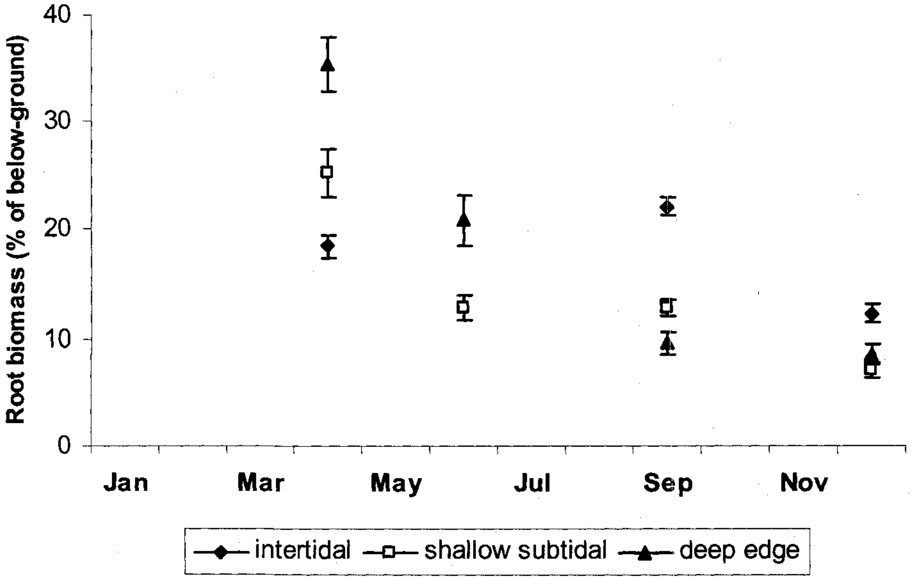
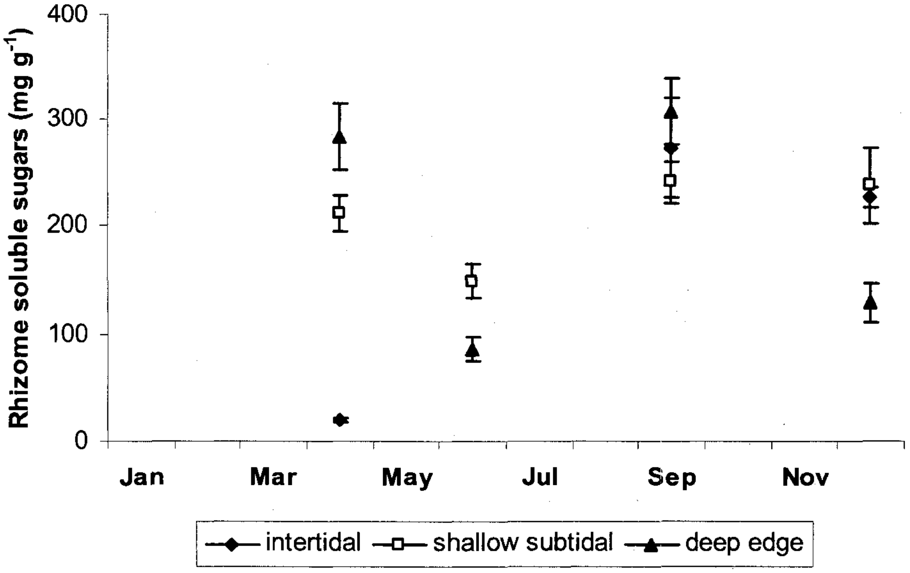


Figure 4.6. Variation with month of (a) weight per shoot, (b) total plant biomass, (c) shoot to root ratio, (d) rhizome weight to length ratio and (e) root biomass (as % of below-ground biomass) of eelgrass plants along a depth gradient at Fishing Island. Values are means ± SE, *n* = 3.

a.



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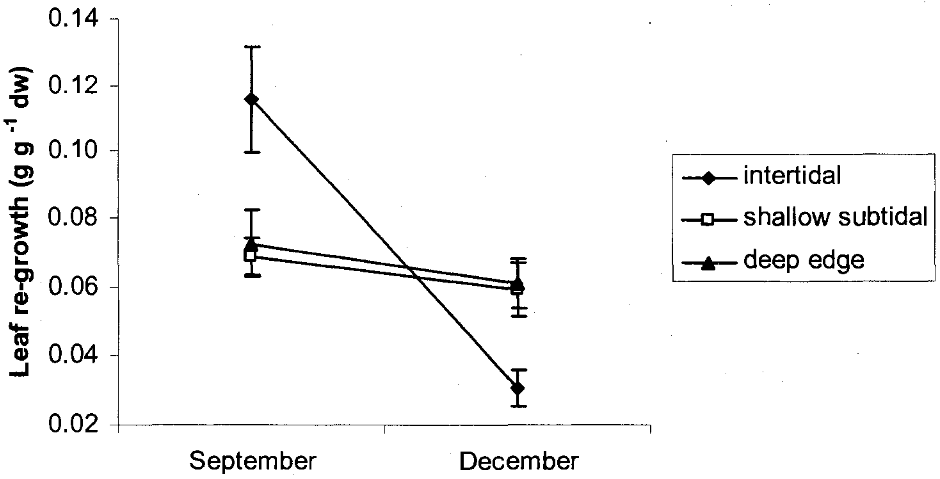

Figure 4.7. (see next page for figure caption)

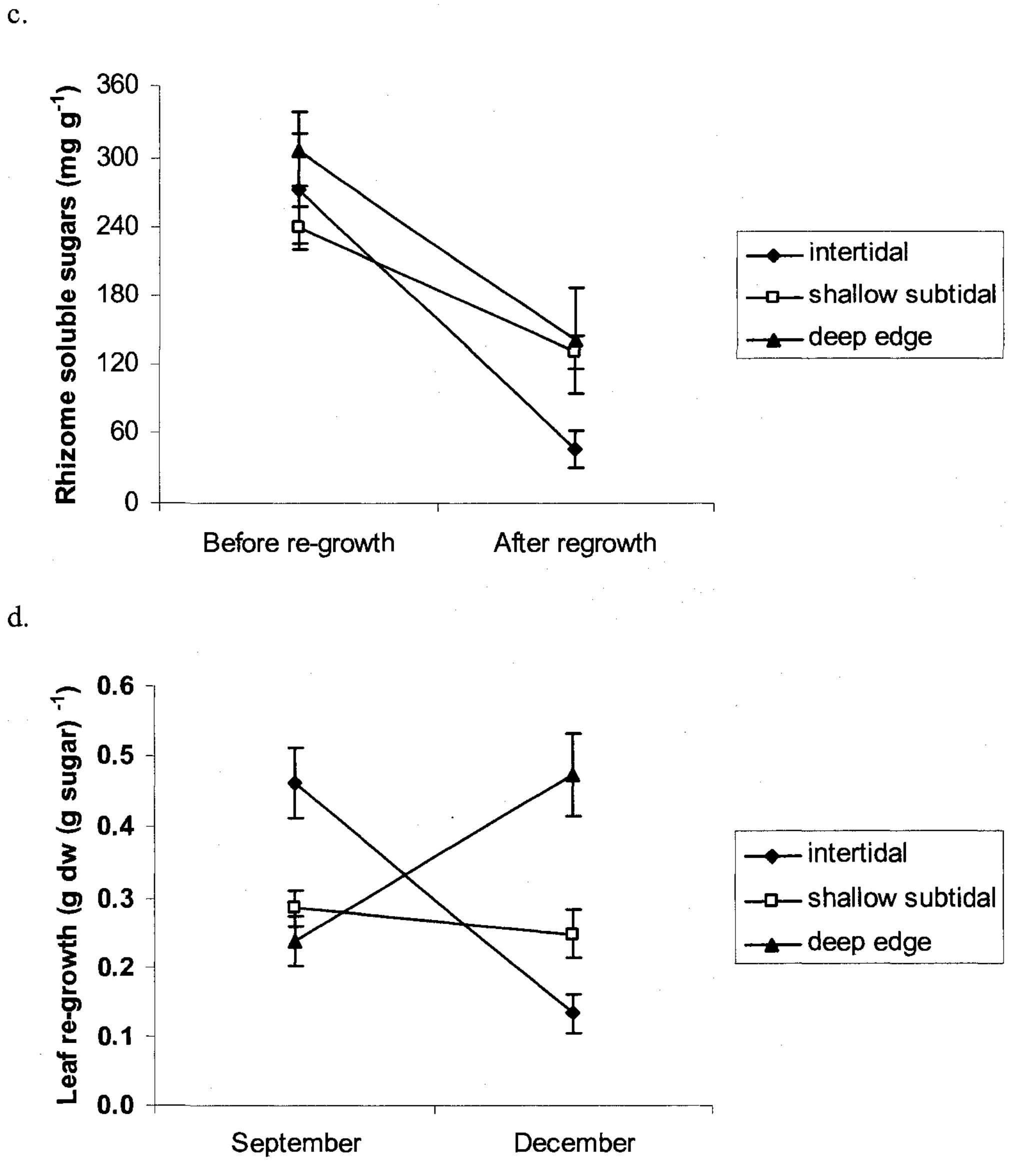

Figure 4.7. Rhizome sugar concentration and leaf re-growth in eelgrass along a depth gradient at Fishing Island, showing: (a) variation with month of rhizome soluble sugar concentration; (b) leaf re-growth from rhizome fragments incubated in the dark; (c) rhizome soluble sugar concentration before and after re-growth (measured in September); and (d) leaf re-growth estimated from rhizome sugars..Values are means ± SE, *n* = 3.

CHAPTER V

SUMMARY AND SYNTHESIS

Light reduction and eelgrass plasticity

The seagrass *Zostera marina* L. (eelgrass) is mainly distributed in temperate coastal estuarine environments often bordering heavily industrialized and developed areas of the northern hemisphere (Short and Burdick, 1996; Moore and Short, 2006). The reduction in light availability to eelgrass through eutrophication and turbidity, which has been shown to result in widespread loss of eelgrass across its distributional range (Chapter II), remains a real concern to scientists and environmental managers. In addition to anthropogenically-induced decreases in light availability, eelgrass's broad latitudinal distribution range implies that it occupies areas with differing light regimes dictated by differences in day length, but also by fluctuations in light availability due to seasonal changes and naturally occurring depth gradients. Natural and anthropogenic factors that reduce light availability to eelgrass meadows have been summarized in Chapter II. Because eelgrass is found in a highly variable and complex underwater light environment, it displays substantial phenotypic plasticity (the ability to adjust to changes in the environment, within a natural range of variation).

Minimum light requirements

When light limitation is severe in duration, intensity, or both, eelgrass decline or loss ensues. Several reports of eelgrass loss linked to such reduction in light availability are presented in Chapter II. In an attempt to offer managers a tool to identify critical thresholds and predict the impact of short-term human perturbations, and thereby prevent further eelgrass loss, a number of models have been developed by scientists to describe the minimum light requirements for growth and survival. Three of such models, the depth limits model (for plant growth and survival), the light compensation point model (for plant metabolism) and the H_{sat} model (for plant carbon balance), each with its advantages and disadvantages, are reviewed in Chapter II. Physiological studies, field observations of the maximum depth of eelgrass colonization and light at that depth, shading experiments and statistical models have contributed extensively to a better understanding of MLR, with models and shading experiments yielding the most useful results (Batiuk et al., 2000). However, useful numbers for light requirements or actual thresholds cannot often be provided due to difficulties in setting up replicates of more than a few light levels. Practical applications of minimum light requirement models for eelgrass management are few. The fact that there are at least three different models to assess minimum light requirements of eelgrass makes a clear understanding of MLR difficult and may have contributed to the limited application of these models by managers to date.

There is a wide variability in reported values for the minimum light requirements (MLR) of eelgrass (range 11- 30%, see Chapter II). By relating temporal measurements of photosynthesis with measurements of plant morphology in an outdoor mesocosm experiment to explain eelgrass plant response to a gradient of light conditions (Chapter III), this thesis showed that eelgrass plants in the mesocosms were able to persist at light levels of 58% SI and above, but that 11% SI was inadequate for long-term eelgrass growth and survival and resulted in mortality of 81% of the plants. Although significant growth occurred at 34% SI, with surviving seedlings reaching maturity and even flowering within three months, plant vigor at this light level was compromised, suggesting that 34% SI represents a sub-optimal condition for eelgrass growth. The mesocosm results suggest that the MLR for eelgrass falls between 11 and 34% SI.

Continuous light measurements during one year in 2004 at the maximum depth limit of eelgrass growth, the "deep edge" (Chapter IV), where plants grow at or near their minimum light requirements, revealed that light availability was 13% SI. A two-year monitoring of light at the same sites gave a close estimate of 12 ± 4% SI (Rivers, 2006), confirming the minimum light requirements of eelgrass in Great Bay Estuary. The results of these deep edge study fall within the range of values (11 – 30% SI) reported in the literature for MLR (Chapter II) and of those found in the mesocosm experiment (between 11 and 34% SI) (Chapter III).

Eelgrass responses to light reduction

The studies presented in this thesis highlight the importance of light intensity, as shown by the mesocosm shading study (Chapter III), and its duration, as shown by the study at the maximum depth limit (Chapter IV), on the response mechanisms of eelgrass. Shading to 34% SI (a level above the MLR reported in the literature) induced both physiological and morphological responses in the plants. Physiological responses included significant reductions in photosynthetic capacity and the minimum saturating irradiance, which were detectable only after 19 days of shading, even at the lowest experimental light level (11% SI), at which substantial plant mortality (81%) was recorded after 63 days of shading (Chapter III). Our mesocosm study included the first attempt to quantify rhizome growth and lateral branching of individual eelgrass seedlings. By tracking individual ramets (genetically identical shoots formed by rhizome branching) within experimental treatments, the shading experiment revealed that at the shoot level, light reduction to 34% induced significant reductions in rhizome growth, lateral branching, shoot production, number of nodes per plant and plant weight, and an increase in shoot to root ratio. In this study, rhizome elongation was the most sensitive of all measured parameters, displaying significant differences even at light levels where plants thrived.

At the maximum depth limit (Chapter IV), seasonal light reduction induced similar responses in which photosynthetic capacity, the minimum saturating light, above-ground shoot weight and total biomass decreased, while shoot to root ratio increased. On the contrary, leaf size at the deep edge declined with light reduction in winter, while this parameter showed no significant changes with shading in the mesocosm experiment. Light reduction with depth (Chapter IV) induced clear patterns (similar as above) in shoot density and shoot to root ratio, the two morphological parameters that have been acknowledged in the literature as the dominant response mechanisms to increase tolerance to reduced light (Zimmerman et al., 1989; Olesen and Sand-Jensen, 1993; Dalla Via et al., 2003; Hauxwell et al., 2003; Middelboe et al., 2003). Eelgrass plants along the depth gradient exhibited reducing shoot density with depth, which has been recognized as an adaptation to reduce self-shading and increase the proportion of light received by individual plants (Krause-Jensen et al., 2000; Olesen et al., 2002). At the same time, an increase in S:R with a decrease in light availability, as shown in the mesocosm and deep edge studies (Chapters III and IV) indicates a phenotypic adjustment in the allocation of photosynthate in an attempt to reduce the respiratory burden of below-ground biomass so as to maintain a positive carbon balance (Kraemer and Alberte, 1993; Olesen and Sand-Jensen 1994; Zimmerman et al., 1995; Olesen et al., 2002).

In the present thesis, it was demonstrated clearly that the mechanism by which eelgrass meadows in the field reduce their shoot densities in response to reduced light is through a reduction in lateral banching. The results of the mesocosm study provided evidence linking lower shoot densities with moderate shading to a slow growth rate of horizontal rhizomes and a total lack of lateral expansion in extreme shading. Inhibited lateral branching at low light provides a mechanism to explain the lower shoot densities reported from eelgrass growing in more turbid environments (Moore et al., 1997; Hauxwell et al., 2006) or at greater depths (Chapter 4; Krause-Jensen et al., 2000; Middelboe et al., 2003). Thus, the similarity in the response of eelgrass to light reduction demonstrated in three different studies in this thesis (shading, season, depth) confirm light availability as a limiting factor to eelgrass growth and that eelgrass bed structure may be controlled by light availability in estuarine waters (Dennison and Alberte, 1985).

Acclimation and timing

The response of eelgrass plants to light reduction depends not only on the magnitude of the light reduction, but also on the nature of the event (sudden vs. gradual), its duration and the season in which it occurs. A shading experiment can be considered analogous to a pulse turbidity event (e.g. a storm or a dredging plume), but not to a gradual change in water clarity or to chronic light reduction at depth. When light reduction happens gradually, plants may be able to photoacclimate to changes in light levels by physiological and morphological adjustments, thereby enabling effective use of ambient light paramount to plant survival under light stress. Such acclimations to light reduction with season were clearly demonstrated in the deep edge study (Chapter IV).

On the other hand, when plants are exposed to a sudden change in light availability (e.g. the mesocosm shading experiment – Chapter III), plant responses may be more drastic, depending on the magnitude of light reduction. The mesocosm study indeed showed low survival of plants shaded to 11% SI, a light level which may be comparable in magnitude to the 13% SI measured at the deep edge. At 11% SI in mesocosms, a decreasing photosynthetic capacity with time (Chapter III) implied that these plants were actually dying and would not survive the winter. In addition, there was no lateral expansion at 11% SI, suggesting a total lack of storage of carbohydrate reserves. At the maximum depth (13% SI) plants built up carbohydrate reserves in concentrations that were comparable to those in high light plants growing in shallow subtidal and intertidal areas. Plants at the deep edge (13% SI) were able to adjust the shoot to root ratio with decreasing light, but plants in mesocosms (11% SI) did not produce substantial above or below-ground tissue such that the S:R was not different from control. Thus, the nature of light reduction could explain the differences in response between chronically light-limited plants in the field (Chapter IV) and those shaded in mesocosm to a comparable light level (Chapter III). The fact that eelgrass is a clonal plant implies that some of the shoots at the maximum depth may have been supported in part by connected shoots growing at shallower depths, but there are no data from the deep edge study (presented in Chapter IV) that confirm this.

The timing of pulse events may determine how eelgrass plants respond (Chapter II). For example, Dennison and Alberte (1985) observed significant reductions in photosynthetic rates, growth and biomass with reduced light - shortened H_{sat} - only in June but not in August, suggesting that seasonal features of the eelgrass habitat may influence photosynthetic adjustment to the light environment. Carbohydrate reserves stored during favorable periods provide a supplemental source of energy for growth and respiration during periods of negative carbon balance. Shading in the period when plants are normally building up carbohydrate reserves could therefore potentially reduce survival (Burke et al., 1996). Periods when eelgrass plants experience negative carbon balance are not the same in all eelgrass areas, but seem to vary with latitude (Colarusso, 2007). Chapter IV of this thesis shows that in Great Bay Estuary, such periods occur during spring and autumn rather than during winter. Contrary to expectation, the periods of negative carbon balance (inferred) (i.e. spring (April) and autumn (September)) were not periods of lowest ambient light (irradiance measured as mol photons m^{-2} s^{-1}). Instead, these periods of negative carbon balance occurred when H_{sat} was lowest: April (2.5 hrs) and September (3 hrs), and were followed by significant drops in rhizome plant soluble sugar concentrations, suggesting mobilization of stored carbohydrate reserves.

Winter survival at the deep edge

Despite very low light levels in winter, survival of eelgrass plants at the deep edge during this period was found to depend largely on wintertime photosynthesis, with mobilization of stored reserves playing only a minor role. The minimum saturating irradiance (I_k), which is sensitive to temperature changes (see Marsh et al., 1986; Kirk, 1994), was very low in winter due to low water temperatures. Despite low ambient light, a very low I_k resulted in a relatively long daily period of photosynthesis-saturating light (H_{sat} = 5.5 hours). An 80% reduction in I_k from September to December effectively lengthened H_{sat} availability from 3 hours in September to 5.5 hrs in December. Thus, lowering of I_k was an adaptation by the plants to lengthen H_{sat} availability, extending the period of root aerobiosis and allowing for sustained photosynthesis under conditions of very low light availability. In addition, a lower respiratory demand expected as a result of the low winter temperatures and a greater S:R ratio may have contributed further to ensuring that the seemingly low winter light was sufficient for plant survival. Light-limited deep edge eelgrass plants exhibited a greater degree of morphological acclimations to light reduction in winter than plants at shallower depths.

Contribution of carbohydrate reserves

The present thesis made the first attempt to quantify leaf re-growth (g dw (g sugar)$^{-1}$) from eelgrass rhizomes reserves as well as the contribution of rhizome sugars to winter growth (Chapter IV). The study demonstrated that eelgrass plants consumed up to 68% of the sugars stored in the rhizomes to generate new leaf tissue in the absence of light, with no more re-growth occurring after 48 days. The relative amount of sugar mobilized from rhizome reserves increased with depth, but the contribution of these reserves to winter eelgrass growth at the deep edge was relatively small (6%), underscoring the importance of wintertime photosynthesis in plant survival. The average contribution of soluble sugar reserves of 6% could be scaled up to a maximum of 13% by including an estimate of the contribution of rhizome starch and total non-structural carbohydrates (sugars and starch) from leaves.

Conclusion

The findings of the various studies presented in this dissertation show that eelgrass portrays a considerable degree of phenotypic plasticity, which enables it to adjust to temporal as well as gradual changes in light availability through morphological and photo-physiological acclimations. This plasticity allows for its survival in environments with a highly variable and complex underwater light environment, such as coastal and estuarine areas of the northern hemisphere, which are under increasing pressure from anthropogenic activities that reduce light availability to eelgrass. The greater insight offered by the present thesis into the survival mechanisms of eelgrass in response to light reduction, especially with regard to the factors influencing eelgrass minimum light requirements and the seasonal acclimation of eelgrass plants at the deep edge to changing light conditions, is valuable information that can guide coastal resource managers in their efforts to conserve eelgrass resources.

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