Accepted Manuscript

Title: Light availability and temperature, not increased CO₂, will structure future meadows of *Posidonia oceanica*

Authors: Iris E. Hendriks, Ylva S. Olsen, Carlos M. Duarte

PII: S0304-3770(17)30048-7
DOI: <http://dx.doi.org/doi:10.1016/j.aquabot.2017.02.004>
Reference: AQBOT 2942

To appear in: *Aquatic Botany*

Received date: 16-5-2016
Revised date: 10-2-2017
Accepted date: 10-2-2017

Please cite this article as: Hendriks, Iris E., Olsen, Ylva S., Duarte, Carlos M., Light availability and temperature, not increased CO₂, will structure future meadows of *Posidonia oceanica*. *Aquatic Botany* <http://dx.doi.org/10.1016/j.aquabot.2017.02.004>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Light availability and temperature, not increased CO₂, will structure future meadows of *Posidonia oceanica*

Iris E. Hendriks^{1*}, Ylva S. Olsen², Carlos M. Duarte^{1,3}

¹ Global Change Department, IMEDEA (CSIC-UIB), Instituto Mediterráneo de Estudios Avanzados, C/ Miquel Marqués 21, 07190 Esporles (Mallorca), Spain

² The UWA Oceans Institute and School of Biological Sciences, The University of Western Australia, 35 Stirling Highway, Crawley 6009, Australia

³ King Abdullah University of Science and Technology (KAUST), Red Sea Research Center, Thuwal 23955-6900, Saudia Arabia

* **Correspondence:** Iris E. Hendriks, Global Change Department, IMEDEA (CSIC-UIB), Instituto Mediterráneo de Estudios Avanzados, C/ Miquel Marqués 21, 07190 Esporles (Mallorca), Spain. iris@imedea.uib-csic.es

Highlights

- Temperature negatively affects growth of *P. oceanica* in short-term experiments
- Photosynthesis does not differ in high CO₂ treatments after short-term exposure
- Light, more than CO₂ or temperature, is a critical factor for photosynthesis

Abstract

We evaluated the photosynthetic performance of *Posidonia oceanica* during short-term laboratory exposures to ambient and elevated temperatures (24-25 °C and 29-30 °C)

warming and $p\text{CO}_2$ (380, 750 and 1000 ppm $p\text{CO}_2$) under normal and low light conditions (200 and 40 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ respectively). Plant growth was measured at the low light regime and showed a negative response to warming. Light was a critical factor for photosynthetic performance, although we found no evidence of compensation of photosynthetic quantum efficiency in high light.. Relative Electron Rate Transport (rETR_{max}) was higher in plants incubated in high light, but not affected by $p\text{CO}_2$ or temperature. The saturation irradiance (I_k) was negatively affected by temperature. We conclude that elevated CO_2 does not enhance photosynthetic activity and growth, in the short term for *P. oceanica*, while temperature has a direct negative effect on growth. Low light availability also negatively affected photosynthetic performance during the short experimental period examined here. Therefore increasing concentrations of CO_2 may not compensate for predicted future conditions of warmer water and higher turbidity for seagrass meadows.

Keywords: Seagrass; Global Warming; Temperature; Ocean Acidification; Light Limitation

1. Introduction

Climate change, in particular warming, is emerging as a challenge to seagrasses worldwide (Short and Neckles, 1999; Waycott et al., 2009; Marbà and Duarte, 2010) and predicted increases in dissolved CO_2 can have significant and direct effects on marine plant communities (Short and Neckles, 1999), but the responses of different plants depend on their degree of carbon limitation and on carbon uptake mechanisms.

Seagrass photosynthesis is frequently limited by the availability of dissolved inorganic carbon (DIC) under natural conditions, in particular in areas of high light availability (Beer and Koch, 1996; Madsen et al., 1996). Experimental evidence (e.g. Jiang et al. 2010; Ow et al. 2015) indicated that marine plants increase photosynthetic rates under elevated CO₂ concentrations (Koch et al. 2013; Brodie et al. 2014). The increased photosynthetic activity with increasing CO₂ concentrations translates into an increase growth and biomass (Zimmerman et al., 1997). However plant response might change from species to species (Apostolaki et al., 2014) and might vary according to the duration of the exposure. Long-term responses are likely to depend on the degree of physiological and morphological acclimation to increased CO₂ (Drake, 1992; Dahlman, 1993), especially when associated with an increase in temperature. Chronic exposure to elevated temperatures, for example, changes physiological processes (Campbell et al., 2006) leading to a decrease in the photosynthesis: respiration ratios (Marsh Jr et al., 1986). It will also affect critical life history events such as reproduction (Diaz-Almela et al., 2007) as flowering intensity and seed production in seagrass meadows are correlated to sea temperature, with increasing reproduction at higher temperatures (Van Vierssen et al., 1984; Hootsmans et al., 1987). Second, increased amplitude and duration of heat waves as predicted by the IPCC (2014) will enhance shoot mortality and trigger population decline when critical temperature thresholds are exceeded (Marbà and Duarte, 2010; Jordà et al., 2012). These changes will affect the growth and distribution of seagrasses and may lead to the local extinction or displacement of species with low thermal tolerance (Short and Neckles, 1999). The Mediterranean Sea is particularly vulnerable to warming as temperature increases faster compared to the global ocean (Vargas-Yáñez et al., 2010; Calvo et al., 2011) and model projections forecast an increase in summer sea surface temperatures in excess of 4–5 °C in the western

Mediterranean (Sánchez et al., 2004; Jordà et al., 2012). This gradual increase in seawater temperature, coupled with the predicted increase in the frequency of heat waves, is expected to have a strong impact on the long term persistence of Mediterranean seagrass meadows (Jordà et al. 2012). The expected decrease in meadow cover and shoot density is enhanced by additional, non-climatic causes of meadow loss, especially in coastal, shallow areas. Seagrass beds require, on average, 2.4 mol photons $\text{m}^{-2} \text{d}^{-1}$ to survive (Gattuso et al., 2006) and underwater irradiance sets their depth distribution (Duarte, 1991), composition and morphology (Short and Wyllie-Echeverria, 1996). High turbidity associated with sedimentation and eutrophication has been suggested as a main cause of seagrass decline in coastal shallow waters (Cambridge et al., 1986; Erftemeijer et al., 2006), although plants might be able to enhance light harvesting efficiencies through photo-acclimation (Olesen and Sand-Jensen, 1993; Abal et al., 1994; Lee et al., 2007).

Here, we experimentally evaluate the effect of temperature and $p\text{CO}_2$ on growth of *P. oceanica* shoots during short-term laboratory incubations and light availability and temperature and $p\text{CO}_2$ on photosynthetic efficiency. Light reduction can be indicated by photosynthetic measures like rETR (relative Electron Transport Rate) and the irradiance where the P-I curve reaches the light-saturated photosynthetic rate (rETR_{max}), denominated I_k ($\text{rETR}_{\text{max}}/\alpha$) as well as leaf growth (McMahon et al 2013). We expected a higher photosynthetic rate (as rETR) in higher CO_2 treatments to compensate for the effect of low light intensities. Seagrass growth, biomass, and measurements of I_k are also predicted to respond positively to ocean acidification (e.g. Jiang et al. 2010). We discuss whether observed effects of temperature, light and CO_2 on photosynthetic efficiency could affect the energy balance and/or depth distribution of *P. oceanica* in a future warmer and more acidic Mediterranean Sea compared to present day conditions.

2. Methods

We performed experiments evaluating the response of *Posidonia oceanica* to increased temperature and $p\text{CO}_2$ and reduced light. The set-up consisted of treatments simulating factorial combinations of present and future temperature and atmospheric $p\text{CO}_2$ levels (IPCC, 2007) at two light conditions. We bubbled seawater collected from the bay of Palma with gas with ambient $p\text{CO}_2$ values (ca. 380 ppm $p\text{CO}_2$), medium (ca. 750 ppm) and high-end scenario $p\text{CO}_2$ (ca. 1000 ppm $p\text{CO}_2$) to simulate the range of conditions predicted for the end of the century. These were combined factorially with two temperatures, the ambient temperature at the time of collection (24 - 25 °C) and 4 - 6°C warmer (29 – 30 °C), simulating future warming scenarios. The warm temperature treatments are 0.6 to 1.6 °C above the maximum summer temperatures averaged for the period 2008-2013 in the bay of Palma (28.4 ± 0.34 °C for Magalluf, N. Marbà pers. comm). The experiments were done in a temperature-controlled room and each temperature treatment was achieved by placing the aquaria in tanks with circulating water in which programmed thermostats were placed.

We used three replicate 6 L-aquaria per treatment. Plants and seawater were collected from the Bay of Palma. In each aquarium 3 - 10 shoots of *Posidonia oceanica* with a part of their rhizome attached were inserted. The cut end of the horizontal rhizome of each plant was sealed using a non-toxic polyvinylsiloxane silicone elastomer to maintain gas pressure inside the rhizome.

Incubations lasted 1-3 weeks and aquaria were illuminated by two T5 lamps with two 54-W fluorescent bulbs set to a photoperiod of 12:12 h and delivering incident PAR light levels around 40 μmol (low light treatment) or 200 μmol (high light treatment) photons $\text{m}^{-2} \text{s}^{-1}$ at the seagrass canopy.

Measurements of the carbonate system

The partial pressure of CO₂ was adjusted to the desired level by bubbling with an air-CO₂ mixture. Atmospheric air was first scrubbed by soda lime to remove all CO₂ then mixed with pure CO₂ from a bottle using mass flow controllers (MFCs; AALBORG GFC-17). To achieve present-day and future *p*CO₂ levels, gases were mixed to 380, 750 and 1000 ppm *p*CO₂ in mixing bottles (filled with marbles to increase the surface area) and gently bubbled from the base of each tank with a neoprene bubble curtain. pH was continuously monitored and recorded at 1 minute intervals using Metrohm pH sensors calibrated using 4.0, 7.0 and 10.0 pH_{NBS} standards and connected to a D130 and D230 data logger (Consort) and computer. Due to a limited number of pH sensors, not all replicates could be measured simultaneously. We therefore measured one replicate per treatment for one week, after which, the pH electrodes were recalibrated and inserted in another (randomly chosen) replicate. Experimental duration was in total 1-3 weeks, with one week of pH data for each replicate tank. Periodically, pH_T was evaluated by the spectrophotometric method (Dickson 1993, Spectrophotometer JASCO 7800) to verify accuracy of the electrodes. Samples for total alkalinity (TA) analyses were collected every week from the aquaria. Seawater was poisoned with 0.02 ml of saturated solution of HgCl₂ (Merck, Analar) to avoid biological alteration. TA was determined by open cell titration as described in Dickson 2007 with a Metrohm Titrando 808, with Tiamo™ titration software 2.2. Certified CO₂ seawater reference material (CRM Batch 101) from the Andrew Dickson lab at UC San Diego was used to evaluate the accuracy of the results. The remaining parameters of the carbonate system (bicarbonate, carbonate, saturation states for aragonite and calcite) were calculated from pH_{NBS}, temperature and total

alkalinity values using the CO2SYS program (Pierrot et al. 2006), with dissociation constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and HSO_4^- using Dickson (1990).

Measurements of responses

Growth under light limited conditions was measured by the hole punch method (Short and Duarte, 2001). Briefly, at the beginning of the experiment, a hole was punched in each shoot at the height of the meristem using a hypodermic needle. Growth of each leaf was measured at the end of the incubation as the distance from the meristem to the hole.

Photosynthetic efficiency was evaluated by Pulse Amplitude Modulated fluorometry (Diving PAM, from Walz, Germany). We measured electron transport rate (ETR) under a series of increasing light intensities at intervals of 10s to produce Rapid Light Curves (RLCs). Relative ETR (rETR) was calculated as $\text{rETR} = \text{Yield} \times \text{PAR} \times 0.5$. We used rETR, as we did not have a reliable measure of absorptance factor (AF), however, as all plants came from the same location, they should be comparable. We fitted recorded rETR to PAR in RStudio (v0.99.902) according to the model of Eilers and Peeters (1988) to obtain model constants b_0 , b_1 and b_2 as:

$$rETR = \frac{1}{(b_0 \times PAR^2 + b_1 \times PAR + b_2)}$$

We subsequently calculated $\text{rETR}_{\max}$ as $\frac{1}{b_1 + 2\sqrt{b_0 \times b_2}}$, and $\alpha = 1/b_2$ and $I_k = \frac{b_2}{b_1 + 2\sqrt{b_0 \times b_2}}$.

Effects of temperature, light and $p\text{CO}_2$ on photosynthetic parameters were analysed with linear mixed-effects models (lmer package) in RStudio (v0.99.902), using aquaria as random factor and including all interactions. Data were log transformed when not

directly normally distributed. Growth data was only available for the light limited treatment, and was evaluated with a simple linear model (lm).

3. Results

Growth

There was no effect of week of measurement (1, 2, or 3: $t=-1.15$, $p=0.27$) on growth at limited light levels. Growth rates decreased significantly with increasing temperature ($t=-2.14$, $p<0.05$), but were not affected by $p\text{CO}_2$ ($t=-1.23$, $p=0.24$; Figure 1).

Photosynthetic efficiency

The Yield of the plants did not change with temperature ($F=0.02$, $p=0.90$), $p\text{CO}_2$ ($F=0.17$, $p=0.84$), or with light ($F=1.40$, $p=0.25$). Log transformed rETR_{max} was affected by light regime ($F=5.60$, $p<0.05$), with higher rETR_{max} under saturating light conditions. Relative ETR_{max} was, however, not affected by temperature ($F=0.03$, $p=0.88$), or $p\text{CO}_2$ ($F=1.34$, $p=0.28$) and there were no significant interactions among factors, see Table 1. The saturation irradiance (I_k), increased with increasing temperature ($F=4.16$, $p<0.05$) as expected, but did not differ among $p\text{CO}_2$ levels ($F=0.06$, $p=0.94$) or light ($F=1.05$, $p=0.31$). We expected α to be affected by light, however there was only a trend visible ($F=3.81$, $p=0.059$) and contrary to expectations α decreased, not increased, for lower light conditions, therefore no compensation of apparent photosynthetic quantum efficiency was observed between our light treatments. This lower α is mainly due to the lower values in the ambient temperature treatments under saturating light conditions (Table 1). There was no change in α with temperature ($F=3.08$, $p=0.09$), or $p\text{CO}_2$ ($F=2.41$, $p=0.11$).

Discussion

Few long-term experimental studies have been published up to date on the effect of OA on seagrasses, as keeping large clonal plants long under controlled conditions is difficult, and so is maintaining the experimental treatment conditions as plant photosynthesis affects CO₂ levels. In a 5-month long experiment with *Zostera noltii* Alexandre et al. (2012) showed that both the maximum photosynthetic rate (P_m) and photosynthetic efficiency (α) were higher (1.3- and 4.1-fold, respectively) in plants exposed to CO₂-enriched conditions. However, no significant effects of CO₂ enrichment on leaf growth rates were observed, probably due to nitrogen limitation as revealed by the low nitrogen content of leaves. The leaf nitrate uptake rate of plants exposed to CO₂-enriched conditions was four-fold lower than the uptake of plants exposed to current CO₂ level. In many terrestrial C3 plants lower leaf nitrogen content has been demonstrated when grown at higher CO₂ concentrations (Peterson et al. 1999), which may hold for *P. oceanica*, being a C3 species as well (Pergent et al. 2014). Not all seagrasses are C3 plants and (Koch et al. 2013, Pergent et al. 2014) it is possible that effects of CO₂ on seagrass production may depend on the specific nitrogen availability in each system.

In our short-term experiment, nutrient limitation is unlikely to have played a major role. Yet, we did not observe any effect of $p\text{CO}_2$, on growth or photosynthetic performance of the plant. Growth was negatively affected by increasing temperature, as shown in the past (Olsen et al., 2012) and as predicted for *Posidonia oceanica* meadows under future warming scenarios (Jordà et al., 2012). As we did not measure growth under high-light conditions it is not possible to compare growth between light regimes. Additional insight into OA effects on seagrasses has come from examining populations growing in natural CO₂ vents (Hall-Spencer et al., 2008; Fabricius et al 2011; Porzio et

al., 2011). These results should be interpreted with caution as the pH at the sites can be highly variable and cannot be controlled, the areas are open to influences from non-OA sites (e.g. recruitment) and the historical biochemistry is not completely understood (Koch et al., 2013). Nonetheless, these studies consistently show loss of crustose coralline algal epiphytes on seagrass leaves, and greater seagrass density close to seeps with a lower pH (Hall-Spencer et al., 2008; Martin et al., 2008; Porzio et al., 2011). Lower epiphyte load can have positive consequences for seagrasses as it reduces shading and competition for nutrients from epiphytes. Vent site studies have failed to conclusively show up- or down regulation of photosynthesis in seagrass by elevated CO₂. For example, no differences in rETR_{max} or Fv/Fm were found between control and acidified vent sites (Fabricius et al., 2011). Higher CO₂ availability should in theory reduce the light requirements of seagrasses (Zimmerman et al., 1997), however we have not found an indication that in short-term experiments this effect is sufficient to compensate for low light regimes. The low light treatment supplied 3.46 mol photons m⁻² day⁻¹, while the high light treatment delivered 17.28 mol photons m⁻² day⁻¹, both above the average daily compensation irradiance for seagrasses of 2.4 mol photons m⁻² day⁻¹ (Gattuso et al., 2006), and well over the minimum light requirements of *P. oceanica*, ranging from 0.1 to 2.8 mol photons m⁻² day⁻¹ (Gattuso et al., 2006). The low light treatment (40 μmol photons m⁻² s⁻¹) provided significantly less light than what is typical for *P. oceanica* meadows in the natural environment, ranging from higher end values of approximately 700 μmol photons m⁻² s⁻¹ in summer for shallow depths, to around 100-150 μmol photons m⁻² s⁻¹ in deeper areas and winter conditions (Dattolo et al., 2014). In summary, elevated CO₂ did not seem to enhance short-term photosynthetic activity or growth for *P. oceanica*, while warming had a direct negative effect on growth. Light limitation also affected photosynthetic performance during the short

experimental period tested here. Future meadows of *P. oceanica* are therefore likely to be structured by light availability and temperature more than increased $p\text{CO}_2$. The possible benefits to seagrasses from increased $p\text{CO}_2$ may thus not be able to compensate for predicted future conditions of warming and for lower light levels associated with increased turbidity.

Acknowledgements

This research was supported by the MedSeA project (www.medsea-project.eu, contract number 265103 of the Framework Program 7 of the European Union), and ESTRESX (ref. CTM2012-32603), funded by the Spanish Ministry of Economy and Competitiveness. I.E.H. was supported by a JAE-DOC fellowship (CSIC, Spain). YSO was funded by a Marie Curie IEF from the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement no. 254297: FP7-PEOPLE-2009-IEF.

References

- Abal, E., Loneragan, N., Bowen, P., Perry, C., Udy, J., Dennison, W., 1994. Physiological and morphological responses of the seagrass *Zostera capricorni* Aschers, to light intensity. *Journal of Experimental Marine Biology and Ecology* 178, 113-129.
- Alexandre, A., Silva, J., Buapet, P., Bjork, M., Santos, R., 2012. Effects of CO_2 enrichment on photosynthesis, growth, and nitrogen metabolism of the seagrass *Zostera noltii*. *Ecology and Evolution* 2, 2620-2630.

- Apostolaki, E., Vizzini, S., Hendriks, I., Olsen, Y., 2014. Seagrass ecosystem response to long-term high CO₂ in a Mediterranean volcanic vent. *Marine environmental research* 99, 9-15.
- Beer, S., Koch, E.W., 1996. Photosynthesis of marine macroalgae and seagrass in globally changing CO₂ environments. *Marine Ecology Progress Series* 141, 199-204.
- Calvo, E., Simó, R., Coma, R., Ribes, M., Pascual, J., Sabatés, A., Gili, J., Pelejero, C., 2011. Effects of climate change on Mediterranean marine ecosystems: the case of the Catalan Sea. *Clim. Res.* 50, 1-29.
- Cambridge, M., Chiffings, A., Brittan, C., Moore, L., McCOMB, A.J., 1986. The loss of seagrass in Cockburn Sound, Western Australia. II. Possible causes of seagrass decline. *Aquatic Botany* 24, 269-285.
- Campbell, S.J., McKenzie, L.J., Kerville, S.P., 2006. Photosynthetic responses of seven tropical seagrasses to elevated seawater temperature. *Journal of Experimental Marine Biology and Ecology* 330, 455-468.
- Dahlman, R.C., 1993. CO₂ and plants: Revisited. *Vegetatio* 104/105, 339-355.
- Dattolo, E., Ruocco, M., Brunet, C., Lorenti, M., Lauritano, C., D'Esposito, D., De Luca, P., Sanges, R., Mazzuca, S., Procaccini, G., 2014. Response of the seagrass *Posidonia oceanica* to different light environments: Insights from a combined molecular and photo-physiological study. *Mar. Environ. Res.* 101, 225-236.
- Diaz-Almela, E., Marbà, N., Duarte, C.M., 2007. Consequences of Mediterranean warming events in seagrass (*Posidonia oceanica*) flowering records. *Global Change Biology* 13, 224-235.

- Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep-Sea Research* 34, 1733–1743
- Dickson, A.G., 1990. Standard potential of the reaction: $\text{AgCl(s)} + \frac{1}{2}\text{H}_2\text{(g)} = \text{Ag(s)} + \text{HCl(aq)}$, and the standard acidity constant of the ion HSO_4^- in synthetic sea water from 273.15 to 318.15 K. *J. Chem. Thermodyn.* 22: 113–127
- Dickson, A.G., 1993. The measurement of sea water pH. *Mar. Chem.* 44: 131–142.
- Dickson, A.G., Sabine, C.L. and Christian, J.R., (Eds.) 2007. Guide to Best Practices for Ocean CO₂ Measurements. PICES Special Publication 3, 191 pp.
- Drake, B.G., 1992. The impact of rising CO₂ on ecosystem production. In: Wisniewski, J., Lugo, A.E. (Eds.), *Natural Sinks of CO₂*. Kluwer Academic Press, Dordrecht, pp. 25-44.
- Duarte, C.M., 1991. Seagrass Depth Limits. *Aquatic Botany* 40, 363-377.
- Eilers, P.H.C. and Peeters, J.C.H., 1988. A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton. *Ecological modelling*, 42(3), 199-215.
- Erftemeijer, P.L., Lewis III, R., Roy, R., 2006. Environmental impacts of dredging on seagrasses: A review. *Marine Pollution Bulletin* 52, 1553-1572.
- Fabircius, K.E., Langdon, C., Uthicke, S., Humphrey, C., Noonan, S., De'ath, G., Okazaki, R., Muehllehner, N., Glas, M.S., Lough, J.M., 2011. Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. *Nature Climate Change* 1, 165-169.
- Gattuso, J.P., Gentili, B., Duarte, C. M., Kleypas, J. A., Middelburg, J. J., Antoine, D., 2006. Light availability in the coastal ocean: impact on the distribution of

- benthic photosynthetic organisms and their contribution to primary production. *Biogeosciences* 3, 489-513.
- Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S.M., Rowley, S.J., Tedesco, D., Buia, M.-C., 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* 454, 96-99.
- Hootsmans, M., Vermaat, J., Van Vierssen, W., 1987. Seed-bank development, germination and early seedling survival of two seagrass species from The Netherlands: *Zostera marina* L. and *Zostera noltii* hornem. *Aquatic Botany* 28, 275-285.
- IPCC, 2007, Climate Change 2007. The Physical Science Basis. Working Group I Contribution to the Fourth Assessment Report of the IPCC Intergovernmental Panel on Climate Change. , Cambridge University Press, Cambridge.
- IPCC, 2014, Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: Barros, V.R., C.B. Field, D.J. Dokken, M.D. Mastrandrea, K.J. Mach, T.E. Bilir, M. Chatterjee, K.L. Ebi, Y.O. Estrada, R.C. Genova, B. Girma, E.S. Kissel, A.N. Levy, S. MacCracken, P.R. Mastrandrea, White, L.L. (Eds.), Cambridge, United Kingdom and New York, NY, USA, p. 688.
- Jiang, Z.J., Huang, X.-P., Zhang, J.-P., 2010. Effects of CO₂ Enrichment on Photosynthesis, Growth, and Biochemical Composition of Seagrass *Thalassia hemprichii* (Ehrenb.) Aschers. *Journal of Integrative Plant Biology* 52, 904-913.
- Jordà, G., Marbà, N., Duarte, C.M., 2012. Mediterranean seagrass vulnerable to regional climate warming. *Nature Climate Change* 2, 821–824.

- Koch, M., Bowes, G., Ross, C., Zhang, X.-H., 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology* 19, 103-132.
- Lee, K.-S., Park, S.R., Kim, Y.K., 2007. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A review. *Journal of Experimental Marine Biology and Ecology* 350, 144-175.
- Madsen, T.V., Maberly, S.C., Bowes, G., 1996. Photosynthetic acclimation of submersed angiosperms to CO₂ and HCO₃⁻. *Aquatic Botany* 53, 15-30.
- Marbà, N., Duarte, C.M., 2010. Mediterranean warming triggers seagrass (*Posidonia oceanica*) shoot mortality. *Global Change Biology* 16, 2366-2375.
- Marsh Jr, J.A., Dennison, W.C., Alberte, R.S., 1986a. Effects of temperature on photosynthesis and respiration in eelgrass (*Zostera marina* L.). *Journal of Experimental Marine Biology and Ecology* 101, 257-267.
- Martin, S., Rodolfo-Metalpa, R., Ransome, E., Rowley, S., Buia, M.-C., Gattuso, J.-P., Hall-Spencer, J., 2008. Effects of naturally acidified seawater on seagrass calcareous epibionts. *Biol. Lett.* 4, 689-692.
- McMahon, K., Collier, C., Lavery, P.S., 2013. Identifying robust bioindicators of light stress in seagrasses: A meta-analysis. *Ecological Indicators* 30, 7-15.
- Mehrbach, C., Culbertson, C.H., Hawley, J.E., Pytkowicz, R.N., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography* 18, 897-907.
- Porzio, L., Buia, M.C., Hall-Spencer, J.M., 2011. Effects of ocean acidification on macroalgal communities. *Journal of Experimental Marine Biology and Ecology* 400, 278-287.

- Olesen, B., Sand-Jensen, K., 1993. Seasonal acclimatization of eelgrass *Zostera marina* growth to light. Marine Ecology Progress Series 94, 91-99.
- Olsen, Y.S., Sánchez-Camacho, M., Marbà, N., Duarte, C.M., 2012. Mediterranean Seagrass Growth and Demography Responses to Experimental Warming. Estuaries and Coasts 35, 1205-1213.
- Ow, Y.X., Collier, C.J. and Uthicke, S., 2015. Responses of three tropical seagrass species to CO₂ enrichment. Marine Biology, 162, 1005-1017.
- Pierrot, D., Lewis, E., Wallace, D.W.R., 2006. MS Excel Program Developed for CO₂ System Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee. doi: 10.3334/CDIAC/otg.CO2SYS_XLS_CDIAC105a
- Sánchez, E., Gallardo, C., Gaertner, M., Arribas, A., Castro, M., 2004. Future climate extreme events in the Mediterranean simulated by a regional climate model: a first approach. Global and Planetary Change 44, 163-180.
- Short, F.T., Duarte, C.M., 2001. Methods for the measurement of seagrass growth and production. Global seagrass research methods, 155-182.
- Short, F.T., Neckles, H.A., 1999. The effects of global climate change on seagrasses. Aquatic Botany 63, 169-196.
- Short, F.T., Wyllie-Echeverria, S., 1996. Natural and human-induced disturbance of seagrasses. Environ. Conserv. 23, 17-27.
- Van Vierssen, W., Van Kessel, C., Van Der Zee, J., 1984. On the germination of *Ruppia* taxa in western Europe. Aquatic Botany 19, 381-393.
- Vargas-Yáñez, M., Moya, F., García-Martínez, M., Tel, E., Zunino, P., Plaza, F., Salat, J., Pascual, J., López-Jurado, J.L., Serra, M., 2010. Climate change in the Western Mediterranean sea 1900–2008. Journal of Marine Systems 82, 171-176.

- Waycott, M., Duarte, C.M., Carruthers, T.J.B., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck, J., K.L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Short, F.T., Williams, S.L., 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. *Proc. Natl. Acad. Sci. U. S. A.* 106, 12377-12381.
- Zimmerman, R.C., Kohrs, D.G., Steller, D.L., Alberte, R.S., 1997. Impacts of CO₂ Enrichment on Productivity and Light Requirements of Eelgrass. *Plant Physiology* 115, 599-607.

Figure legends

Figure 1. Daily leaf growth (mm day⁻¹) at ambient $p\text{CO}_2$ (green diamonds), intermediate (blue squares) and high $p\text{CO}_2$ (red triangles) treatments. Error bars are Standard Error of $n=3$ shoots.

Table 1. Average Yield ($\Delta F/F_m'$), ETR_{max} ($\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$), α (photosynthetic quantum efficiency), and I_k (saturation irradiance in $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$) of three replicate shoots per aquarium and standard error (SE) for two light conditions, three $p\text{CO}_2$ treatments and two temperature treatments.

Light	$p\text{CO}_2$	Temperature	Yield	SE	$rETR_{max}$	SE	α	SE	I_k	SE
Saturating	High	High	0.75	0.01	21.9	3.21	0.61	0.03	37.2	5.49
		Ambient	0.74	0.01	22.6	2.32	0.57	0.04	40.9	3.33
	Control	High	0.74	0.01	19.3	1.62	0.54	0.02	36.4	2.87
		Ambient	0.76	0.01	22.6	2.26	0.65	0.10	41.0	3.57
Limited	High	High	0.75	0.01	20.2	2.18	0.59	0.04	34.6	2.33
		Ambient	0.77	0.00	19.1	1.02	0.59	0.05	35.0	1.99
	Intermediate	High	0.76	0.00	21.2	2.54	0.62	0.03	36.2	5.52
		Ambient	0.74	0.01	16.9	2.08	0.49	0.04	35.4	2.71
	Control	High	0.75	0.01	15.4	1.22	0.52	0.03	29.8	1.51
		Ambient	0.76	0.01	16.6	2.12	0.44	0.04	37.7	2.19