

# Chlorophyll fluorescence measures of seagrasses *Halophila ovalis* and *Zostera capricorni* reveal differences in response to experimental shading

Juanita S. Bité · Stuart J. Campbell ·  
Len. J. McKenzie · Robert G. Coles

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**Abstract** In coastal waters and estuaries, seagrass meadows are often subject to light deprivation over short time scales (days to weeks) in response to increased turbidity from anthropogenic disturbances. Seagrasses may exhibit negative physiological responses to light deprivation and suffer stress, or tolerate such stresses through photo-adaptation of physiological processes allowing more efficient use of low light. Pulse Amplitude Modulated (PAM) fluorometry has been used to rapidly assess changes in photosynthetic responses along in situ gradients in light. In this study, however, light is experimentally manipulated in the field to examine the photosynthesis of *Halophila ovalis* and *Zostera capricorni*. We aimed to evaluate the tolerance of these seagrasses to short-term light reductions. The seagrasses were subject to four light treatments, 0, 5, 60, and 90% shading, for a period of 14 days. In both species, as shading increased the photosynthetic variables significantly ( $P < 0.05$ ) decreased by up to 40% for maximum electron transport rates ( $ETR_{max}$ ) and 70% for saturating irradiances ( $E_k$ ). Photosynthetic efficiencies ( $\alpha$ ) and effective quantum yields ( $\Delta F/Fm'$ )

increased significantly ( $P < 0.05$ ), in both species, for 90% shaded plants compared with 0% shaded plants. *H. ovalis* was more sensitive to 90% shading than *Z. capricorni*, showing greater reductions in  $ETR_{max}$ , indicative of a reduced photosynthetic capacity. An increase in  $E_k$ ,  $Fm'$  and  $\Delta F/Fm'$  for *H. ovalis* and *Z. capricorni* under 90% shading suggested an increase in photochemical efficiency and a more efficient use of low-photon flux, consistent with photo-acclimation to shading. Similar responses were found along a depth gradient from 0 to 10 m, where depth related changes in  $ETR_{max}$  and  $E_k$  in *H. ovalis* implied a strong difference of irradiance history between depths of 0 and 5–10 m. The results suggest that *H. ovalis* is more vulnerable to light deprivation than *Z. capricorni* and that *H. ovalis*, at depths of 5–10 m, would be more vulnerable to light deprivation than intertidal populations. Both species showed a strong degree of photo-adaptation to light manipulation that may enable them to tolerate and adapt to short-term reductions in light. These consistent responses to changes in light suggest that photosynthetic variables can be used to rapidly assess the status of seagrasses when subjected to sudden and prolonged periods of reduced light.

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J. S. Bité (✉)

School of Tropical Environment Studies and Geography,  
James Cook University, Townsville, QLD 4810, Australia  
e-mail: Juanita.Bite@jcu.edu.au

S. J. Campbell · Len. J. McKenzie · R. G. Coles  
Queensland Fisheries Service, Department of Primary Industries  
and Fisheries, Northern Fisheries Centre, PO Box 5396,  
Cairns, QLD 4870, Australia

S. J. Campbell · Len. J. McKenzie  
CRC Reef Research Centre, P.O. Box 772,  
Townsville, QLD 4810, Australia

## Introduction

Coastal waters and estuaries are highly productive and ecologically valuable ecosystems. These systems are under increasing stress from anthropogenic disturbances due to sediment dredging, catchment runoff and urbanisation. These disturbances and the increasing frequencies of natural disturbances (e.g. flooding and cyclones) (Preen et al. 1995; Campbell and McKenzie 2004) directly reduce the distribution of ecologically important primary producers

and inhibits the maintenance of healthy marine ecosystems (Dennison 1987; Duarte 1991; Alcoverro et al. 2001).

Seagrasses are often the dominant primary producers in coastal ecosystems and contribute to maintaining water quality by increasing the stability of sediments, biogeochemical cycling and trophic dynamics. The distribution of seagrasses is widely accepted as a barometer of coastal water quality (Duarte 1991; Gallegos and Kenworthy 1996; Biber et al. 2005). However, loss of seagrass biomass and suboptimal seagrass growth are usually detected when coastal ecosystems are already degraded and their poor condition is unable to be reversed by management actions. Consequently, for seagrass ecosystems, the development of a set of rapid and predictable sublethal stress indicators that respond to low-light fluxes have been the focus of intense research efforts in recent times (Kraemer and Hanisak 2000; Biber et al. 2005).

In response to the need for measures of sublethal stress thresholds for seagrasses, there have been many efforts to quantify the physiological responses of seagrasses in response to light deprivation over short time scales (days to weeks) commensurate with timescales of turbidity events in coastal waters (Longstaff et al. 1999; Ibarra-Obando et al. 2004; Biber et al. 2005). In situ and rapid assessments of sublethal stress have generally been restricted to morphological and structural photo-adaptive responses of seagrasses to light reduction, including measures of canopy height, shoot densities and leaf area indices (Longstaff and Dennison 1999).

Chlorophyll fluorescence techniques, such as Pulse Amplitude Modulated (PAM) fluorometry, offer a rapid in situ tool to develop sublethal physiological indicators of stress, detecting a response in seagrasses to light reduction within days. Chlorophyll fluorescence (fluorescence yield) can be measured and used to calculate the proportion of photons absorbed by the photosynthetic PSII reaction centres (i.e. the quantum yield), since fluorescence is inversely correlated to photosynthetic efficiency. In light-adapted plants, these photons are used for photosynthetic electron transport, described as the effective quantum yield ( $\Delta F/F_m'$ ). The effective quantum yield of a plant's photosystem II (PSII) is  $\Delta F/F_m' = F - F_m'/F_m'$ ; where the minimum fluorescence ( $F$ ), as measured immediately before the saturating pulse, and subtracted from the maximal fluorescence ( $F_m'$ ), measured immediately after a saturating pulse of light and then divided by  $F_m'$ .  $F - F_m'$  is described as the variable fluorescence ( $\Delta F$ ) (Beer et al. 2001; Ralph and Gademann 2005).

Research has focused on maximum quantum yield in dark adapted plants, but this has not been found to be a sensitive indicator of plant stress (Longstaff and Dennison 1999; Biber et al. 2005). In naturally fluctuating light climates there are predictable changes in maximal electron

transport rates (ETRs), photosynthetic efficiencies and saturating irradiances (Beer et al. 1998; Ralph et al. 1998; Campbell et al. 2003). Such measures can be simply derived from rapid light curves (RLCs), using PAM fluorescence, and offer a more effective means of evaluating the photosynthetic performance of seagrasses as affected by their previous light history (Beer et al. 2001; Ralph and Gademann 2005). Nonetheless, there is an absence of manipulative studies that have investigated photosynthetic derivatives of chlorophyll fluorescence (e.g.  $ETR_{max}$ , photosynthetic efficiency and saturating irradiance), as physiological indicators, that respond to changes in light availability over relatively short timescales of days to weeks, before seagrass mortality.

In recent times, seagrasses in Hervey Bay have fluctuated considerably in response to anthropogenic disturbance (Preen et al. 1995; Campbell and McKenzie 2004). Hervey Bay is a subtropical embayment containing ~2,000 km<sup>2</sup> of fast growing seagrass species including *Halophila ovalis*, *Halodule uninervis* and *Zostera capricorni*. These species have relatively small rhizomes and roots with low carbohydrate storage capacity (Abal et al. 1994), making them susceptible to light reduction over short timescales (i.e. days to weeks) (Longstaff and Dennison 1999).

Currently there are no rapid methods to detect stress, in seagrasses, in response to light reduction before morphological changes or mortality occurs. In this study, we examined differences in the photosynthetic responses of two co-existing species of seagrass to 2-week light reduction, a period that seagrass meadows are often subject to severe depletion of light caused by anthropogenic and natural disturbances. Photo-adaptation was also investigated as a mechanism potentially employed by both species to survive severe light depletion. Chlorophyll fluorescence and, in particular, RLCs were used to rapidly assess if photo-adaptation could be detected under different short-term shading manipulations and before seagrass mortality.

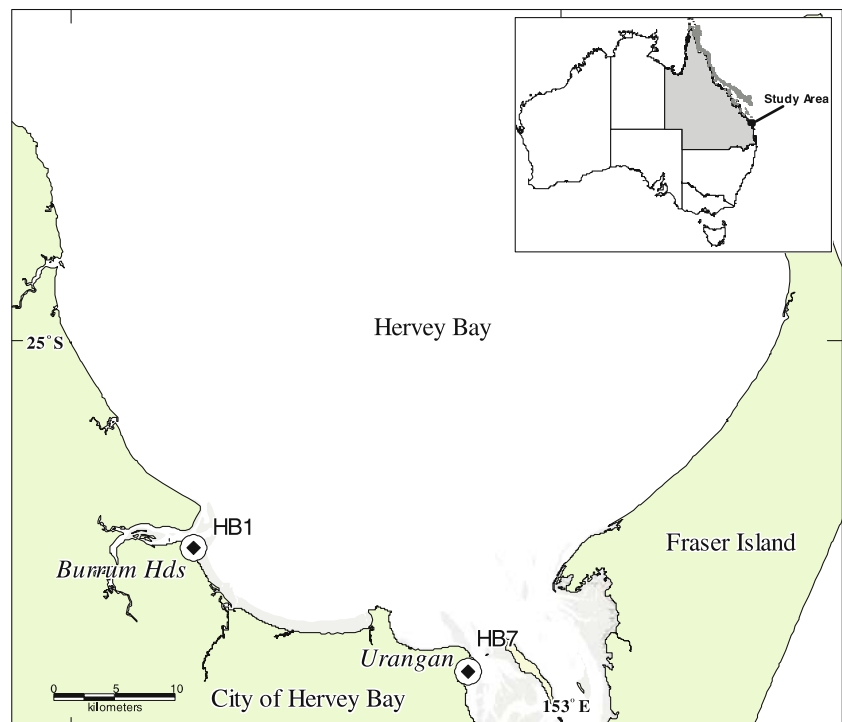
## Methods

### Shading experiment

#### Site location

Two, 0 m depth, intertidal sites were chosen in Hervey Bay. The sites were located at Urangan (25°18.249' 152°54.394'), and Burrum Heads (25°11.349' 152°37.559') (Fig. 1). Both sites consisted of mixed meadows of *H. ovalis* and *Z. capricorni* and were exposed at low tide for 2–3 h during a tidal cycle. The maximum tidal height ranges from 2 to 3 m.

**Fig. 1** Location of experimental shading experiments in Hervey Bay, Australia



At both sites three  $20 \times 20 \text{ cm}^2$  replicate plots were exposed to three different shade treatments. Three replicate control plots with no shade treatment were also marked, giving a total of 12 plots. The shade treatments consisted of  $100 \times 100 \text{ cm}^2$  mesh cloths suspended on an aluminium structure 15 cm above the sediment. The *Z. capricorni* canopy reached a maximum of 10 cm at Urangan and 6 cm at Burrum Heads, while *H. ovalis* plants had a maximum length of 3 cm. The shade cloths were positioned so that plants received attenuated light during daylight. A  $25 \text{ cm} \times 25 \text{ cm}$  square was cut into the centre of each shaded plot, 15 cm into the sediment, ensuring all rhizomes were cut. Cutting prevented the shaded seagrass receiving photosynthates translocated, via the rhizomes, from non-shaded parts of the plant (Longstaff and Dennison 1999). The cuts were repeated several times during the duration of shading. The three treatments consisted of 90, 60 and 5% shading. Control plots of seagrass, without shade cloth, were also marked. The shade cloths were deployed for a total of 14 days, being changed every 3 days to reduce the effect of algal fouling.

At day 0, between 11:00 and 13:00 h during low tide and under sunny conditions, measures of RLCs, using a diving PAM, were made on light adapted leaves of *H. ovalis* and *Z. capricorni* from the centre of nine replicate plots to be treated and each of three control plots. Healthy green leaves were placed in a plastic 'leaf clip' in situ (Walz, Effeltrich, Germany) to record RLCs. The PAM light probe was attached to the leaf clip at a fixed distance from the leaf. The diving PAM automatically generated

RLCs using an incremental sequence of 10 s actinic illumination periods, with light intensities increasing in the eight steps 0, 50, 150, 340, 580, 850, 1,180 and 1,760  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  photosynthetically active radiation (PAR). For each illumination period initial fluorescence ( $F$ ) and following a saturating pulse of white light (800 ms of  $8,000 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  PAR), maximum fluorescence ( $F_m'$ ), were measured. The same procedure was followed after 14 days (14 days) of continuous shading.

#### Depth gradient experiment

##### Site location

Two sites were chosen at each depth of interest (0, 5 and 10 m) for analyses of photosynthetic variables of *H. ovalis* along a depth gradient.

Using the diving PAM and the procedure described above, the photosynthetic performance of *H. ovalis* was examined at three depths (0, 5 and 10 m), with two replicate sites for each depth (total of six sites). At each site, RLCs were measured, as described above, for ten replicate light adapted *H. ovalis* plants, between 11:00 and 13:00 h, under sunny conditions.

##### In situ underwater light measurements

Ambient underwater light was measured as photon flux using the diving PAM, which was calibrated underwater with a Li-189 light meter (LiCoR, Lincoln, NE, USA). Five

photon flux measurements were made under every shade cloth at days 0 and 14 during PAM measurements ( $n = 75$ ). Three to five measurements were made at each depth during PAM measures along the depth gradient. All light measurements were made under sunny conditions, avoiding cloudy periods. Mean values of light measures are presented.

#### Season

The shade experiment and depth gradient photosynthetic measures were completed within the month of November 2003, immediately prior to the onset of the wet season. The water temperature was 23°C for the duration of the shade experiment and the depth gradient photosynthetic measures.

#### Calculation of photosynthetic variables

Apparent photosynthetic ETRs were calculated as the product of effective quantum yield ( $F - F_m'/F_m' = \Delta F/F_m'$ ; where  $F$  is initial fluorescence,  $F_m'$  is maximum fluorescence and  $\Delta F$  is variable fluorescence), the incubation irradiance ( $I$ ) and the absorbance factor (AF), i.e. the fraction of light absorbed by the leaf. This product was further multiplied by 0.5 because it was assumed that half the photons required for the movement of electrons along the photosystem pathways are absorbed by PSII (Schreibers et al. 1995). Effective quantum yield ( $\Delta F/F_m'$ ) was calculated from the RLC for the first of the eight actinic illuminations. AF values for *H. ovalis* and *Z. capricorni* were derived by measuring the proportion of light absorbed by single leaves according to the method described by Beer et al. (2001). The maximum ETR ( $ETR_{max}$ ) and photosynthetic efficiency ( $\alpha$ ) were calculated by fitting the RLC data to an exponential function;  $ETR = ETR_{max} \times (1 - \exp[-\alpha(I)/ETR])$  (Jassby and Platt 1976) where  $ETR$  = electron transport rate and  $I$  = irradiance. The onset of light saturation ( $E_k$ ) was calculated as  $ETR_{max}/\alpha$ . In the few cases where a down-regulation drop in ETR was recorded, measures from high photon flux (e.g. 1,760  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  PAR) were removed before fitting data to the exponential model.

#### Data analysis

##### Shading experiments

One-way ANOVAs were used to determine the effects of plots prior to shading treatments on  $ETR_{max}$ , photosynthetic efficiency ( $\alpha$ ), minimum irradiance at which the plant is photosynthetically saturated ( $E_k$ ), initial fluorescence

yield ( $F$ ), maximum fluorescence yield ( $F_m'$ ), effective quantum yield ( $\Delta F/F_m'$ ) and AFs. Effective quantum yield and AF data from Urangan and Burrum Heads were arcsin square-root transformed prior to analysis.

Three-way ANOVAs were used to determine the effects of species, shade treatment and site on  $ETR_{max}$ ,  $\alpha$ ,  $E_k$ ,  $F$ ,  $F_m'$ ,  $\Delta F/F_m'$  and AF. Photosynthetic efficiency,  $\Delta F/F_m'$  and AF data from Urangan and Burrum Heads were arcsin square-root transformed prior to analysis.  $ETR_{max}$  and  $F_m'$  data from Burrum Heads were  $\log_e$  transformed.

##### Depth gradient and shading

ANOVAs were used to test for significant differences in mean photosynthetic variables in *Halophila ovalis* ( $ETR_{max}$ ,  $\alpha$ ,  $E_k$ ,  $F$ ,  $F_m'$ ,  $\Delta F/F_m'$  and AF) ( $n = 3-10$ ) among both sites (Urangan and Burrum Heads) and depths (0, 5 and 10 m), and among all shade treatments (control, 5, 60 and 90%) at the two sites, at 14 days ( $n = 14$ ). Post hoc Bonferroni tests were used to test for significant differences among means.  $ETR_{max}$  and  $E_k$  data were  $\log_e(x + 1)$  transformed prior to analysis. All analyses were performed using SYSTAT (Version 10.2).

## Results

### Before shading

Immediately prior to the shading manipulations at day 0, there were no significant differences ( $P < 0.05$ ) for photosynthetic variables among each of the treatment plots at either site, Urangan and Burrum Heads.

### Shading manipulations

Three-way ANOVA revealed a significant interaction between site, species and shade treatment for  $ETR_{max}$  and saturating light intensity ( $E_k$ ) (Table 1). Post hoc, Bonferroni, analysis showed the interaction was due to a significantly higher mean ( $\pm$ SE)  $ETR_{max}$  ( $178.24 \pm 17.53$ ) for non-shaded (control) *Z. capricorni* at Burrum Heads compared with the means of non-shaded *Z. capricorni* ( $96.87 \pm 20.61$ ) and *Halophila ovalis* plants ( $81.21 \pm 5.32$ ) at the Urangan site (Fig. 2). At Burrum Heads the mean  $ETR_{max}$  for *Z. capricorni* non-shaded plants ( $178.24 \pm 17.53$ ) was also significantly higher than 60% ( $94.62 \pm 23.69$ ) and 90% ( $83.78 \pm 7.64$ ) shaded plants (Fig. 2). At Urangan, means of  $ETR_{max}$  for 5% ( $103.95 \pm 12.16$ ) and 60% ( $91.26 \pm 10.00$ ) shaded *Z. capricorni* were higher than the mean of 90% shaded *Z. capricorni* ( $74.49 \pm 2.29$ ) plants (Fig. 2). Mean  $ETR_{max}$  of 90% shaded *H. ovalis* at Burrum Heads

**Table 1** Three-way ANOVA of the effect of site (Urangan and Burrum Heads), species (*Halophila ovalis* and *Zostera capricorni*) and shade treatment (0, 5, 60 and 90% light reduction) on photosynthetic variables in Hervey Bay,  $n = 3$

Photosynthetic variable	df	MS	F	P
<b>ETR<sub>max</sub></b>				
Site	1	1.68	34.258	0.001
Species	1	0.873	17.811	0.002
Shading	3	0.622	12.691	0.001
Site × species	1	0.027	0.555	0.462
Site × shade	3	0.169	3.452	0.028
Species × shade	3	0.062	1.274	0.300
Site × species × shade	3	0.176	3.591	0.024
Error	32	0.049		
<b>A</b>				
Site	1	0.034	18.911	0.001
Species	1	0.003	1.633	0.211
Shading	3	0.021	11.491	0.001
Site × species	1	0.002	0.881	0.355
Site × shade	3	0.006	3.409	0.029
Species × shade	3	0.005	2.519	0.076
Site × species × shade	3	0.001	0.249	0.861
Error	32	0.002		
<b>E<sub>k</sub></b>				
Site	1	603,386.68	42.883	0.001
Species	1	84,445.329	6.002	0.020
Shading	3	274,982.58	19.543	0.001
Site × species	1	1,220.384	0.087	0.770
Site × shade	3	38,327.461	2.724	0.061
Species × shade	3	66,161.828	4.702	0.008
Site × species × shade	3	50,470.265	3.587	0.024
Error	32	14,070.504		
<b>F</b>				
Site	1	155,838.02	49.19	0.001
Species	1	59,854.687	18.89	0.001
Shading	3	8,406.854	2.654	0.065
Site × species	1	623.521	0.197	0.660
Site × shade	3	750.021	0.237	0.870
Species × shade	3	3,945.91	1.246	0.309
Site × species × shade	3	6,087.854	1.922	0.146
Error	32	3,167.854		
<b>Fm'</b>				
Site	1	603,386.68	42.883	0.001
Species	1	84,445.329	6.002	0.020
Shading	3	274,982.58	19.543	0.001
Site × species	1	1,220.384	0.087	0.770
Site × shade	3	38,327.461	2.724	0.061
Species × shade	3	66,161.828	4.702	0.008
Site × species × shade	3	50,470.265	3.587	0.024
Error	32	14,070.504		

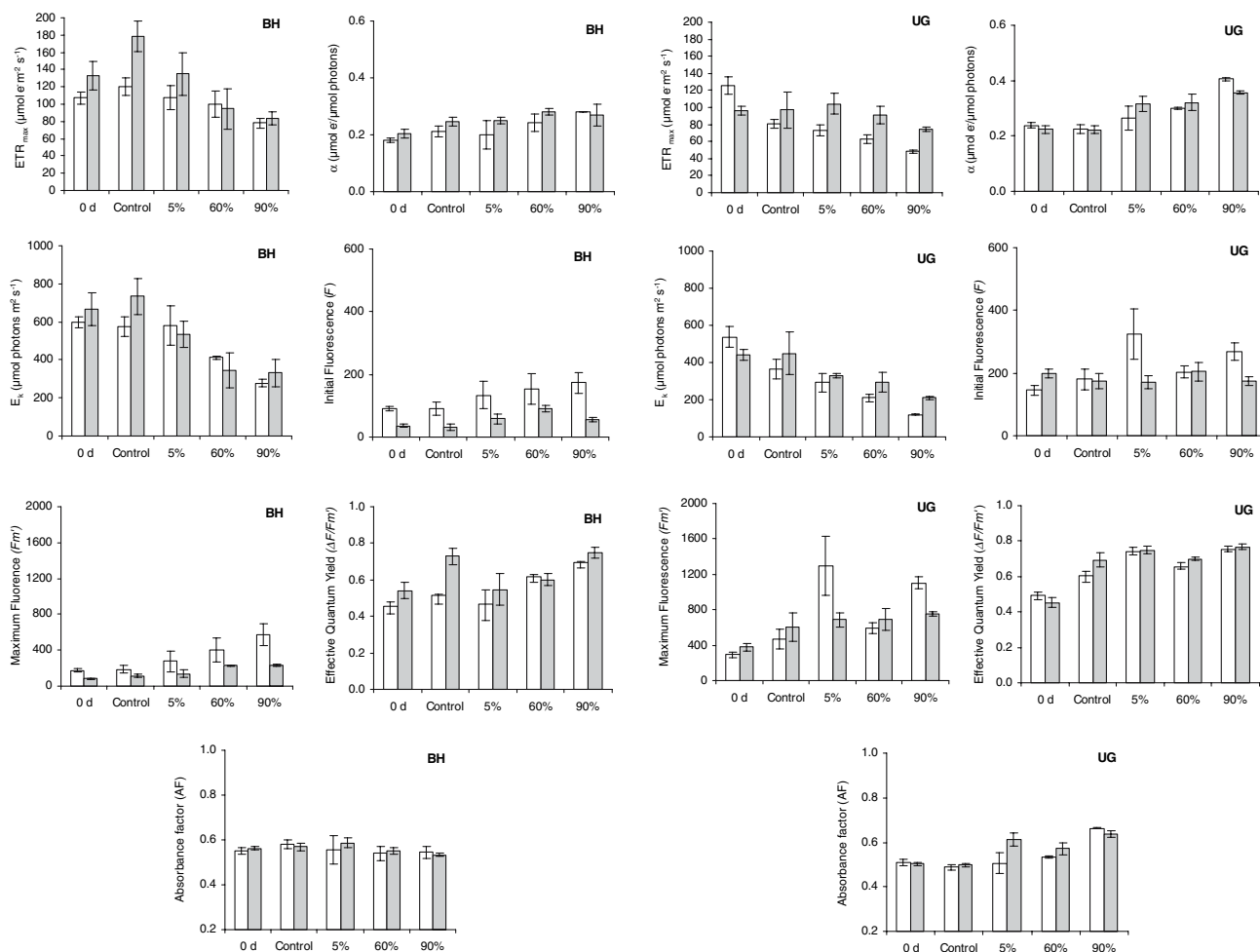
**Table 1** continued

Photosynthetic variable	df	MS	F	P
<b>ΔF/Fm'</b>				
Site	1	0.116	24.348	0.001
Species	1	0.052	10.812	0.002
Shading	3	0.040	8.322	0.001
Site × species	1	0.007	1.446	0.238
Site × shade	3	0.024	5.028	0.006
Species × shade	3	0.015	3.163	0.038
Site × species × shade	3	0.011	2.239	0.103
Error	32	0.005		
<b>Absorbance factor</b>				
Site	1	0.001	0.297	0.590
Species	1	0.004	1.777	0.192
Shading	3	0.008	3.524	0.026
Site × species	1	0.002	0.983	0.329
Site × shade	3	0.019	8.639	0.001
Species × shade	3	0.004	1.568	0.216
Site × species × shade	3	0.003	1.165	0.338
Error	32	0.002		

(78.02 ± 14.86) and Urangan (48.61 ± 1.92) was significantly lower compared with non-shaded and 5% shaded *H. ovalis* at both sites.

For E<sub>k</sub> the significant three-way interaction (Table 1) was best explained by significantly higher mean E<sub>k</sub> of non-shaded *Z. capricorni* (733.07 ± 92.40) and *H. ovalis* (573.23 ± 51.51) at Burrum Heads compared with non-shaded *Z. capricorni* (449.21 ± 113.64) and *H. ovalis* plants (365.06 ± 50.58) at Urangan (Fig. 2). Species interactions were best explained by higher mean E<sub>k</sub> for 60% (294.73 ± 54.93) and 90% (209.70 ± 9.07) shaded *Z. capricorni* compared with mean E<sub>k</sub> for 60% (209.70 ± 21.32) and 90% (120.07 ± 3.88) shaded *H. ovalis*, respectively, at Urangan, but no such differences were found at Burrum Heads (Fig. 2). At Burrum Heads there was no difference between mean E<sub>k</sub> values in 60% shaded (342.86 ± 92.70) and 90% shaded *Z. capricorni* (331.03 ± 72.68), but at Urangan mean E<sub>k</sub> of 60% shaded *Z. capricorni* (294.73 ± 54.93) was significantly higher than the mean E<sub>k</sub> of 90% shaded plants (209.70 ± 9.07) (Fig. 2). At Burrum Heads the mean E<sub>k</sub> of 5% shaded *Z. capricorni* (534.65 ± 69.62) was significantly higher than 60% shaded (342.86 ± 92.70) *Z. capricorni*, whereas at Urangan no difference was found between mean E<sub>k</sub> of 5% shaded (328.66 ± 10.49) and 60% shaded (294.73 ± 54.93) *Z. capricorni* (Fig. 2).

A shading effect was evident for all parameters measured, except *F*. At both sites, 90% shade treated *H. ovalis* (1,100.67 ± 69.20 at Urangan; 230.33 ± 12.35 at Burrum Heads) showed higher mean Fm' compared



**Fig. 2** Effect of shading on photosynthetic variables  $ETR_{max}$ ,  $\alpha$ ,  $E_k$ ,  $F$ ,  $Fm'$ ,  $\Delta F/Fm'$  and  $AF$ , at Urgan (*UG*) and Burrum Heads (*BH*) sites. Values are shown at 0 day and after 14 days shading for control,

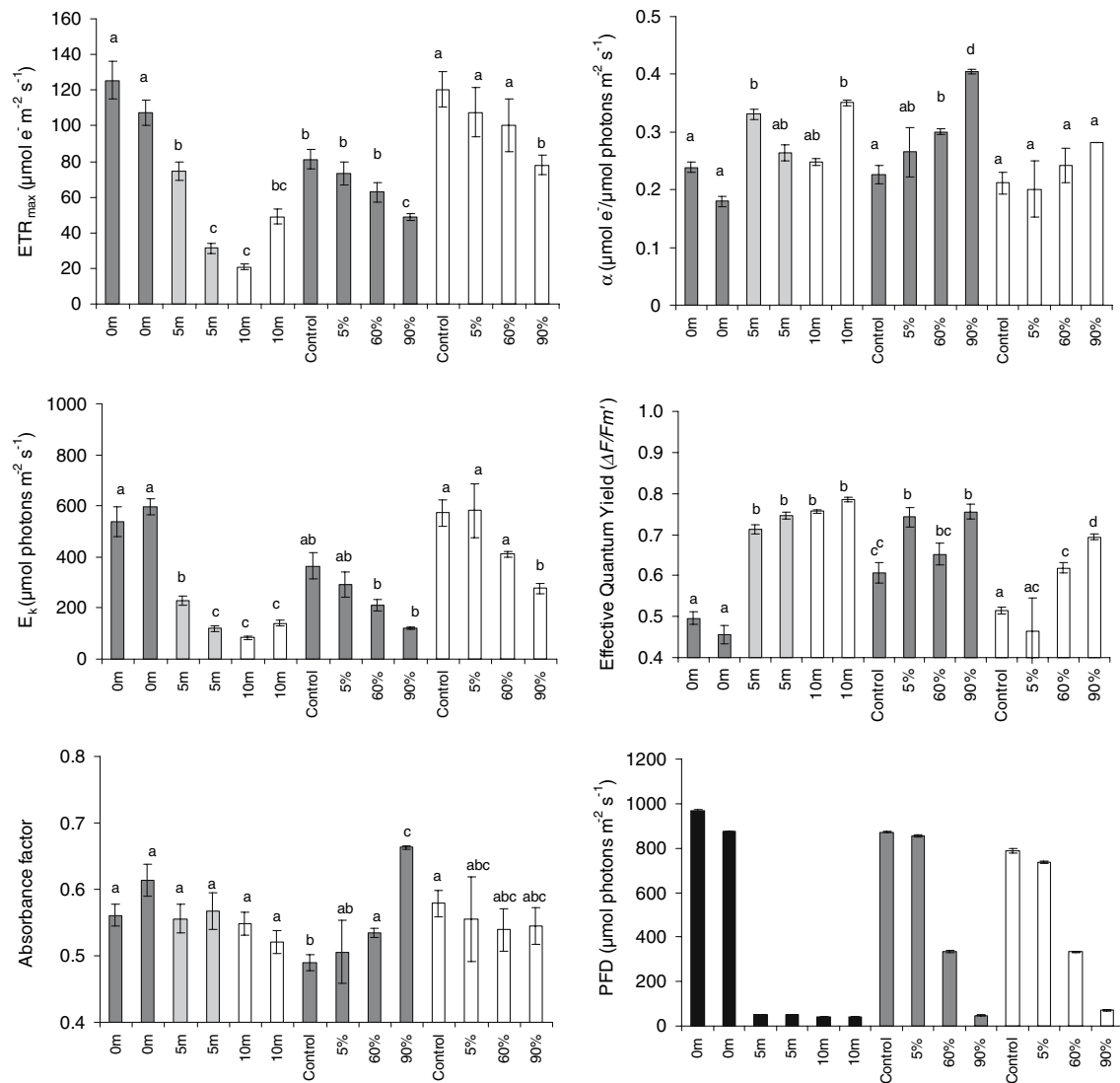
5, 60 and 90% shade treated plots for *H. ovalis* (white bars) and *Z. capricorni* (shaded bars) (mean  $\pm$  SE,  $n = 3$ )

with control *H. ovalis* ( $47.33 \pm 113.87$  at Urgan;  $187.33 \pm 42.29$  at Burrum Heads) plants (Fig. 2, Table 1). At both sites, 90% shaded *H. ovalis* had significantly higher mean  $\alpha$  ( $0.2813 \pm 0.0003$  at Burrum Heads;  $0.4047 \pm 0.0045$  at Urgan) and mean  $\Delta F/Fm'$  ( $0.6943 \pm 0.0068$  at Burrum Heads;  $0.7553 \pm 0.0180$  at Urgan) compared with non-shaded ( $\alpha - 0.2120 \pm 0.0189$  at Burrum Heads and  $0.2267 \pm 0.0156$  at Urgan;  $\Delta F/Fm' - 0.5143 \pm 0.0088$  at Burrum Heads and  $0.6070 \pm 0.0255$  at Urgan) and 5% shaded ( $\alpha - 0.2010 \pm 0.0492$  at Burrum Heads and  $0.2650 \pm 0.0420$  at Urgan;  $\Delta F/Fm' - 0.4647 \pm 0.0799$  at Burrum Heads and  $0.7423 \pm 0.0234$  at Urgan) *H. ovalis* plants, at respective sites. At Urgan, 90% shaded *Z. capricorni* had significantly higher mean  $\alpha$  ( $0.3557 \pm 0.0062$ ) compared with non-shaded ( $0.2220 \pm 0.0140$ ), 5% ( $0.3147 \pm 0.0278$ ) and 60% ( $0.3207 \pm 0.0296$ ) shaded *Z. capricorni*. At Urgan,  $\Delta F/Fm'$  was significantly higher for 90% shaded

*Z. capricorni* ( $0.7683 \pm 0.0143$ ) compared with non-shaded *Z. capricorni* ( $0.6940 \pm 0.0381$ ). For  $ETR_{max}$  and saturating light intensity ( $E_k$ ), the opposite was generally observed for both species at both sites, where 90% shaded plants had significantly lower values than control and 5% shaded plants, especially in *H. ovalis* (Fig. 2). At Urgan 90% shaded *Z. capricorni* ( $0.6368 \pm 0.0154$ ) and *H. ovalis* ( $0.6632 \pm 0.0023$ ) had higher  $AF$ s than respective 5% shaded *Z. capricorni* ( $0.6120 \pm 0.0297$ ) and *H. ovalis* ( $0.5060 \pm 0.0481$ ) plants (Fig. 2).

#### *Halophila ovalis*: depth gradient and shading experiment

Plots of  $ETR_{max}$ , photosynthetic efficiency ( $\alpha$ ), saturating irradiance ( $E_k$ ), effective quantum yield ( $\Delta F/Fm'$ ) and  $AF$  against depth and shading treatments revealed a decline in



**Fig. 3** Effect of depth (0, 5 and 10 m) on photosynthetic variables ( $ETR_{max}$ ,  $\alpha$ ,  $E_k$ ,  $F_m'$ ,  $\Delta F/F_m'$  and AF) for *H. ovalis*. Each graph includes the effect of shading treatments at 14 days (control, 5, 60 and 90% shade plots) at Urangan (grey bars) and Burrum Heads (white

bars). Photon flux density at each depth and shade treatment are also shown (mean  $\pm$  SE,  $n = 3$ ). Different letters above bars indicate significant differences (Bonferroni) between treatment means at  $P < 0.05$

$ETR_{max}$  and  $E_k$  and a general increase in  $\alpha$  and  $\Delta F/F_m'$  with increasing depth and shade (Fig. 3). Significant differences among means of each variable were found (Table 2). Mean  $ETR_{max}$  of *H. ovalis* in non-shaded and 5% shaded plots at Urangan were significantly lower than plants at 0 m but not significantly different than  $ETR_{max}$  of plants at 5 m (Fig. 3). In contrast  $ETR_{max}$  and  $E_k$  of non-shaded plants at Burrum Heads were no different to plants at 0 m, while 90% shaded *H. ovalis* had mean  $ETR_{max}$  and  $E_k$  values that were significantly lower than plants at 0 m but not significantly different compared with plants growing at 5 and 10 m (Fig. 3). Mean  $\alpha$  and  $\Delta F/F_m'$  values in control and 5% shaded plots were not significantly different compared with mean values at 0 m. Similarly mean  $\alpha$

and  $\Delta F/F_m'$  recorded for 60% shaded *H. ovalis* were not significantly different when compared with mean  $\alpha$  and  $\Delta F/F_m'$  of *H. ovalis* at 5 m (Fig. 3). Mean  $\alpha$  and  $\Delta F/F_m'$  values in 90% shaded *H. ovalis* were significantly lower than plants at 0 m but not significantly different compared with *H. ovalis* at 5 and 10 m.

### Discussion and conclusions

Light is a key factor controlling the photosynthetic performance of seagrasses. Both long-term and short-term light deprivation directly influence seagrass photosynthesis and survival (Longstaff et al. 1999; Ruiz and Romero 2001;

**Table 2** *Halophila ovalis*: ANOVA of the effect of shade plots (control, 5, 60 and 90% at two sites) and depths (0, 5 and 10 m) in Hervey Bay

Photosynthetic variable	df	MS	F	P
<i>F</i>				
Treatment	13	26,290.1	8.891	0.001
Error	69	2,956.8		
<i>Fm'</i>				
Treatment	13	810,173.8	19.331	0.001
Error	69	41,911.4		
<i>ETR<sub>max</sub></i>				
Treatment	13	2.172	44.212	0.001
Error	69	0.049		
$\alpha$				
Treatment	13	0.021	16.129	0.001
Error	69	0.001		
<i>E<sub>k</sub></i>				
Treatment	13	3.119	59.406	0.001
Error	69	0.052		
$\Delta F/Fm'$				
Treatment	13	0.097	44.357	0.001
Error	69	0.002		
Absorbance factor				
Treatment	13	0.007	2.281	0.022
Error	41	0.003		

*ETR<sub>max</sub>* and *E<sub>k</sub>* values were log<sub>e</sub> transformed prior to analysis ( $n = 3-10$ )

Peralta et al. 2002). We found short-term changes in the physiology of seagrasses by manipulating light during experimental shading. Of all the photosynthetic variables tested (*ETR<sub>max</sub>*, *E<sub>k</sub>*,  $\alpha$ , *Fm'*,  $\Delta F/Fm'$  and AF) *ETR<sub>max</sub>* and *E<sub>k</sub>* showed the most consistent responses among shade treatments and depth gradients, characterised by reductions in *ETR<sub>max</sub>* and increases in *E<sub>k</sub>*, with light depletion. Both *Halophila ovalis* and *Z. capricorni* exhibited typical responses to shading yet *H. ovalis* had a more conspicuous response, suggesting that this species is more sensitive to reduced transient light than *Z. capricorni*. The results suggest a tolerance and acclimation to light deprivation in both species of seagrass between 5 and 60% of surface irradiance and are consistent with findings that *H. ovalis* has a limited tolerance to light deprivation when compared with morphologically larger species of seagrass (Longstaff et al. 1999).

There was a consistent trend among species and sites, as shown by the decreasing *ETR<sub>max</sub>* and *E<sub>k</sub>* and increasing  $\alpha$  and  $\Delta F/Fm'$  with light depletion, and a strong separation of photosynthetic responses between high and low light treatments. These photosynthetic responses are evidence that photosynthetic parameters derived from RLCs (Beer

et al. 2001) are a useful tool that can be used to evaluate short-term responses of seagrasses between highly differentiated light climates. The parameters sensitive to 90% shading in *H. ovalis* were reduced *ETR<sub>max</sub>*, increased  $\alpha$ , reduced saturating irradiance (*E<sub>k</sub>*) and increased effective quantum yield ( $\Delta F/Fm'$ ). While a reduction in both *ETR<sub>max</sub>* and *E<sub>k</sub>* suggests a limited photosynthetic capacity such reduction indicates photo-acclimation and improved photon capture for conversion to chemical energy. Similarly, the increase in both  $\alpha$  and  $\Delta F/Fm'$  in response to shading indicates a greater proportion of photons were used in photosynthesis and a more efficient use of light (Beer et al. 2001). These photo-adaptive responses to irradiance or photon flux deprivation are analogous with other photo-adaptive responses such as chlorophyll increases under low light conditions (Dennison and Alberte 1986; Longstaff and Dennison 1999). These findings are consistent with reports of reductions in photosynthetic performance and mortality in seagrasses in response to shading (Longstaff and Dennison 1999; Longstaff et al. 1999) and depth (Schwarz and Hellblom 2002; Durako et al. 2003).

The differences in photosynthetic performance of both seagrass species among shade treatments at different sites are easily explained by site features influencing light fluxes and the species responses to these differences. At low tide there was little obvious difference in light climates between sites, yet during tidal inundation high amounts of suspended fine 'silty' sediments at Urangan were evident more so than at Burrum Heads, where the sediments have a high sand composition and are less inclined to be re-suspended during tidal flux. These high silt loads at Urangan resulted in lower light availability and more pronounced shade-type responses (e.g. lower *E<sub>k</sub>*) in both seagrass species at Urangan, compared with seagrasses at Burrum Heads. In addition, the fouling of shade screens appeared heavier at the Urangan site, despite regular cleaning, and may also have contributed to lower photon fluxes under the shade screens. At both sites *Halophila ovalis* was more sensitive to changes in light availability than *Zostera capricorni* and thus exhibited the greatest reduction in *ETR<sub>max</sub>* and *E<sub>k</sub>* and the highest increases in photosynthetic efficiencies in response to shading and in situ light depletion at Urangan. A likely explanation is that *H. ovalis* is less structurally complex and faster growing than *Z. capricorni*, with low storage capacity for carbohydrates that can be used for growth during periods of low light (Longstaff et al. 1999).

The increase in effective quantum yield ( $\Delta F/Fm'$ ) of both seagrass species under 90% shading, relative to controls, was due to elevated *Fm'* values. Elevation of *Fm'* suggests an increase in photochemical efficiency, a response that is consistent with photo-acclimation of plants to shading. This contrasts with Longstaff et al. (1999) who found that quantum yield in dark adapted



*H. ovalis* remained unchanged during complete light deprivation for 24 days. However, experimental shading units deployed by Longstaff and Dennison (1999) and Longstaff et al. (1999) showed photo-adaptive responses of reduced chlorophyll *a* and sugar concentration in *H. ovalis* leaves after 15 days of shading that are consistent with findings of reduced  $ETR_{max}$ , increased  $\alpha$  and increased  $E_k$  in the present study. In the present study no change in  $\Delta F/Fm'$  was found for *Z. capricorni*. Biber et al. (2005) concluded that the maximum quantum yield of *Z. marina* in response to light deprivation was not a sensitive indicator of chronic stress as *Z. marina* was able to acclimate to ambient light conditions. A close examination of Biber et al. (2005) shows a clear stress response in healthy leaves, with quantum yield values of 0.5 at 4 weeks, 1 week prior to an observed decline in shoot numbers and leaf area. Similarly, they show that quantum yield values in *Halodule wrightii*, in dark conditions, declined to 0.6 after 3 weeks and 0.2 after 4 weeks, at least a week prior to noticeable declines in shoot numbers. In the context of the clear photosynthetic responses to light reduction found in the present study, this result demonstrates the importance of using a suite of photosynthetic variables, such as  $ETR_{max}$ ,  $\alpha$ ,  $E_k$  and  $\Delta F/Fm'$ , derived from RLCs to assess short-term responses of seagrasses to light deprivation, not measures of maximum quantum yield alone.

The influence of depth on  $ETR_{max}$  and  $E_k$  implies a strong separation of irradiance history between depths of 0 and 5–10 m. After 2 weeks of exposure to 90% reduced light availability, *H. ovalis* exhibited shade-type physiological responses, i.e. lower  $ETR_{max}$  and  $E_k$ , similar to plants growing at 5–10 m. The similarity in the light climates between 90% shaded plants and ambient light at 5–10 m is the likely explanation. The 40% lower  $ETR_{max}$  and 67% lower  $E_k$  values in 90% shaded *H. ovalis* compared with controls, was generally lower than depth related reductions of  $ETR_{max}$  and  $E_k$  from 0 to 5 m and from 0 to 10 m. Intertidal plants may be more susceptible to light reduction than subtidal plants and exhibit greater photo-adaptive responses in order to optimise light acquisition and maximise the use of low-light climates. In addition, fouling of shade cloths in between cleaning may have lowered available photon flux density (PFD) below recorded levels and contributed to a heightened photo-adaptive response. Comparative lowering of  $ETR_{max}$  and  $E_k$  values has been recorded for *Halophila stipulacea* (Schwarz and Hellblom 2002) and *H. ovalis* (Ralph 1996). In order to optimise light acquisition and maximise their use of low-light climates, these plants can diurnally regulate their saturating irradiance as detected in a number of seagrass species (Ralph et al. 1998; Campbell et al. 2003). We have also measured a three to fourfold increase in light

saturating irradiance, between 0600 and 1200 hours, in deepwater *H. ovalis* (unpublished data).

Absorbance factors have been shown to vary considerably with geographic location (Silva and Santos 2003), depth (Schwarz and Hellblom 2002), species (Beer et al. 2001; Campbell et al. 2003; Lan et al. 2005) and leaf age (Enríquez et al. 2002). In Hervey Bay there was no change in AF with depth, but the high AFs of dark shaded (90%) *H. ovalis* at Urangan were indicative of a photo-adaptive response but were unlikely alone to be a reliable indicator of light reduction. The general lack of change in AFs with depth and shading suggests that factors (e.g. time of year, nutrient status) other than light attenuation may be influencing AFs over time scales of weeks to months.

The stronger responses to shading suggest that *H. ovalis* was more vulnerable to light deprivation than *Z. capricorni* and that *H. ovalis* at depths of 5–10 m would be more vulnerable to light deprivation than intertidal populations. Intertidal *H. ovalis* appears to be more vulnerable to reductions in irradiance than intertidal *Z. capricorni*, implicating local light conditions as important influences on species specific photosynthetic performance. The lower photosynthetic responses of *H. ovalis* at depths greater than 5 m compared with intertidal plants, also suggest a greater vulnerability to light deprivation with increased depth. Compared with large seagrasses, the more pronounced responses of *H. ovalis* to light reduction may be due to differences in the capacity of small seagrasses to store carbohydrates and maintain photosynthetic performance (Czerny and Dunton 1995; Kraemer and Alberte 1995; Longstaff et al. 1999). The short-term response of seagrasses to in situ reductions in light availability is likely to result in reduced growth rates and therefore can provide a rapid and precise indicator of sub-lethal stress. The photo-adaptive responses from experimental light reduction and the measures of light attenuation and saturating irradiances along depth gradients, particularly at lower depth limits of survival, could also be used to predict reductions in photosynthetic capacity before mortality eventuates.

Both species showed a photo-adaptive response to light depletion that may help them tolerate short-term yet severe reductions in light commonly encountered in Hervey Bay. Responses of  $ETR_{max}$ , saturating irradiance ( $E_k$ ) and photosynthetic efficiency ( $\alpha$ ) to light reduction were the most consistent and are therefore likely to provide the best indicators of photo-adaptation and possible seagrass stress.

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