When *Zostera marina* is intermixed with *Ulva*, its photosynthesis is reduced by increased pH and lower light, but not by changes in light quality

Esther F. Mvungi [a,b,*], Thomas J. Lyimo [c], Mats Björk [a]

[a] *Department of Botany, Stockholm University, SE 10691 Stockholm, Sweden*
[b] *Department of Botany, University of Dar es Salaam, P.O. Box 35060, Dar es Salaam, Tanzania*
[c] *Department of Molecular Biology and Biotechnology, University of Dar es Salaam, P.O. Box 35179, Dar es Salaam, Tanzania*

ABSTRACT

To evaluate the effects of *Ulva* on the photosynthetic capacities of the temperate seagrass *Zostera marina*, we compared it in the laboratory under normal light and light filtered through layers of *Ulva intestinalis*, and repeated the experiments with the addition of pH-induced changes in carbon speciation and availability. One thallus of *Ulva* reduced photosynthetically available irradiance to underlying seagrass by about 50% and shifted the quality of remaining light towards the green part of the spectrum (approximately 520–570 nm). There was no significant difference in photosynthetic performance between *Z. marina* under normal light and under *Ulva*-filtered light when adjusted to the same irradiance as for the control plants, indicating that the green spectrum transmitted through *Ulva* layers was being absorbed by the seagrass and was as efficient in driving photosynthesis as was the normal light. On the other hand, algae-generated pH shifts which could extend up to pH 10 had significant negative effects on photosynthesis of the seagrass, reducing the electron transport rates with up to 75%.

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1. Introduction

Nutrient over-enrichment (eutrophication) in coastal waters poses serious threats to marine ecosystems and is one of the major causes of global seagrass decline (Short and Wyllie-Echeverria, 1996; Hauxwell et al., 2001; Ralph et al., 2006; Orth et al., 2006; Burkholder et al., 2007). The massive dieback of *Zostera marina* in the Western Wadden Sea, for example, was a result of nutrient loading in 2004, associated with major overgrowth of algae, and thus shading of the seagrass (van Katwijk et al., 2009).

Opportunistic macroalgae like *Ulva intestinalis* frequently occur in masses, forming dense canopies, reducing the amount of light reaching the seagrasses beneath (Brun et al., 2003; McGlathery, 2001). Because the algae layers absorb light mainly in the blue and red regions of the spectrum (Vergara et al., 1997; Drake et al., 2003), light reaching the seagrasses is often within the "green" region of the spectrum (approximately 520–570 nm, Vergara et al., 1997), which has been regarded as less "useful" for photosynthesis due to the plants lower absorption of light at these wavelengths (e.g. Mazzella and Alberte, 1986). Hence, photosynthesis in seagrasses

is thought to be affected by both the quality and the quantity of light as in other photosynthetic organisms (e.g. Morel et al., 1987).

When marine plants like seagrasses and macroalgae co-exist, their photosynthetic activity will elevate the pH of the surrounding water if the removal of CO_2 exceeds its re-dissolution from the air (Björk et al., 2004; Beer et al., 2006): As the pH rises, CO_2 is first replaced by the inorganic carbon forms HCO_3^- and CO_3^{2-} and then, at even higher pH values, also HCO_3^- decreases while CO_3^{2-} continues to increase. These changes in water chemistry will in turn affect the plants, but the negative effect on seagrasses may be more drastic than that on many macroalgae because the former acquire HCO_3^- less efficiently than the latter (Björk et al., 1997; Schwarz et al., 2000; Invers et al., 2001). Thus, under the high-pH conditions that often occur in shallow, densely populated bays, photosynthesis of, e.g. *Z. marina* may become a limiting factor for its growth and development.

In coastal waters pH usually fluctuates, often due to photosynthetic carbon uptake of submerged aquatic vegetation. In a study on the Swedish coast, it was found that pH in open bays with dense vegetation cover normally fluctuated between 8.1 and 8.7 at midday (Buapet et al., unpublished data) and can reach as high as 10.0 during phytoplankton blooms (Hansen, 2002; Hinga, 2002). In enclosed areas, pH can be even higher, ranging from 9.0 in pools with dense stands of seagrass (Beer et al., 2006) to 10.1 in isolated rock pools (Björk et al., 2004). Several studies have examined the effect of pH change on macroalgae (e.g. Beer and Koch, 1996;

* Corresponding author at: University of Dar es salaam, P.O. Box 35060, Dar es Salaam, Tanzania. Tel.: +255 022 2410764; fax: +255 2410078.
  E-mail addresses: mvungie@yahoo.co.uk, emvungi@udsm.ac.tz (E.F. Mvungi).

Menéndez et al., 2001; Middelboe and Hansen, 2007), however, considerably fewer have focussed on the effect of pH change caused by inorganic carbon (Ci) competition (e.g. Liu et al., 2005; Schwarz et al., 2000; Invers et al., 1997) on seagrass photosynthetic capacity. In almost all of these studies buffers were used to simulate pH change and the buffers themselves have been reported to have a negative effect on seagrass photosynthesis (Hellblom et al., 2001; Uku et al., 2005).

Previous studies have shown that green macroalgae such as *U. intestinalis* can affect seagrass productivity through direct shading (Brun et al., 2003; Liu et al., 2005; Lamote and Dunton, 2006) and resource competition (Coffaro and Bocci, 1997; Beer et al., 2006). However, algal blooms, by changing the pH of the surrounding waters, can cause carbon competition between seagrasses and macroalgae, and there is little research available on the effect of this on the photosynthetic capacity of seagrass. Many studies have examined the effect of light quantity on the photosynthesis of seagrass (e.g. Moore and Wetzel, 2000; Peralta et al., 2002; Ralph et al., 2007; Mackey et al., 2007), but to the best of our knowledge, none to date have assessed the effect of *Ulva*-filtered light on the photosynthesis activity of seagrass. Light quality and pH changes each have negative impacts on seagrass photosynthetic performance; however, they often occur in combination. Therefore, in this laboratory study we set out to investigate both the effect of *Ulva*-filtered light on the photosynthetic performance of *Z. marina* and the effect of the *Ulva*-filtered light in combination with *Ulva*-induced Ci and pH changes.

2. Materials and methods

Z. marina ramets and *Ulva* thalli were collected from Kristineberg Marine Research Station, Fiskebäckskil, on the West coast of Sweden in November, 2009, stored in different containers and transported to the Botany Department, Stockholm University. At the laboratory, both seagrasses and *Ulva* thalli were kept separately in cold (10–12 °C) aerated natural seawater, with fluorescent light of ca. 60 μmol photon m^{-2} s^{-1}, and left to acclimate for one week prior to initiation of the experiment.

2.1. Leaf optical properties

The optical properties of *Z. marina* leaves under normal and *Ulva*-filtered light were determined at wavelengths from 400 to 700 nm using a fibre optic spectrophotometer (Ocean optics Inc., USB2000) following the procedures described by Runcie and Durako (2004), with some modifications. Leaves were placed on the spectrometer's sensor stand and a "cool" light source (KL 1500 LCD, Zeiss) with a colour temperature of about 3000 K was placed directly above and perpendicular to the leaf on the sensor. Transmission data were recorded for the light passing through the sensor, both with the leaf in place and without (100% transmission). The same procedures were repeated with the light source covered with one layer of *Ulva*. When the light source was covered by an *Ulva* layer, the light intensity was adjusted to obtain the same light intensity as for the previous measurement under normal light, i.e. 3500 intensity counts. The amount of light transmitted between wavelengths 400–700 nm (photosynthetically active radiation [PAR]) was averaged and the absorption factor was calculated as a fraction of the light passing through the sensor both with and without the seagrass leaf.

A metallic mirror considered to be 100% reflection (control) and the upper surfaces of the seagrass leaves were measured by spectrophotometer to determine the base reflectivity of the seagrass. This measurement was then used to correct for light reflected from the leaf surface during the experiment. In this case the sensor and the incident light were positioned in seawater, opposite each other at a 45° angle approximately 30 mm above the surface of the leaf or control and the spectrum of the reflected light between wavelengths of 400 and 700 nm was recorded. The reflection factor was calculated by dividing the portion of light reflected from the leaf sample by the light reflected from the control surface. The value obtained was then used to correct the absorption values. All spectra obtained were corrected for electrical noise using dark spectra, and the light was optimized to a maximum signal of 3500 counts. All the optical measurements of *Z. marina* were performed under water.

2.2. Photosynthetic characteristics of the seagrass

The measurements were made in a 500 ml water-cooled jacketed Plexiglas container connected to a water heating/cooling bath (LAUDA) set at 15.0 °C. The seawater in the container was stirred continuously using a magnetic stirrer. Two experiments were conducted separately: the first to analyse the effect of light quality (normal light vs. *Ulva*-filtered light) on the photosynthetic characteristics of the seagrass, *Z. marina*; the second was to assess the combined effects of light quality, as above, and *Ulva*-induced pH change on seagrass photosynthesis.

2.2.1. Photosynthetic characteristics in response to normal and Ulva-filtered light

In this experiment, seagrass shoots were fixed in the incubation chamber on a plastic holder mounted with the pulse amplitude modulated (PAM) fibre optic fluorometer cable and the light sensor, arranged in such a way that the incoming irradiance was more or less perpendicular to the leaf sample. The incubation chamber container contained seawater with pH 8.1. PE curves were performed using the PAM fluorometer, with the external light source initially set at 0 and manually increased to 10, 25, 50, 100, 250, and 500 μmol photons m^{-2} s^{-1}. At each light intensity the measurement was taken after 5 min. After completion of this cycle, the light source was mounted with one layer of *Ulva* (to create *Ulva*-filtered light; there was no contact between the *Ulva* layer and the water containing the seagrass, but the *Ulva* was kept moist) and the above procedure was performed on the same shoot, also after 5 min at 0, 10, 25, 50, 100, 250, and 500 μmol photons m^{-2} s^{-1}. The two cycles were conducted also in reverse sequence giving the same effects. After completion of both cycles, the container was emptied and cleaned prior to next run with a new shoot. A total of 20 seagrass shoots were analysed for five consecutive days. Before measurement, the light meter of the PAM was calibrated with the light meter ILT1400A (International Light Technologies). The electron transport rates (ETR) at each light intensity was calculated using the formula: ETR = PAR $\times$ Y $\times$ AF $\times$ 0.5, where PAR is photosynthetically active radiation reaching the leaf, Y is effective quantum yield, AF is the absorption factor which is the fraction of incident light absorbed by the leaf (determined in this experiment) and 0.5 is the fraction of absorbed light assumed to be used by PSII (Beer and Björk, 2000).

2.2.2. Photosynthetic response to combined effects of light quality and change in pH

This experiment was carried out the same way as the first one but, the light curves measurements were done first when seagrass shoot was placed in normal seawater (pH 8.0–8.2), and thereafter 10 g of *U. intestinalis* thalli were added in the water containing seagrass, left at 15 °C, then measurements were done at 2 h interval after addition of *Ulva* (the algae was placed so as not to shade the seagrasses). The experiment was run for ten consecutive days at which two new seagrass shoots were analysed per day. The pH, which rose quickly as a function of photosynthetic removal of Ci

from the water, was recorded at each measurement interval using pH meter (multi 340i, WTW, Germany). After attaining the maximum pH values, *Ulva* thalli were removed from the chamber and water with seagrass was then briefly purged with 5% CO_2 until the ambient pH of the water was restored. This was done to assess post-stress recovery after exposure to these high pH ranges and to test if that there was remaining effect from other substances (e.g. allelochemicals) excreted by the *Ulva* (cf. Gross, 2003). The maximum electron transport rate of the seagrass (ETR_{max}) and the initial slope (α) in both experiments were calculated by fitting the data obtained from the light curves to the non-linear relationship according to Jassby and Platt (1976). The onset of light saturation (Ik) was calculated by dividing the maximum electron rate by the initial slope.

2.3. Inorganic carbon concentration

Concentration of total inorganic carbon in the seawater in a chamber was determined at each measurement interval (described above) following procedures of Anderson and Robinson (1946). Following this procedure, 4 ml of water sample was taken, the pH was measured before addition of 0.01 N HCL, then 1 ml of 0.01N HCL was added and the pH was recorded again. The values obtained before and after addition of HCL were then used to first calculate total alkalinity (TA) and then the total carbon (TC) using the formula of Riley and Skirrow (1965) and constants of Smith and Kinsey (1978). From these values also the relative concentrations of CO_2 and $HCO_3{}^-$ was calculated using the CO_2 sys.xls program of Pelletier et al. (2007). A minimum of 5 measurements were done per day for ten consecutive days.

2.4. Statistical analysis

The variation in maximum electron transport rate, saturating irradiance and the initial slope of seagrass subjected to normal and *Ulva*-filtered light were tested using two tailed *t*-test. Whereas, correlation between photosynthetic characteristics of seagrass under normal and *Ulva*-filtered light in response to pH change were analysed using multiple regression analysis. Similarly the correlation between the concentration of inorganic carbon and the pH changes were analysed using linear regression analysis. Variation in leaf optical properties under normal and *Ulva*-filtered light was determined using one-way analysis of variance with Turkey–Kramer multiple comparison test. Prior to any statistical analysis, the normality and homogeneity of variance assumptions were tested and verified by a Shapiro–Wilk normality test. The statistical significance levels for all tests were set at $p < 0.05$.

3. Results

3.1. Light absorption by seagrass leaves

The overall light absorption of leaves of *Z. marina* is shown in Fig. 1. The percentage absorption by the seagrass leaf varied significantly for the control light and the light that had passed through *Ulva* thalli ($p < 0.0001$). The absorbance of *Z. marina* under both normal light and *Ulva*-filtered lights were highest in the blue (400–500 nm) and red (600–700 nm) regions, and were lowest in the green region with wavelength between 500 nm and 600 nm. The absorption factor (AF) of *Z. marina* in control light which was 0.82 ± 0.08 as compared to in *Ulva*-filtered light when it was 0.67 ± 0.04. The reflection factor of the incident irradiance was 0.04 ± 0.03 (control light). Furthermore, the amount of light reaching the seagrass was reduced to close to half the original value for
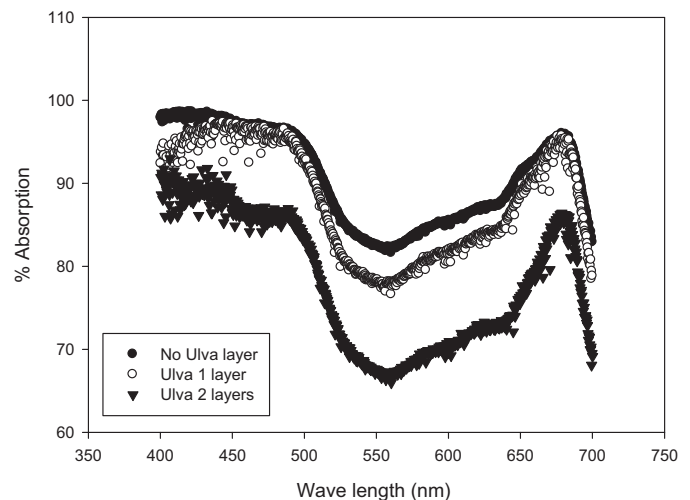

Fig. 1. *Zostera marina*. Percent absorption of different light qualities by *Z. marina* leaves while subjected to (i) normal direct light from the light source (filled circles), (ii) light filtered through 1 *Ulva intestinalis* thallus (open circles), and (iii) light filtered through 2 *Ulva intestinalis* thalli (filled triangles), $n = 72$.
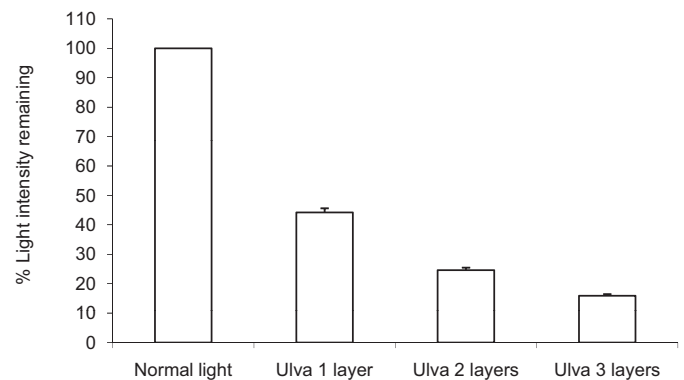

Fig. 2. *Zostera marina*. Percentage reduction of light when passed through *Ulva intestinalis* thalli (mean $\pm$ SD).

each added thallus, and with each additional *Ulva* layer, the light absorbed decreased significantly (Fig. 2).

3.2. Photosynthetic characteristics in response to normal and Ulva-filtered light

The photosynthetic parameters obtained in respect to light quality in normal seawater are presented in Fig. 3. The QY of the seagrasses was higher under *Ulva*-filtered light at each irradiance level than under normal light. However, there was no significant difference between the maximum electron transport rates of the seagrasses subjected to unfiltered light and those subjected to *Ulva*-filtered light ($p > 0.05$) when the rates were adjusted for the absorption factors of the different light qualities. Similarly, there were no significant differences in the Ik of seagrasses subjected to normal light ($100 \pm 55 \,\mu$mol photon m^{-2} s^{-1}) versus those subjected to *Ulva*-filtered light ($116 \pm 59 \,\mu$mol photon m^{-2} s^{-1}). The initial slope (α), however, varied significantly ($p = 0.001$, $t = 3.33$) between the two lights, being higher in the normal light (0.26 ± 0.05) than in the *Ulva*-filtered light (0.22 ± 0.03).

Table 1

Zostera marina. Photosynthetic characteristics of *Z. marina* in normal and *Ulva*-filtered light according to pH changes from the addition of *Ulva* thalli to the water media (mean ± SD, $n = 41$).

pH range	ETR_{max}		Ik		α	
	Normal light	*Ulva* filtered light	Normal light	*Ulva* filtered light	Normal light	*Ulva* filtered light
8.0–8.3	23.3 ± 4.98	19.5 ± 5.77	74.5 ± 25.3	71.7 ± 25.1	0.33 ± 0.06	0.28 ± 0.03
8.4–9.0	17.3 ± 6.28	16.3 ± 6.08	47.5 ± 26.6	60.9 ± 28.2	0.35 ± 0.06	0.27 ± 0.02
9.1–9.5	9.2 ± 5.62	10.3 ± 5.76	34.4 ± 25.3	38.9 ± 24.6	0.32 ± 0.09	0.27 ± 0.04
9.6–10.5	5.5 ± 4.21	9.3 ± 3.52	15.6 ± 18.7	34.6 ± 15.9	0.35 ± 0.09	0.28 ± 0.05
Recovery (8.0–8.2)	18.4 ± 3.30	16.9 ± 2.48	68.7 ± 25.1	68.9 ± 25.9	0.30 ± 0.04	0.29 ± 0.03
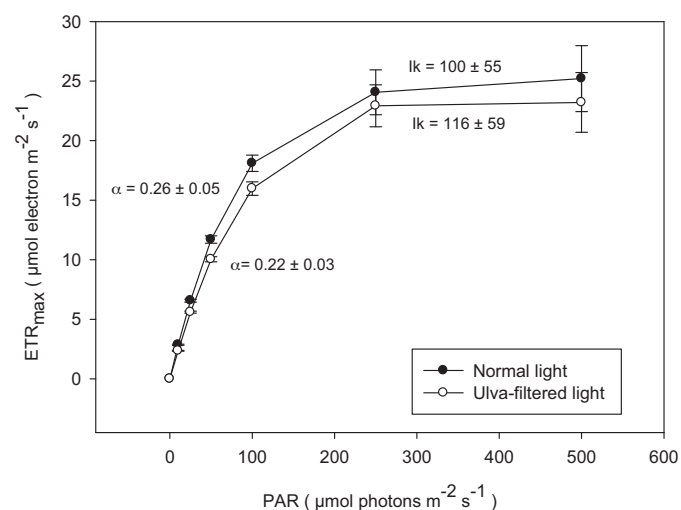
Fig. 3. *Zostera marina.* Electron transport rate in relation to irradiance levels under normal (Filled circles) and *Ulva*-filtered light (Open circles). Vertical bars represent the standard error, $n = 20$. There was no significant difference between the two light treatments.

3.3. Combined effects of light quality and pH change on photosynthetic characteristics

Electron transport rates of seagrass decreased significantly ($p < 0.0001$, $n = 41$) with increasing pH (Fig. 4 and Table 1). There was a significant negative correlation between the ETR_{max} and the pH (r, $p = -0.78$, <0.0001 and -0.60, <0.0001) in both normal light and *Ulva*-filtered light. However, there was no significant difference in ETR_{max} between seagrass subjected to normal or to *Ulva*-filtered
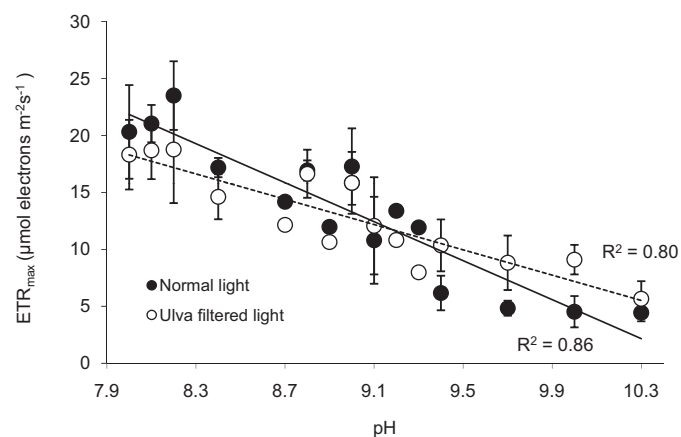
Fig. 4. *Zostera marina.* Photosynthetic rate under normal (closed circles, solid trendline) and *Ulva*-filtered (open circles, dashed trendline) light in relation to the increase in pH. Data points are averages of different measurements ± SE, $n = 41$. There was no significant difference between the "normal" and "*Ulva* filtered" light treatments.

Table 2

Changes in total carbon (TC), CO_2, and HCO_3^- concentrations ($\mu mol \, l^{-1}$) of water in relation to change in pH. The values were calculated following Pelletier et al. (2007) using Excel software. Data are mean ± SD, $n = 60$.

pH range	[TC]	[CO_2]	[HCO_3^-]
8.0–8.4	1816.2 ± 82.9	11.24 ± 2.58	1604.3 ± 100.2
8.5–8.8	1531.5 ± 67.5	4.05 ± 1.56	1201.1 ± 112.3
8.9–9.2	1327.0 ± 136.0	0.69 ± 0.37	713.6 ± 159.7
9.3–9.6	1012.3 ± 171.7	0.15 ± 0.12	346.5 ± 137.8
9.7–10.0	493.2 ± 69.2	0.013 ± 0.01	86.3 ± 24.2
10.1–10.4	251.1 ± 91.3	0.012 ± 0.01	51.9 ± 29.1
Recovery	1805.5 ± 55.5	10.9 ± 0.82	1613.2 ± 53.1

light ($p > 0.05$, Fig. 3). The initial saturating irradiance followed a similar trend as ETR_{max}; with the initial slope (α) remained more or less the same with increasing pH in both normal and *Ulva*-filtered light (Table 1). Furthermore, when the initial pH of the water was restored by purging the chamber with CO_2, electron transport rates recovered until there was no significant difference between recovery and initial electron transport rates of seagrass (Table 1).

The concentrations and speciation of inorganic carbon are presented in Table 2. There was significant reduction in total inorganic carbon ($p < 0.0001$, $r = -0.95$, $n = 60$) in the surrounding seawater as pH increased during the experiment (Table 2). At normal seawater pH, the concentration of total inorganic carbon ranged from 1.65 to 2.17 mmol l^{-1} and decreased with increasing pH to concentration values less than 0.5 mmol l^{-1}. However, after a short purging with 5% CO_2, the initial concentration of inorganic carbon in the water was restored (Table 2).

4. Discussion

An obvious effect of *Ulva* overgrowing *Zostera* is of course the reduction of electron transport rates by the large decrease in the amount of light reaching the seagrass canopy. If available light in this way is reduced for an already light limited plant, this can pose a serious threat to seagrass meadow (e.g. Short et al., 1995; Hauxwell et al., 2003). The light requirements for seagrass survival vary considerably and are species specific, depending on morphology and physiological adaptations to the environments (Lee and Dunton, 1997; Leoni et al., 2008). The minimum light requirement for seagrass survival has been reported to vary between 2% and 37% of the surface irradiance (Duarte, 1991; Dennison et al., 1993; Kenworthy and Fonseca, 1996; Lee et al., 2007). In our study, shading by a single algal layer reduced the available irradiance by close to 50%, and three layers removed over 80% of the light. Overgrowths of even one layer may be expected to have an important influence on the performance of seagrass meadows.

In addition to reducing the amount of light available to the seagrass, *Ulva* thalli affected light quality by absorbing mainly in the blue and red areas of the spectrum, leaving the remaining light dominated by the green parts of the spectrum. It is often assumed that green light is not important in driving photosynthesis. For terrestrial plants, however, this has been shown to be a misconception: while it true that monochromatic green light can be less

effective, light that contains "white" light, but is dominated by the green part of the spectrum may be even more effective in the deeper parts of the photosynthetic tissue (e.g. Sun et al., 1998; Terashima et al., 2009; Nishio, 2000). In the present study, the ability of seagrass to capture such "greener" light was lower under *Ulva*-filtered light than under normal light, but since the QY of electron transfer in the PSII of *Z. marina* was correspondingly higher in *Ulva*-filtered light, the absolute ETR proved to be as effective as the control light in driving photosynthesis (Fig. 3). Thus, it appears that the changes in light quality caused by an overcast of green algae are of little consequence for the photosynthetic rate of the seagrass.

In addition to reductions in amount and alterations in quality of light, the growth of macroalgae and seagrass together resulted in major changes in the chemistry of the surrounding water. When *Ulva* in this study was allowed to decrease the amount of available inorganic carbon, thus increasing the pH, there was a significant reduction in the photosynthetic rates of the seagrass. This could be due to the effective carbon uptake mechanisms of *U. intestinalis* (Björk et al., 2004) that increase the pH, thus depleting the accessible inorganic carbon, leading to lower photosynthetic rates in the seagrass.

In this experiment the seawater pH was raised by *Ulva* to as high as 10.3. When pH levels rose, photosynthetic rates in seagrass decreased. When pH was returned to original levels by the addition of CO_2, electron transport rates recovered. Thus we assume that the observed response is primarily caused by the decrease in available Ci in the media, even though we cannot fully rule out the possibility that other effects (e.g. allelopathic) from the *Ulva* secretion may have contributed to the observed effects on the photosynthetic rate (Gross, 2003).

In conclusion, the effect of *Ulva* layers above the seagrass canopy would mainly be due to decreases in light levels reaching the underlying seagrass leaves, since it appears that given the same level of light intensity reaching the seagrass surface, the *Ulva*-filtered light drives photosynthetic activity of *Z. marina* as efficiently as does normal light. However, the photosynthetic capacity of *Z. marina* (and probably other seagrass species) is also highly influenced by the increases in pH and reductions in Ci induced by photosynthetically efficient, opportunistic macroalgae such as *Ulva*, that often occur in masses in eutrophic shallow waters. *Z. marina* has also, however, a high potential for recovery when the pH of the surrounding water is reduced, as occurs naturally during the cycle of low-tide shallows and high tide current exchange.

Acknowledgements

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