Contribution to the Theme Section 'Primary production in seagrasses and macroalgae'

Photosynthetic responses of *Halophila stipulacea* to a light gradient. II. Acclimations following transplantation

Yoni Sharon[1,2,*], João Silva[3], Rui Santos[3], John W. Runcie[4], Mark Chernihovsky[2], Sven Beer[1]

[1]Department of Plant Sciences, Tel Aviv University, Tel Aviv 60078, Israel

[2]The Interuniversity Institute for Marine Sciences, POB 469, Eilat 88103, Israel

[3]ALGAE - Marine Plant Ecology Research Group, Centre of Marine Sciences (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

[4]School of Biological Sciences, University of Sydney, New South Wales 2006, Australia

ABSTRACT: *Halophila stipulacea* is the dominant seagrass in the Gulf of Aqaba (northern Red Sea), where it grows from the intertidal to depths exceeding 50 m. Its successful growth under such a broad irradiance gradient shows either a high plasticity or is caused by longer-term adaptations to the various depths, possibly resulting in the formation of ecotypes. In April 2008 we transplanted shoots of this seagrass between the extreme depths of its distribution at the study site (8 and 33 m) in order to evaluate its acclimation potential to various irradiances. We compared photosynthetic parameters derived from light response curves generated by PAM fluorometry (so-called rapid light curves, RLC) and measured chlorophyll *a* and *b* concentrations. RLCs from the shallow (~400 µmol photons m^{-2} s^{-1} at midday) and deep (~35 µmol photons m^{-2} s^{-1} at midday) sites were characteristic for high- and low-light growing plants, respectively, and the transplanted seagrasses acclimated to their new environments within 6 d, at which time their RLCs resembled those of the original plants growing at the depths to which they had been transplanted. Concentrations of both chlorophyll *a* and *b* decreased or increased when the plants were transferred to high- vs. low-light environments, respectively, but the chlorophyll *a:b* ratios remained constant. These fast changes in photosynthetic responses and light absorption characteristics in response to changing light environments points to *Halophila stipulacea* as being a highly plastic seagrass with regard to irradiance, which may partly explain its abundance across a wide range of irradiances along the depth gradient that it occupies.

KEY WORDS: Acclimation · Depth gradient · *Halophila stipulacea* · Irradiance · Photosynthesis · Pigments · Seagrass

INTRODUCTION

In addition to diurnal changes in insolation, the marine environment is characterised by strongly attenuated irradiances along a relatively short depth gradient. The ability of marine macrophytes to develop mechanisms to utilise and/or cope with different irradiances may therefore be crucial for their functioning at temporally dynamic as well as spatially different surroundings (Sultan 2000). Some marine macroalgae have in-

deed been shown to possess the capacity to cope with variation in irradiance as well as spectral composition in a plastic way (e.g. Monro & Poore 2005, Mata et al. 2006). Regarding submerged marine angiosperms (seagrasses), much information has been acquired regarding their photophysiological responses to irradiance (Olesen et al. 2002, Durako et al. 2003, Silva & Santos 2003, recently reviewed by Ralph et al. 2007), but whether these responses are the result of adaptations (long-term selection processes resulting in eco-

types) or acclimation processes (short-term plastic responses) are largely unknown.

Halophila stipulacea is the dominant seagrass in the clear waters (light attenuation coefficient K_d [400 to 700 nm] = 0.1) of the Gulf of Aqaba (northern Red Sea). There it grows abundantly in fine sediments from the intertidal down to depths >50 m (Lipkin 1979, Y. Sharon pers. obs.) where, unlike shallow-growing species, it is not affected by hydrostatic pressure (Beer & Waisel 1982). Its photosynthetic responses to irradiance at various depths are characterised by decreasing maximal photosynthetic rates (measured as relative electron transport rates, rETR) and decreasing onsets of saturating irradiances (I_k) at increasing depths from 7 to 30 m (Schwarz & Hellblom 2002). For plants grown at higher irradiances, midday chloroplast clumping was quantified by Sharon & Beer (2008), and it was suggested that this is an important photoprotective mechanism for shallow-growing plants. Thus, while acclimation to daily temporal changes in irradiance was shown for high-light plants of *Halophila stipulacea*, it is not known whether the lower rETRs of this seagrass at low irradiances such as demonstrated by Schwarz & Hellblom (2002) are also due to acclimation processes or can only be explained by longer-term adaptations.

As part of the recent Group for Aquatic Primary Productivity (GAP, see www.gap-aquatic.org/) workshop in Eilat (April 2008), we studied *in situ* various photophysiological aspects of the seagrass *Halophila stipulacea*. In the present study, we tested the photosynthetic plasticity of this plant as a response to *in situ* irradiance. This was done by comparing pigment contents and photosynthetic parameters derived from pulse-amplitude modulated (PAM) fluorometry following reciprocal transplantations from the 2 edges of an *H. stipulacea* meadow.

MATERIALS AND METHODS

The *Halophila stipulacea* seagrass bed studied was located ca. 200 m south of the InterUniversity Institute (IUI) in Eilat, the Gulf of Aqaba, northern Red Sea (29° 30' N, 34° 54' E). The vertical extension of the bed varies between seasons, and was between 8 and 33 m at the time of the present study (April 2008). Midday irradiances at the 2 experimental depths at this time were ~400 and ~35 µmol photons m^{-2} s^{-1} at 8 and 33 m, respectively; most days were cloudless during the 2 wk experimental period. The water temperature was 23°C. All plants were accessed by SCUBA diving using NITROX.

In order to measure the plasticity of the photosynthetic responses of *Halophila stipulacea* to irradiance, we transplanted plants reciprocally from one site to another. Some 30 to 50 shoots, including rhizomes

(possibly belonging to the same clone) and roots, from the 2 depths were removed with their sediments (ca. 3 l) into mesh bags, and 3 such bags were placed into dug-out depressions at each of the sites (both vertically and horizontally for same-depth controls). Photosynthesis and pigment measurements were performed just prior to transplantation and following 6 and 14 d of exposure to the various sites.

Chlorophyll fluorescence was measured on cloudless days at 09:30 to 10:30 h at the 2 sampling depths using an underwater fluorometer (Diving-PAM). Individual leaves (from different shoots, n = 5) were placed into a leaf-clip and rapid light curves (RLCs) were initiated immediately. Each leaf was irradiated with increasing pre-set photosynthetically active radiation (PAR) steps (0, 37, 56, 104, 160, 227, 339, 512 and 818 µmol photons m^{-2} s^{-1}) for 5 s, and the effective quantum yield of Photosystem II (PSII) was recorded after each step. It was found that 5, 10 and 30 s of exposure to each level of irradiance yielded the same results, so 5 s was chosen in order to minimise the diving time. Electron transport rates (ETR) were calculated as ETR = $Y \times I \times$ AF $\times$ 0.5 where Y is the effective quantum yield of PSII, I is the incident irradiance, AF is the absorption factor and 0.5 is the assumed proportion of light absorbed between PSII and PSI. AFs were estimated *in situ* by measuring the light absorbed by the leaves as described elsewhere (Beer & Björk 2000, except that ambient light was used instead of a lamp), and assuming that all light was absorbed by the photosynthetic pigments of the leaves (Sharon & Beer 2008). ETR vs. PAR curves were generated after correcting incident PAR with AF so as to depict absorbed PAR on the *x*-axes (according to Saroussi & Beer 2007; this is logically called for since the ETRs of the *y*-axis also include AF values). The maximal photosynthetic rate (P_{max}) and onset of light saturation (I_k) were calculated after fitting the ETR data to equations by Platt et al. (1980). The maximal effective quantum yield (Y_0) was taken as the first yield measurement of the RLC (according to Saroussi & Beer 2007; this value is equal to α as commonly calculated from the initial slope of the RLC). Initially, in order to find out if the plants from the deep and shallow sites had the same potential photosynthetic capacity, PSII maximum quantum yield of photochemistry in PSII (F_v/F_m) of dark-adapted plants were recorded for those plants at dawn (06:00 h).

Chlorophylls *a* and *b* (chl *a + b*) were extracted in *N'N*-dimethylformamide from cut-out leaf sections within 20 min of collection (n = 5). The absorbance was measured in a spectrophotometer at 664, 647 and 625 nm and concentrations were calculated according to Moran (1982).

Statistical analyses were performed using Sigma Stat 3.00. Differences were considered significant at $p < 0.05$.

RESULTS

In situ F_v/F_m values (mean ± SD) of native plants were found to be 0.76 ± 0.02 (n = 10) and 0.76 ± 0.01 (n = 10) for shallow (8 m) and deep (33 m) plants, respectively. Thus, no significant difference was found in the maximum photosynthetic efficiency of PSII in *Halophila stipulacea* at the different bathymetric sites during the time of these measurements.

The average RLCs (n = 5) of *Halophila stipulacea* from the 2 different sites are depicted in Fig. 1. The P_{max}, I_k and Y_0 (equalling α) values derived from these curves were 39.7 ± 3.8 µmol electrons m^{-2} s^{-1}, 65.0 ± 7.1 µmol photons m^{-2} s^{-1} and 0.61 ± 0.03 mol electrons mol photons^{-1}, respectively, for the shallow plants and 20.6 ± 1.4 µmol electrons m^{-2} s^{-1}, 41.1 ± 4.4 µmol photons m^{-2} s^{-1} and 0.50 ± 0.04 mol electrons mol photons^{-1}, respectively, for the deep plants. All parameters were significantly different between the shallow and deep plants (Mann-Whitney *U*-test, p < 0.001).

P_{max} of the shallow plants decreased significantly (Mann-Whitney *U*-test, p < 0.001) 6 d after transplantation to the deep site, and kept decreasing slightly after 14 d of exposure to the dimmer environment (Fig. 2). Conversely, deep plants increased their P_{max} significantly (p < 0.001) 6 d after transplantation, and did not change over the remaining 8 d. Thus, P_{max} values of shallow plants became quantitatively similar to those of deep plants after 6 d and vice versa. The control plants, which were dug up and then replanted at the same depths in order to appraise whether mechanical uprooting had affected them, showed much less of a change in P_{max} over time.

I_k changed similarly to P_{max} at the different environments to which the plants were transferred

(Fig. 3). Also, I_k of the transplanted seagrasses became quantitatively similar to that of the plants originally growing at the 2 depths. While the Y_0 values of the transplanted plants also showed a pattern of change with time such that they came to resemble those of the plants originally growing at the 2 depths (Fig. 4), this change was slower than for the other parameters.

The chl *a* + *b* content of shallow plants increased between 6 and 14 d following transplantation to the deeper site (Fig. 5). Conversely, the deep plants showed a decreased chl *a* + *b* content after 6 d following transplantation to the shallow site. There was no significant difference in chl *a*:*b* ratios following the transplantations (Fig. 6).

Fig. 2. *Halophila stipulacea*. Maximal photosynthetic rates (P_{max}) at 0, 6 and 14 d after transplantation from 8 to 33 m depth (○, ——), from 33 to 8 m (●, ——) and the corresponding controls (– – –). Data points are means ± SD of 4 replicates
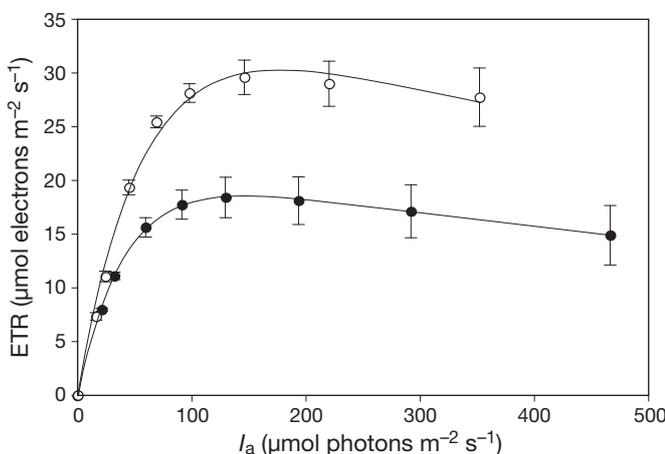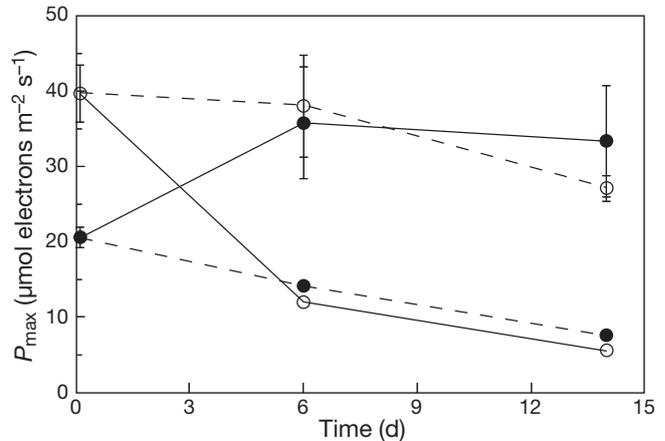

Fig. 1. *Halophila stipulacea*. Electron transport rates (ETR) as a function of absorbed irradiances (I_a) derived from rapid light curves of plants growing at 8 (○) and 33 m (●) depth. Data points are means ± SD of 5 replicates
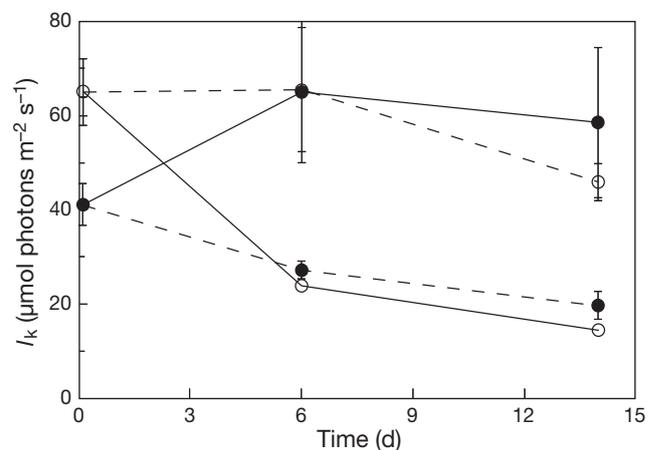

Fig. 3. *Halophila stipulacea*. Onset of light saturation (I_k) at 0, 6 and 14 d after transplantation from 8 to 33 m depth (○, ——), from 33 to 8 m (●, ——) and the corresponding controls (– – –). Data points are means ± SD of 4 replicates
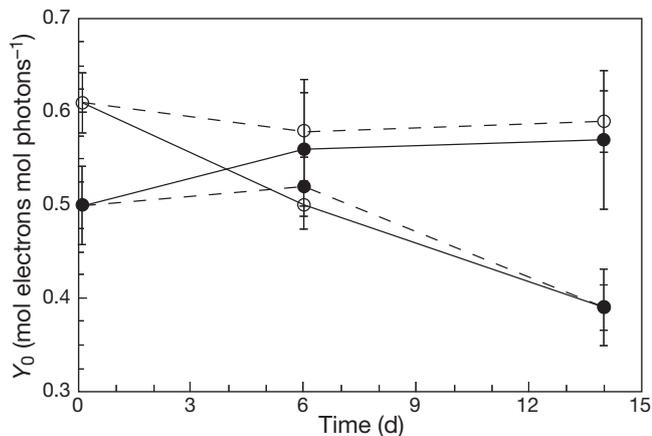
Fig. 4. *Halophila stipulacea*. Maximal effective quantum yield
(Y_0) at 0, 6 and 14 d after transplantation from 8 to 33 m depth
(○, ——), from 33 to 8 m (●, ——) and the corresponding controls
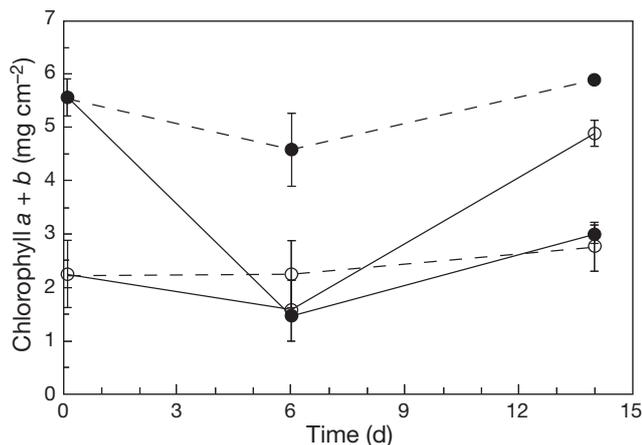(– – –). Data points are means ± SD of 4 replicates

Fig. 5. *Halophila stipulacea*. Chlorophyll *a* + *b* concentrations
at 0, 6 and 14 d after transplantation from 8 to 33 m depth
(○, ——), from 33 to 8 m (●, ——) and the corresponding controls
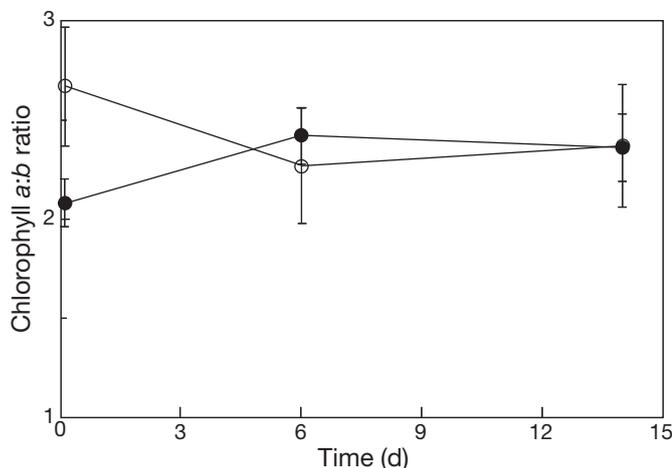(– – –). Data points are means ± SD of 4 replicates

Fig. 6. *Halophila stipulacea*. Chlorophyll *a*:*b* ratio at 0, 6 and
14 d after transplantation from 8 to 33 m depth (○, ——), from
33 to 8 m (●, ——). Data points are means ± SD of 4 replicates

DISCUSSION

Our results indicate that *Halophila stipulacea* is a
highly plastic seagrass that can acclimate to various
light environments quickly. This was shown before
for plants grown under high-light conditions, where
chloroplast movements were suggested as a means to
both capture photons under diurnal low-light periods
(when chloroplasts are dispersed) and apparently pro-
tect the leaves from photodamage during midday (by
clumping the chloroplasts, Sharon & Beer 2008). In the
present study, we showed that the photosynthetic
apparatus also features plasticity and it can acclimate
to various irradiances along a depth gradient accord-
ing to classical adaptation strategies of high- and low-
light plants. Further, this plasticity is in line with the
thought that specimens of *H. stipulacea* growing at
various depths in a meadow are not ecotypes, although
the maximal irradiance during midday spans at least
1 order of magnitude.

The apparent lack of ecotype formation at various
depths within a *Halophila stipulacea* meadow may stem
from its largely vegetative growth pattern (through rhi-
zome elongation) resulting in large areas belonging to
the same clone. Further, we have observed that the ver-
tical extension of the meadow differs between seasons.
During the summer, the studied meadow extends from
5 m to a depth of 45 m (Y. Sharon pers. obs.), while dur-
ing the winter the meadow's lower limit only extends to
~30 m. This may be due to irradiance and/or tempera-
ture; the lowest irradiance in the Gulf of Aqaba is in Jan-
uary and the lowest temperature in March (Winters et al.
2006). The present study suggests that the plasticity in
the response to seasonal irradiance levels would allow
for the successful acclimation to the different depths as
observed throughout the year. This would then explain
the dynamic seasonal meadow extensions as based on
clonal growth, although *H. stipulacea* meadows were
found to be highly polymorphic between depths in the
Mediterranean (Procaccini et al. 1999).

On the cellular level, the fast (within 6 d) adjustment of
P_{max} to irradiance points towards rapid changes in the
carbon fixation reactions during the acclimation to high
or low midday irradiances. These changes could involve
key enzymes of the Calvin cycle, e.g. Rubisco, or other
reactions following those of the primary photochemistry.
The fact that P_{max} values in the transplanted seagrasses
became similar to those of the plants growing originally
at the transplant depths shows that these acclimations
are not only qualitative but also quantitative. The finding
that I_k values varied plastically in concord with P_{max} is
also in agreement with acclimation to high and low irra-
diances. While chl *a* + *b* content also showed plasticity,
the response in the plants transplanted from the high- to
the low-light environment was slower than in the low- to

high-light transplants, indicating a longer time is required for the production of new chlorophyll than its loss. The lack of changes in chl *a*:*b* ratios in all treatments suggests that this parameter has no role in *in situ* acclimation processes, such as reported by Durako et al. (2003) for 2 other *Halophila* species. While terrestrial shade-adapted plants often increase chl *b* contents relative to chl *a*, it may be that the spectral changes with depth prevent such a change in *H. stipulacea*.

In addition to the differences in photosynthetic responses at different irradiances, *Halophila stipulacea* has been reported to feature various leaf morphologies with depth (e.g. longer and wider leaves in deeper waters, Lipkin 1979). Also, changes in P_{max} and I_k along a depth gradient have been reported (Schwarz & Hellblom 2002). However, in neither case was it verified whether those changes could be due to acclimation processes or were the outcome of longer-term adaptations possibly resulting in ecotype formations. Olesen et al. (2002) have suggested that some seagrasses (i.e. *Cymodocea nodosa*) can acclimate and respond differently to changing light conditions than others (i.e. *Posidonia oceanica*). While *C. nodosa* displayed reduced light requirements with depth, both species acclimated mainly at the population structure level by reducing self-shading in deeper waters.

This is the first work reporting that *Halophila stipulacea*'s wide depth distribution across a broad range of irradiances can be explained by its plastic photosynthetic abilities allowing for successful acclimation both spatially and seasonally. While most of the differences between shallow (i.e. high-light) and deeper (low-light) growing plants measured in the present study follow classical sun- vs. shade-acclimation patterns, others do not. For the latter, the higher Y_0 (i.e. α) in shallow-growing plants and the stable chl *a*:*b* ratios may be a result of spectral changes along the depth gradient in the water column, which still need to be elucidated.

LITERATURE CITED

Beer S, Björk M (2000) Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated (PAM) fluorometry. Aquat Bot 66:69–76

Beer S, Waisel Y (1982) Effects of light and pressure on photosynthesis in two seagrasses. Aquat Bot 13:331–337

Durako MJ, Kunzelman JI, Kenworthy WJ, Hammerstrom WJ (2003) Depth-related variability in the photobiology of two populations of *Halophila johnsonii* and *Halophila decipiens*. Mar Biol 142:1219–1228

Lipkin Y (1979) Quantitative aspects of seagrass communities, particularly of those dominated by *Halophila stipulacea*, in Sinai (Northern Red Sea). Aquat Bot 7:119–128

Mata L, Silva J, Schuenhoff A, Santos R (2006) The effects of light and temperature on the photosynthesis of the *Asparagopsis armata* tetrasporophyte (*Falkenbergia rufolanosa*), cultivated in tanks. Aquaculture 252:12–19

Monro K, Poore AGB (2005) Light quantity and quality induce shade-avoiding plasticity in a marine macroalga. J Evol Biol 18:426–435

Moran R (1982) Formulas for determination of chlorophyllous pigments extracted with *N,N*-dimethylformamide. Plant Physiol 69:1376–1381

Olesen B, Enriquez S, Duarte CN, Sand-Jensen K (2002) Depth-acclimation of photosynthesis, morphology and demography of *Posidonia oceanica* and *Cymodocea nodosa* in the Spanish Mediterranean Sea. Mar Ecol Prog Ser 236: 89–97

Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J Mar Res 38:687–701

Procaccini G, Acunto S, Fama P, Maltagliati F (1999) Structural, morphological and genetic variability in *Halophila stipulacea* (Hydrocharitaceae) populations in the western Mediterranean. Mar Biol 135:181–189

Ralph PJ, Durako MJ, Enriquez S, Collier CJ, Doblin MA (2007) Impact of light limitation on seagrasses. J Exp Mar Biol Ecol 350:176–193

Saroussi S, Beer S (2007) Alpha and quantum yield of aquatic plants derived from PAM fluorometry: uses and misuses. Aquat Bot 86:89–92

Schwarz AM, Hellblom F (2002) The photosynthetic light response of *Halophila stipulacea* growing along a depth gradient in the Gulf of Aqaba, the Red Sea. Aquat Bot 74:263–272

Sharon Y, Beer S (2008) Diurnal movements of chloroplasts in *Halophila stipulacea* and their effect on PAM fluorometric measurements of photosynthetic rates. Aquat Bot 88:273–276

Silva J, Santos R (2003) Daily variation patterns in seagrass photosynthesis along a vertical gradient. Mar Ecol Prog Ser 257:37–44

Sultan SE (2000) Phenotypic plasticity for plant development, function and life history. Trends Plant Sci 5: 537–542

Winters G, Loya Y, Beer S (2006) *In situ* measured seasonal variations in F_v/F_m of two common Red Sea corals. Coral Reefs 25:593–598