



Short-term growth and biomechanical responses of the temperate seagrass *Cymodocea nodosa* to CO₂ enrichment

Carmen B. de los Santos^{1,*}, Jasmin A. Godbold^{2,3}, Martin Solan²

¹Centre of Marine Sciences (CCMAR), University of Algarve – Campus de Gambelas, 8005-139 Faro, Portugal

²Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton, Waterfront campus, European Way, Southampton, SO14 3ZH, UK

³Biological Sciences, University of Southampton, Life Sciences Building, Highfield campus, Southampton, SO17 1BJ, UK

ABSTRACT: Seagrasses are often regarded as climate change ‘winners’ because they exhibit higher rates of photosynthesis, carbon fixation and growth when exposed to increasing levels of ocean acidification. However, questions remain whether such growth enhancement compromises the biomechanical properties of the plants, altering their vulnerability to structural damage and leaf loss. Here, we investigated the short-term (6 wk) effects of decreasing pH by CO₂ enrichment on the growth, morphology and leaf-breaking force of the temperate seagrass *Cymodocea nodosa*. We found that the plant biomass balance under levels of acidification representative of short-term climate change projections (pH 8.04) was positive and led to an increase in leaf abundance in the shoots. However, we also found that plant biomass balance was negative under levels of acidification experienced presently (pH 8.29) and those projected over the long-term (pH 7.82). Leaf morphology (mean leaf length, thickness and width) was invariant across our imposed acidification gradient, although leaves were slightly stronger under [CO₂] representative of short-term climate change. Taken together, these findings indicate that a subtle increase in growth and mechanical resistance of *C. nodosa* is likely to occur following short- to medium-term changes in ocean chemistry, but that these positive effects are unlikely to be maintained over the longer term. Our study emphasises the need to account for the interdependencies between environmental conditions and variations in multiple aspects of the structure and functioning of seagrass communities when considering the likely consequences of climate change.

KEY WORDS: Biomechanics · Breaking force · Structural change · Ocean acidification · Carbon dioxide · Response traits · Growth · *Cymodocea nodosa*

INTRODUCTION

Ocean pH levels are predicted to decrease by approximately 0.4 units by 2100 due to increasing anthropogenic CO₂ partial pressure in the seawater (Stocker et al. 2013), and there is a clear consensus that ocean acidification will form a major challenge for marine biota and ecosystems (Fabry et al. 2008). However, experimental manipulations of ocean acidification reveal that species responses take time to

develop and to be expressed (Form & Riebesell 2012, Godbold & Solan 2013) and that there are significant variations in the vulnerability of species, ranging from negative responses (e.g. growth, calcification, reproduction and survival), through resistance, to positive effects (growth, photosynthesis) in some non-calcifying species (Kroeker et al. 2010, 2013). For marine macrophytes, the increased availability of bicarbonate (HCO₃⁻) and dissolved inorganic carbon commonly enhances primary productivity and

*Corresponding author: carmen.bdelossantos@gmail.com

growth (reviewed by Koch et al. 2013), providing that there is sufficient light and nutrients. Under these circumstances, seagrass communities can modify the carbonate chemistry of the entire local environment and, in turn, influence species–environment interactions. For instance, seagrasses indirectly protect associated invertebrate communities from exposure to acidification by buffering changes in ocean chemistry, as reported for tropical seagrass communities (Unsworth et al. 2012) and Mediterranean *Posidonia oceanica* meadows (Garrard et al. 2014, Hendriks et al. 2014).

Growth enhancement by ocean acidification may involve associated responses in the organisms, such as the alteration of mechanical properties, although this remains a less prominent area of research. Mechanical resistance is an important feature in protecting marine plants from biotic (herbivory, e.g. Vergés et al. 2007) and abiotic forcing (drag, e.g. de los Santos et al. 2016a). The force needed to abscise the leaf at the ligule, the joint between the leaf sheath and blade in most seagrass species, is an important leaf trait that retains the structural integrity of the plant. Leaf shedding is ultimately controlled by the balance of the leaf-breaking force at the abscission area and an external environmental force, such as hydrodynamic forces associated with the action of waves and currents, especially during storms. Yet, studies on the effects of ocean acidification on the mechanical properties of marine flora are scarce, despite expectations that the severity and frequency of storms will increase with climate change. The few studies that have been conducted have predominantly focussed on calcified algae (e.g. Ragazzola et al. 2012, Newcomb et al. 2015), but the extent to which the biomechanics of seagrasses respond to ocean acidification has not been fully investigated.

There are reasons, however, to expect changes in the mechanical properties of seagrass leaves in acidified waters. First, seagrasses show considerable phenotypic plasticity in the mechanical traits of leaves, allowing adjustments to their physical environment and nutrient or light supply (Kopp 1999, La Nafie et al. 2012, 2013, de los Santos et al. 2013, 2016a). Secondly, high levels of carbon fixation associated with elevated $[\text{CO}_2]$ lead to both an increased C:N ratio and an increased concentration of non-structural carbohydrates in seagrasses that have to be relocated by the plant (Jiang et al. 2010). Similarly, at elevated $[\text{CO}_2]$, excess carbon may be available, which may allow individual plants to invest in structural carbon-based components allocated to the cell wall constituting the fibre bundles, which are a major sink for car-

bohydrates. The allocation of supporting tissues in the leaf contributes substantially to its material strength (de los Santos et al. 2016a), which determines, along with the leaf cross-sectional area, the leaf-breaking force (i.e. the maximum capacity of a leaf to withstand a physical force), a trait reflecting the tolerance of plants against mechanical damage (Niklas 1992). Hence, under elevated $[\text{CO}_2]$, seagrasses may experience structural reinforcement of the leaves that is associated with growth enhancement and of a magnitude sufficient to increase their tolerance to physical forces. Thus, physiological and morphological acclimation of seagrasses to elevated $[\text{CO}_2]$ has the potential to alter plant physical performance and/or to modify entry to the food web through herbivory and/or decomposition (Read & Stokes 2006).

Here, we assessed the short-term response (6 wk) in growth, leaf morphology, and leaf-breaking force of the seagrass *Cymodocea nodosa* (Ucria) Ascherson to elevated $[\text{CO}_2]$. We also examined the correlation between leaf growth and breaking force responses to investigate whether growth enhancement compromises plant biomechanical properties.

MATERIALS AND METHODS

Test species and plant material

Cymodocea nodosa is a small, fast-growing species with low storage capacity and high turnover time (Duarte 1991). It also exhibits high intra-specific variability in its leaf morphology and mechanical traits (de los Santos et al. 2013) as well as in its physiology (e.g. Olivé et al. 2013). In addition, *C. nodosa* shows rapid growth responses to a wide range of environmental drivers within a time frame of several weeks (e.g. nutrients, Pérez et al. 1991; light, Malta et al. 2006; salinity, Pagès et al. 2010), including CO_2 -induced pH reduction (Invers et al. 2001, Tomas et al. 2015).

C. nodosa plants were collected by hand from a continuous intertidal meadow in the inner Cádiz Bay (southern Spain, 36.48° N, 6.26° W) on 15 October 2012. Cádiz Bay is a sheltered shallow tidal lagoon (3 m mean water depth) dominated by unidirectional flows, and is abundantly covered by benthic macrophytes (de los Santos et al. 2013). Mean annual water temperature and salinity in the bay are 19.3°C (range 13.7–24.4°C) and 34, respectively (Vergara et al. 2012). The light regime in Cádiz Bay varies greatly with the low/high tide, wind regime and other environmental factors (Morris et al. 2009), but a typical

range of irradiance would be 150 to >1500 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ in the upper subtidal areas during low tide in summer (Malta et al. 2006).

Plants were rinsed with seawater at the collection site, carefully packed in wet and dark conditions in a cool box and transported to the Biodiversity and Ecosystem Futures Facility at the National Oceanographic Centre Southampton, University of Southampton, UK. Within <36 h of collection, plants were submersed in aerated seawater (temperature 19°C, salinity 34) for a 10 d acclimation period under saturating light conditions (380 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$; Olivé et al. 2013), with a photoperiod of 18 h of light and 6 h of darkness.

Experimental set-up

The plants of *C. nodosa* used for the experiment consisted of a single lateral shoot with a bundle of leaves attached to a rhizome portion including 2 nodes, an internode of ca. 2–3 cm and corresponding roots. After tagging and measuring the initial architectural traits (described below) of each plant, they were transferred in pairs to thin-walled (5 mm) transparent Perspex aquaria (internal dimensions, length \times width \times height = 10 \times 10 \times 33 cm) filled with 10 cm of sediment overlain by ~20 cm of seawater. Sediment was collected from an area that hosts significant populations of seagrass (*Zostera marina*, Calshot intertidal flat, 50° 48' N, 1° 19' W, UK), sieved (500 μm mesh) in a filtered seawater bath to remove macrofauna and mixed with commercially sourced natural gravel (granules, size class 2–4 mm) in a 3:2 volume ratio. Aquaria were filled with 2 l of natural seawater (UV sterilised, 10 μm filtered, salinity 34) and randomly placed within a water bath maintained at 19.6 \pm 0.2°C. No nutrients were added. Plants were acclimated to laboratory conditions for an additional 5 d before the start of the experiment. During this acclimation period, no plant dieback was observed.

Aquaria were individually, directly and continuously bubbled with ambient air or CO₂-enriched air during the entire experiment, using a custom built CO₂-air mixing system (Godbold & Solan 2013) to represent atmospheric [CO₂] indicative of present day (380 ppm, 'present day') versus projected (750 and 1000 ppm, 'short-term projected' and 'long-term projected', respectively) conditions (Stocker et al. 2013). For each [CO₂] treatment, air and CO₂ (BOC, CP Grade CO₂ 99.995%) were mixed in an airtight and pressure-resistant vessel before being fed through a flow-controller into a Licor LI-840A in-

frared gas analyser and distributed to the aquaria. Aquaria were continuously bubbled and sealed with a transparent Perspex lid (1 mm thick) following established methods and best practice guidelines (Riebesell et al. 2010, Godbold & Solan 2013). Each lid was cleaned of evaporated salts twice a day to minimise restriction of light. Each [CO₂] treatment was replicated 10 times. In addition, 3 aquaria were maintained without plants under the same experimental conditions to monitor seawater pH without the modifying effects of seagrass productivity.

Aquaria were illuminated with a modular light source (Mazarra LED Lighting System, P-series) consisting of 16 modules (230 \times 230 mm), each housing 16 LEDs: colour temperature/wavelength/LED model: 4 \times cool white/7000–8000 K/Cree Xlamp XM-L; 4 \times blue/460–490 nm/Philips Luxeon Rebel; 4 \times royal blue/440–460 nm/Philips Luxeon Rebel; 1 \times super actinic/400–410 nm/Epileds Dual-Core; 1 \times super actinic/410–420 nm/Epileds Dual-Core; and 2 \times warm white/3000 K/Cree Xlamp XP-G. This light arrangement was designed to deliver the light at an intensity of photosynthetically active radiation sufficient to saturate photosynthesis, with a spectrum similar to the spectral distribution of sunlight. The photoperiod and down-welling plane surface irradiance (measured at the water surface with a Li-Cor LI-190R light meter connected to a logger unit LI-250A) were set in a dawn–daylight–dusk–night cycle: dawn lasted for 0.5 h with a gradual increase in irradiance from 21.3 \pm 3.2 to 383.3 \pm 33.6 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, and then remained constant for a further 17 h; dusk also lasted for 0.5 h, gradually decreasing light to total darkness; and the night period (6 h) received no illumination. Under this regime, daylight irradiance was above the saturating irradiance for *C. nodosa* from the collection site (<70 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$, Olivé et al. 2013). Aquaria were randomly repositioned every 5 d (8 \times during the experiment) to eliminate any unknown effects related to spatial position within the light system.

To avoid accumulation of nutrients and metabolites, we performed a partial (80%) seawater change on each aquarium every 4 d using natural seawater (UV sterilised, 10 μm filtered) held in reservoirs maintained at the appropriate [CO₂] to avoid changes in the water chemistry during water replacement. Salinity, temperature (Mettler-Toledo InLab 737 IP67 temperature–conductivity combination electrode) and pH (NBS scale, Mettler-Toledo InLab Expert Pro temperature–pH combination electrode; calibrated prior to every use) were monitored in each aquarium every 2 d (23 time-point measurements) and were

taken 6.5 h after the initiation of the dawn cycle. Seawater samples (20 ml, 0.45 µm filtered) were taken 5 times during the experiment from the seawater reservoir and from 6 random aquaria per [CO₂] treatment the day after water exchange for monitoring water column nutrient concentrations (NH₄-N, NO_x-N [= NO₂ + NO₃] and PO₄-P, mg l⁻¹). Growth of epiphytes was minimal, but allowed throughout the experiment. The total experimental period was 42 d.

Plant measurements

Prior to transplantation, each *C. nodosa* plant was blotted dry with paper towel and weighed (initial fresh weight; FW₀, g). The number of leaves was counted per shoot (leaf abundance), and the length, width and thickness of all leaves were determined using a ruler (±0.1 cm), a digital calliper (±0.01 mm) and a thickness gauge (±0.01 mm), respectively. Photosynthetic shoot size or shoot leaf area (cm² shoot⁻¹) was calculated as the sum of the 1-side area (leaf length × leaf width) of each leaf within a shoot. The youngest leaf in each shoot was hole punched 1 cm above the sheath to monitor leaf growth (Peralta et al. 2000), and each rhizome was tagged with a numbered tape for plant identification and to track the potential production of new shoots and internodes. To avoid underestimation of the leaf-breaking force, we did not pierce the whole shoot above the leaf sheath, because this could have resulted in leaves breaking in this area during the breaking test. Initially, plants had a mean (±1 SE, n = 60) fresh biomass of 1.13 ± 0.05 g FW plant⁻¹, leaf abundance of 2.76 ± 0.07 leaves shoot⁻¹, total leaf area of 10.87 ± 0.40 cm² shoot⁻¹ and the following morphological characteristics: leaf length = 13.44 ± 0.37 cm, leaf thickness = 0.23 ± 0.01 mm, leaf width = 2.98 ± 0.04 mm.

At the end of the experiment, each plant unit was harvested, cleaned of sediments and epiphytes, blotted dry with paper towel and immediately weighed for final fresh weight (FW_f, g) determination. The leaf abundance and leaf morphometry (length, width and thickness, averaged for all leaves in a shoot) of each plant were determined again, and the position of the initial marked leaf was recorded as a reference to separate new and old leaves.

Leaf growth in each shoot was estimated using 2 traits, following generally accepted standard methodology (Peralta et al. 2000, 2005). We estimated shoot elongation rate (SER, cm shoot⁻¹ d⁻¹, see Eq. 1 below) as the total increase of leaf length in a shoot per unit of time, computed from the length of the new leaves

in the shoot and the increase in length of the old leaves. Leaf loss rate (LLR, cm leaf shoot⁻¹ d⁻¹, Eq. 2) was estimated as the total loss of leaf length in a shoot per unit of time, computed from the sum of the length of the initial leaves lost over the experimental period.

$$\text{SER}(\text{cm shoot}^{-1} \text{ d}^{-1}) = \frac{\sum_{i=1}^n [(L_{i,f} - L_{i,0}) > 0]}{t_f - t_0} \quad (1)$$

$$\text{LLR}(\text{cm shoot}^{-1} \text{ d}^{-1}) = \frac{\sum_{i=1}^n [(L_{i,f} - L_{i,0}) < 0] - \sum_{i=1}^m L_{i,0}}{t_f - t_0} \quad (2)$$

where L = leaf length (cm), subscript i refers to the i^{th} leaf within the shoot, n = the number of leaves at the end of the study period, m = the number of leaves lost during the study period, subscript f and 0 refer, respectively, to final and initial conditions, and $t_f - t_0$ = experimental duration (d). In addition, we determined the plant biomass balance rate (PBR, mg FW shoot⁻¹ d⁻¹), i.e. the gains/losses in biomass of the plant per unit of time, as the difference in epiphyte-cleaned fresh biomass of each plant unit (i.e. shoot, portion of rhizome and roots) between the day of transplantation into the aquaria and the harvest day (Eq. 3):

$$\text{PBR}(\text{mg FW shoot}^{-1} \text{ d}^{-1}) = \frac{\text{FW}_f - \text{FW}_0}{t_f - t_0} \quad (3)$$

Leaf-breaking force at the end of the experiment was measured in a tensometer (Intron® model 5567 and BlueHill® software v. 2.18), using a 5 N-load cell and pneumatic action grips. To avoid effects of leaf age on the measurements (de los Santos et al. 2016b), the first outermost adult leaf (i.e. with a developed sheath) with a healthy appearance (green colouration) was selected from each shoot. Tensile tests were conducted within 48 h of plant harvesting, considered a valid time for measuring mechanical properties in seagrasses (de los Santos et al. 2016b). Leaves were individually clamped into the grips, and the ligule (the junction of the blade and the sheath) was positioned in the centre of the gauge (length 20 mm). Load was set to 0 N at the start of each test and leaves were then stretched at a constant velocity of 10 mm min⁻¹. Displacement (mm) and force (N) were recorded every 0.1 s until the point of leaf fracture, when the breaking force (N) was recorded. Only tests in which the leaves broke at the ligule and did not slip whilst being pulled apart were included in our statistical analyses (90% of tested leaves). After completion of the tensile tests, plants and epiphytes were dried separately at 60°C for 24 h to estimate plant dry biomass (g dry weight [DW] shoot⁻¹) and epiphyte cover (g DW cm⁻² shoot⁻¹).

Statistical analysis

Data are presented as means and associated SEs. Differences in seawater properties (temperature, salinity and nutrient concentration) among treatments were examined using a 1-way analysis of variance (ANOVA, fixed factor with 4 levels: the 3 [CO₂] projected scenarios plus the seawater reservoir). We used 2-way analysis of covariance for pH with the independent nominal variable [CO₂] projected scenarios (3 levels: 'present day', 'short-term projected' and 'long-term projected') and time as the covariate. Differences in pH between the procedural control (*C. nodosa* absent) and experimental aquaria (*C. nodosa* present) were examined using a 2-way ANOVA with [CO₂] projected scenarios and the presence/absence of *C. nodosa* as independent nominal variables. We defined the time span for pH stabilisation in the system using the slope estimate from linear regression. The effect of atmospheric CO₂ enrichment on leaf morphometry (length, thickness and width), plant dynamics (biomass balance, shoot elongation/loss rates), and leaf-breaking force were examined using a linear mixed effects regression model; [CO₂] was included as a fixed factor (3 levels: 'present day', 'short-term projected' and 'long-term projected') and aquarium as a random factor, since some variation may exist amongst aquaria, and the focus of our study concerns the conditions they represent rather than the aquaria themselves. The model was fit by maximum likelihood, and Type III Wald χ^2 tests were used to assess the significance of the fixed effect in the model. Pairwise comparisons to identify homogenous groups were identified using Tukey's multiple comparison tests. Visual inspection of residual plots did not reveal any obvious deviation from homoscedasticity or normality in the linear models. To test the hypothesis that leaf growth enhancement is associated with leaf strengthening (breaking force), we used Pearson's correlation coefficient and linear regression analy-

sis. All statistical analysis was conducted in R (R Development Core Team 2014) using the 'lme4' library (Bates et al. 2015)

RESULTS

Physico-chemical parameters in the water column

The mean seawater temperature and salinity in the aquaria were $19.3 \pm 0.3^\circ\text{C}$ and 34.8 ± 0.6 over the experimental period, without differences among treatments ($p > 0.05$; data not shown). [NH₄-N], [NO_x-N] and [PO₄-P] in the seawater were high, and did not differ among [CO₂] treatments (Table 1). Nutrient concentration was lower in the experimental aquaria than in the seawater reservoir, with the exception of NH₄-N, indicating the occurrence of nutrient uptake by the plants and associated epiphytes (Table 1).

Seawater pH in the aquaria differed between [CO₂] scenarios (interaction: [CO₂] \times seagrass presence/absence, $F_{2,741} = 4.77$, $p = 0.009$; Fig. 1a). Relative to present day conditions (pH = 8.81 ± 0.02), pH was lower in the short-term projected (pH = 8.37 ± 0.01) and long-term projected (pH = 8.13 ± 0.01) treatments. In the absence of plants, pH was lower: 8.29 ± 0.04 (present day conditions), 8.04 ± 0.03 (short-term projected scenario) and 7.82 ± 0.01 (long-term projected scenario). An initial 10 d period was observed in which pH slightly increased over time (pH-time regression slopes significantly different from 0: present day [CO₂], slope = 0.066; short-term projected [CO₂], slope = 0.030; long-term projected [CO₂], slope = 0.018). Following this period, pH stabilised and remained relatively constant within each [CO₂] for the rest of the experimental period (Fig. 1b; slopes insignificantly different from 0: present day [CO₂], slope = 0.006 pH; short-term projected [CO₂], slope = -0.0001 ; long-term projected [CO₂], slope = -0.001).

Table 1. Mean (± 1 SE, n in parentheses) seawater nutrient concentrations (mg l⁻¹) in the seawater reservoir (day of renewal) and experimental aquaria (day after renewal) over the 6 wk experimental period. [CO₂] treatments are present day (pH 8.29), short-term projected (pH 8.04) and long-term projected (pH 7.82). Significance of [CO₂] treatments and reservoir (1-way ANOVA) and associated Tukey's multiple pairwise comparisons (superscript letter denotes groupings) are presented

Nutrient	Reservoir	[CO ₂] projected scenario			ANOVA	
		Present day	Short-term	Long-term	F (df)	p
NH ₄ ⁺ -N	0.09 \pm 0.01 (3)	0.16 \pm 0.03 (20)	0.12 \pm 0.02 (19)	0.13 \pm 0.02 (19)	1.819 (3, 50)	0.1556
NO _x ⁻ -N	4.19 \pm 0.31 (7) ^a	2.03 \pm 0.40 (14) ^b	2.09 \pm 0.49 (11) ^b	2.39 \pm 0.39 (10) ^b	56.6 (3, 20)	<0.001
PO ₄ ³⁻ -P	0.62 \pm 0.02 (7) ^a	0.24 \pm 0.05 (16) ^b	0.35 \pm 0.07 (8) ^b	0.24 \pm 0.06 (13) ^b	333.2 (3, 22)	<0.001

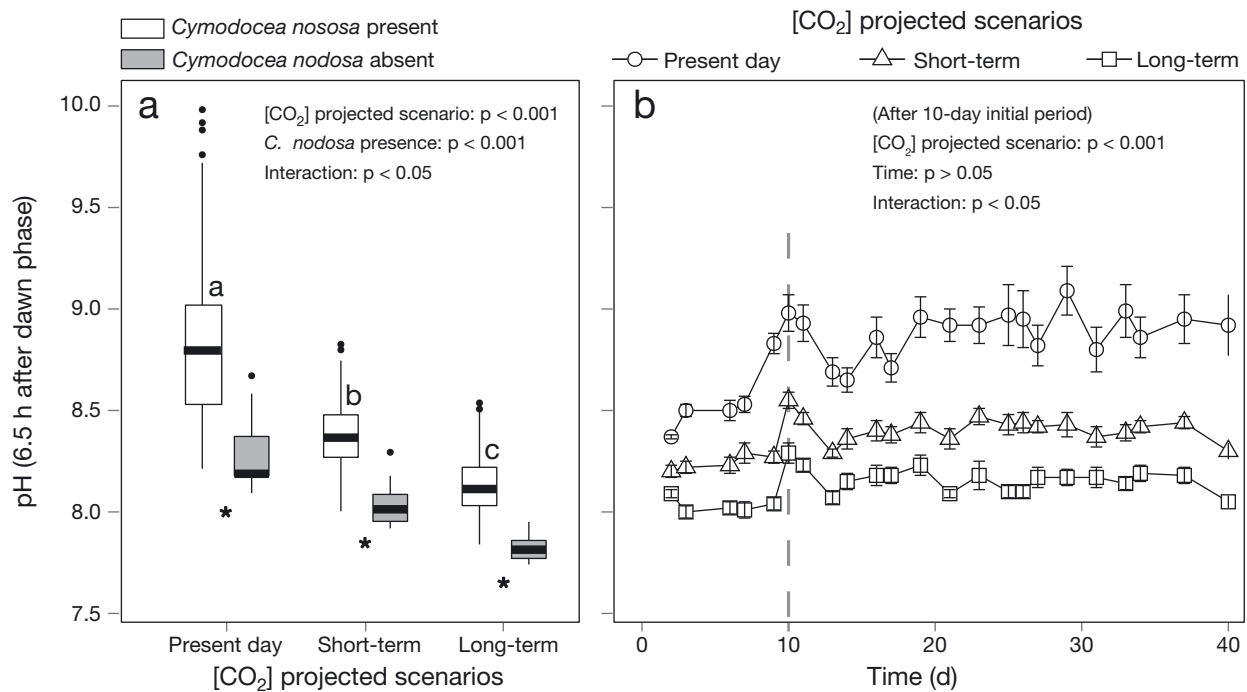


Fig. 1. Seawater pH for (a) aquaria with and without the seagrass *Cymodocea nodosa*, and (b) aquaria containing *C. nodosa* over the 6 wk experimental period (mean \pm 1 SE). Statistical significance (p-values) is given in panel (a) for the 2-way ANOVA ([CO₂] projected scenario, presence/absence of *C. nodosa* and their interaction), and in panel (b) for the 2-way ANCOVA (time, [CO₂] projected scenario and their interaction). In (a), superscript letters represent post hoc Tukey pairwise groupings that indicate differences among [CO₂] projected scenarios; asterisks denote differences between the procedural control (*C. nodosa* absent) and experimental aquaria (*C. nodosa* present). Dashed line in (b) marks the time of pH stabilisation in the system (10 d). Boxplot bar: median; box: interquartile range (IQR); whiskers: max./min. values within the 1.5 \times IQR of the hinge; dots: outliers

Plant responses

Cymodocea nodosa plants appeared healthy throughout the experiment: seagrass tissues did not exhibit any evidence of necrosis, leaves had a bright green colour (with the exception of senescent leaves, which were greyish), and rhizomes had a pinkish colour common for this species. Epiphytic cover was minimal (0.002 g DW cm⁻² of leaf surface per shoot, n = 24) or absent. All plants survived in the short- and long-term projected [CO₂] treatments, but there was some negligible mortality (2 shoots) under present day [CO₂] conditions.

At the onset of the experiment, intense leaf shedding (release of the leaf blade, i.e. above the ligule) was observed, which involved the elimination of the long leaves that were present on the shoots from the field. Plants did not produce new shoots at any of the internodes over the experimental period, and new leaves developed only from the existing shoot. Indeed, 87.6 \pm 15.8% of leaves in a shoot at the end of the experiment were generated during the experimental period (n = 58 shoots). Newly produced replacement tissue had different dimensions. Whilst

initial shoots had an average leaf abundance of 2.76 \pm 0.07 leaves shoot⁻¹ and average leaf length of 13.38 \pm 0.36 cm, at the end of the experiment, leaf abundance subtly increased, ranging from 2.9 to 3.3 leaves shoot⁻¹ and leaf length substantially decreased, ranging from 9.16 to 9.53 cm. The average shoot leaf area was initially 10.87 cm² shoot⁻¹, but was reduced to 6.47–7.91 cm² shoot⁻¹ in the final shoots.

Leaf morphometry did not differ among [CO₂] treatments (linear mixed effects model, df = 2, p > 0.05, χ^2 = 0.41 for length, χ^2 = 1.39 for thickness, and χ^2 = 0.48 for width, Table 2). Leaf abundance was significantly higher in plants grown under short-term projected [CO₂] (3.6 \pm 0.2 leaves shoot⁻¹) than those grown under present day [CO₂] (2.9 \pm 0.1 leaves shoot⁻¹), and both were similar to plants grown under long-term projected [CO₂] (3.3 \pm 0.2 leaves shoot⁻¹) (linear mixed effects model, χ^2 = 9.06, df = 2, p = 0.011, Table 2). Biomass balance was positive in plants grown under short-term projected [CO₂] (2.14 \pm 0.78 mg FW shoot⁻¹ d⁻¹) but negative in plants growing in present day [CO₂] (-2.12 \pm 0.76 mg FW shoot⁻¹ d⁻¹) and long-term projected [CO₂] (-2.27 \pm 1.34 mg FW shoot⁻¹ d⁻¹) (χ^2 = 13.1, df = 2, p = 0.001,

Table 2. Summary of the architectural, mechanical and dynamical traits of *Cymodocea nodosa* obtained at the end of the experiment (mean \pm 1 SE, n = 20; except for the present day [380 ppm] treatment, where n = 18 because 2 shoots died). [CO₂] treatments are present day (pH 8.29), short-term projected (pH 8.04) and long-term projected (pH 7.82). Summaries of the statistical findings (linear mixed effects model, [CO₂] as fixed factor and aquaria as random factor) are presented. Superscript letters represent post hoc Tukey pairwise groupings that indicate differences among [CO₂] treatments; asterisks indicate the level of statistical significance (**p < 0.001, *p < 0.01, *p < 0.05); DW: dry weight, FW: fresh weight

Trait	[CO ₂] projected scenario			Linear mixed effects model (df = 2)	
	Present day	Short-term	Long-term	χ^2	p
Leaf abundance (leaves shoot ⁻¹)	2.89 \pm 0.14 ^a	3.60 \pm 0.20 ^b	3.30 \pm 0.16 ^{ab}	9.06	0.011**
Mean leaf length (cm)	9.63 \pm 0.47	9.53 \pm 0.28	9.16 \pm 0.47	0.41	0.816
Mean leaf thickness (mm)	0.20 \pm 0.01	0.21 \pm 0.01	0.20 \pm 0.01	1.39	0.499
Mean leaf width (mm)	2.27 \pm 0.06	2.28 \pm 0.04	2.24 \pm 0.05	0.48	0.788
Shoot leaf area (cm ² shoot ⁻¹)	6.47 \pm 0.67	7.91 \pm 0.61	6.57 \pm 0.54	3.75	0.153
Plant dry biomass (g DW shoot ⁻¹)	0.15 \pm 0.01 ^a	0.20 \pm 0.02 ^b	0.14 \pm 0.02 ^a	10.21	0.006***
Leaf-breaking force at ligule (N)	1.72 \pm 0.13 ^a	2.30 \pm 0.18 ^b	2.00 \pm 0.18 ^{ab}	6.75	0.034*
Plant biomass balance rate (mg FW shoot ⁻¹ d ⁻¹)	-2.12 \pm 0.76 ^a	2.14 \pm 0.78 ^b	-2.27 \pm 1.34 ^a	13.1	0.001**
Shoot elongation rate (cm shoot ⁻¹ d ⁻¹)	0.60 \pm 0.05	0.71 \pm 0.04	0.64 \pm 0.05	2.94	0.230
Leaf loss rate (cm shoot ⁻¹ d ⁻¹)	0.80 \pm 0.04	0.70 \pm 0.03	0.83 \pm 0.04	5.40	0.067

Fig. 2a, Table 2). Shoot elongation rate did not differ among [CO₂] treatments (linear mixed effects model, $\chi^2 = 2.94$, df = 2, p = 0.230, Fig. 2b) and neither did the leaf loss rate ($\chi^2 = 5.40$, df = 2, p = 0.067, Fig. 2c). In contrast, leaves of plants growing under short-term

projected [CO₂] supported higher tensile forces (2.30 \pm 0.18 N) than those growing under present day [CO₂] (1.72 \pm 0.13 N), whilst the leaf-breaking force of plants grown under long-term projected [CO₂] (2.00 \pm 0.18 N) formed an intermediate grouping (lin-

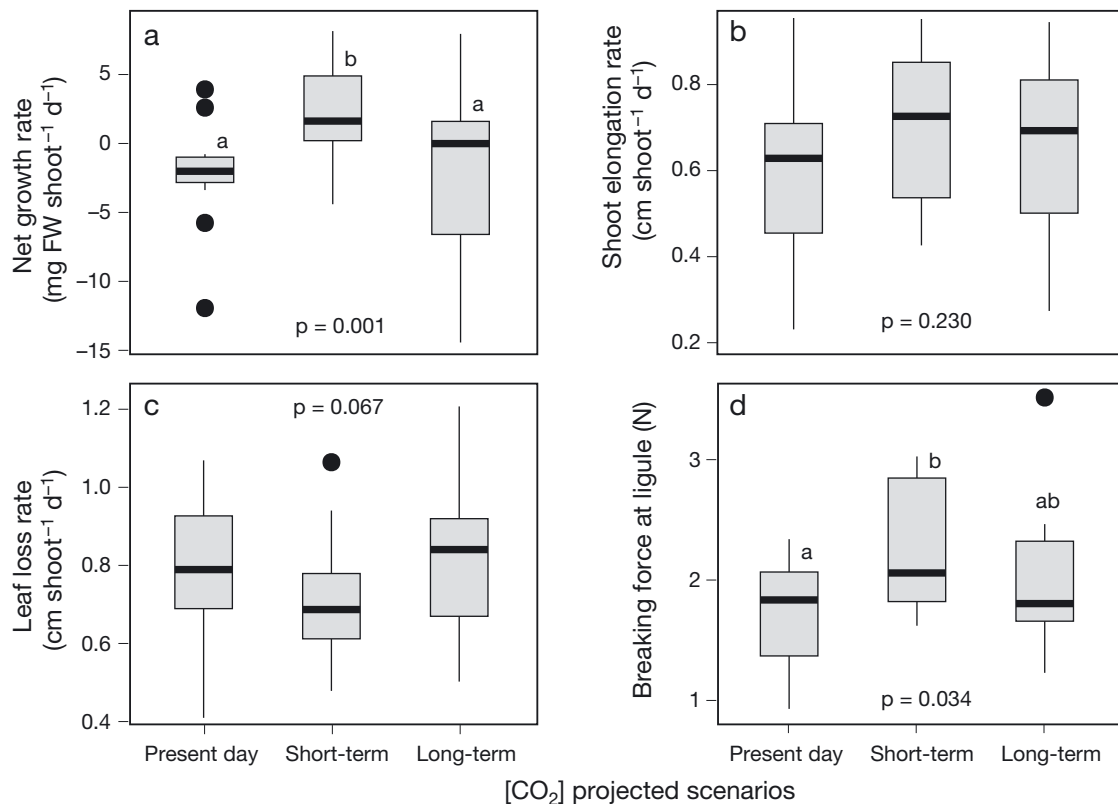


Fig. 2. Response of the seagrass *Cymodocea nodosa* to [CO₂] scenarios (present day versus anticipated short-term and long-term [CO₂] projections). Statistical significance (p values) of the [CO₂] scenario in the mixed-effects linear models is shown, and superscript letters represent post hoc Tukey pairwise groupings for [CO₂]. FW: fresh weight

ear mixed effects model, $\chi^2 = 6.75$, $df = 2$, $p = 0.034$; Fig. 2d). Plants with high elongation rates presented stronger leaves than plants with a low elongation rate, as given by the positive correlation of shoot elongation rate and breaking force (Fig. 3).

DISCUSSION

We have demonstrated that increasing atmospheric $[\text{CO}_2]$ concentration affects the plant biomass balance rate and the leaf-breaking force of the temperate seagrass species *Cymodocea nodosa* and that the mechanical response is correlated with leaf growth. However, our data also indicate that other leaf traits (leaf morphology and leaf dynamics) that reflect different aspects of growth did not respond in the same way. This suggests that responses to increasing $[\text{CO}_2]$ may manifest to a greater extent in the belowground tissues (e.g. Koch et al. 2013) or at the cellular level, since plant growth and development depend largely on the biosynthesis and remodelling of the cell wall (e.g. Sharma et al. 2014). This distinction is important, as it indicates that the evaluation of the effects of ocean acidification on processes that encompass multiple facets of response has the potential to be misleading when conclusions are based on a single,

or a limited set of, response descriptors that are assumed to be representative.

Effects of ocean acidification on the growth of seagrasses have been reported for many species over short- and long-term scales (Table 3). Over the short term, we found no effect on shoot elongation rate, which is consistent with previous short-term studies for the same species (Tomas et al. 2015) and for the congeneric *C. serrulata* (Ow et al. 2015), as well as for other seagrass species exposed to longer experimental periods (Alexandre et al. 2012, Campbell & Fourqurean 2013). The lack of effect in those traits might be explained by the short-term duration of the experiment. We found, however, that *C. nodosa* plants increased in biomass when growing under short-term projected $[\text{CO}_2]$ conditions, but decreased under present day or long-term projected $[\text{CO}_2]$ scenarios. The observed leaf shedding and production of new altered tissue (leaves with reduced leaf area) ultimately contributed to a negative biomass balance in plants from those 2 treatments, i.e. they lost more leaf tissue than they produced. The positive balance in plants growing under short-term projected $[\text{CO}_2]$ conditions could be potentially explained by one or a combination of the following: (1) larger leaves, (2) higher leaf abundance, (3) denser tissues, (4) growth of belowground tissues. We can disregard 'larger leaves' because we did not observe differences in leaf morphology among treatments. We can consider 'more leaves' as an explanation because the plants under CO_2 -enriched conditions showed a significantly higher leaf abundance, but this does not explain the difference in biomass balance between our 2 CO_2 -enriched treatments. The 'denser tissues' and/or 'growth of belowground tissues' were not directly quantified but, based on the observed differences in plant dry biomass (i.e. dry weight of the whole shoot, including both below- and above-ground tissues, Table 2), they form a valid possibility. We observed that plants under the short-term projected $[\text{CO}_2]$ scenario were significantly heavier ($0.20 \pm 0.02 \text{ g DW shoot}^{-1}$) than plants from the other 2 scenarios (0.15 ± 0.01 and $0.14 \pm 0.02 \text{ g DW shoot}^{-1}$). Hence, we explain the differences in the biomass balance by the higher abundance of leaves and heavier shoots in the short-term $[\text{CO}_2]$ scenario. Increased biomass in the belowground tissues has been previously observed in *C. serrulata* (Russell et al. 2013) and *Zostera marina* when exposed to naturally high $[\text{CO}_2]$ (Palacios & Zimmerman 2007). The biomass decline due to leaf shedding is a commonly observed phenomenon in manipulative experiments that involve seagrasses (e.g. de los Santos et al. 2010, Col-

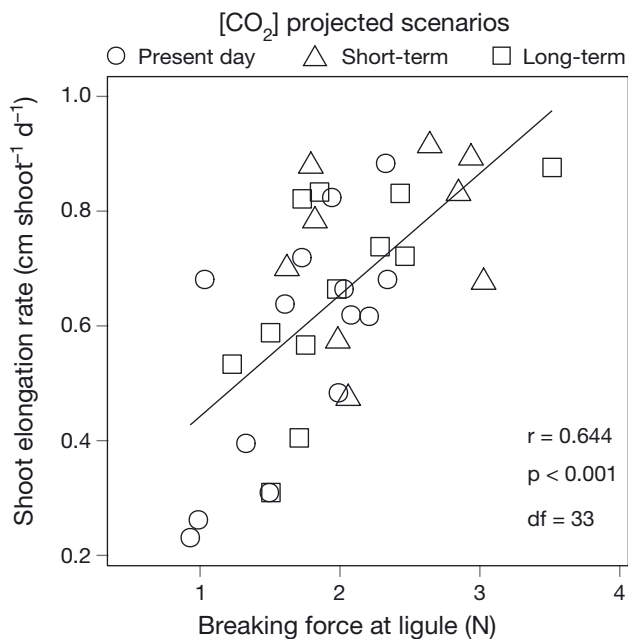


Fig. 3. Correlation between shoot elongation rate and breaking force for the seagrass *Cymodocea nodosa* indicates that breaking force is, at least partly, positively affected by higher rates of shoot elongation. Pearson's correlation coefficient (r) is shown with its significance (p value) and degrees of freedom (df) ($n = 35$)

Table 3. Responses of architectural and dynamic traits of seagrasses experimentally exposed to short- (<6 mo) and long-term (≥6 mo) CO₂ enrichment. Responses are evaluated as ↔: unresponsive, +: increase, -: decrease, ?: inconclusive, na: not available

Species	pH levels	Time frame	Leaf/shoot architecture response	Leaf/shoot growth response	Reference
<i>Zostera noltei</i>	7.91–8.13	Short-term (5 mo)	na	↔ leaf growth rate	Alexandre et al. (2012)
<i>Zostera marina</i>	na	Short-term (7–10 d)	na	? leaf growth rate	Thom (1996)
<i>Zostera marina</i>	6.4–7.5–7.75–8.1	Long-term (1 yr)	+ shoot biomass, + belowground biomass, ↔ leaf biomass, ↔ leaf size, ↔ leaf area	↔ leaf growth rate	Palacios & Zimmerman (2007)
<i>Thalassia testudinum</i>	7.78–8.23–8.24	Long-term (6 mo)	↔ leaf area, ↔ shoot biomass	↔ leaf growth rate	Campbell & Fourqurean (2013)
<i>Thalassia hemprichii</i>	7.76–8.14	Short-term (2 wk)	+ specific leaf area	+ leaf growth rate	Ow et al. (2015)
<i>Thalassia hemprichii</i>	6.20–7.50–7.75–8.10	Short-term (3 wk)	na	+ leaf growth rate (minor)	Jiang et al. (2010)
<i>Halodule uninervis</i>	7.76–8.14	Short-term (2 wk)	↔ specific leaf area	+ leaf growth rate	Ow et al. (2015)
<i>Cymodocea serrulata</i>	7.76–8.14	Short-term (2 wk)	↔ specific leaf area	↔ leaf growth rate	Ow et al. (2015)
<i>Cymodocea nodosa</i>	7.87–8.01	Short-term (6 wk)	↔ leaf abundance (tendency to increase)	↔ leaf growth rate	Tomas et al. (2015)
<i>Cymodocea nodosa</i>	7.82–8.04–8.29	Short-term (6 wk)	↔ leaf length, thickness and width, + shoot biomass (only at pH = 8.04), + leaf abundance (specially at pH = 8.04)	+ plant biomass balance rate (only at pH = 8.04)	This study

lier et al. 2012), including *C. nodosa* (Malta et al. 2006). However, given the absence of measurements of biochemical and physiological responses in our experiment, we cannot distinguish the mechanistic basis of this observation.

It is important to recognise that the loss of biomass over the experimental period could also reflect inappropriate environmental conditions for *C. nodosa*. In general, however, the experimental set-up appeared favourable for the growth of this species, given (1) the high survival at the end of the experiment, (2) the fact that plants produced new leaves in all of the treatments, including those showing a decrease in biomass and (3) the observed pH increase in the aquaria containing plants relative to the controls, indicative of high productivity (Invers et al. 1997, Buapet et al. 2013). Accepting these observations, the negative plant biomass balance we observed might be explained by a shortage in resources. In particular, dissolved inorganic carbon concentrations are limiting under present day [CO₂] because they do not saturate the photosynthetic activity of seagrasses (reviewed by Koch et al. 2013), including *C. nodosa* (Invers et al. 2001). Under higher [CO₂], sub-optimal growth rates have been previously reported and justified by a shortcut in the availability of inorganic nutrients (*Z. noltei*, Alexandre et al. 2012). However, our plants were exposed to ambient high nutrient concentrations, so we discard this hypothesis. Although unlikely, plants could have been light-limited during the experiment despite the irradiance regime set in the mesocosms, which can be considered saturating for *C. nodosa*. These sub-optimal growth conditions appear to be absent at intermediate [CO₂], as plants increased their biomass and exhibited an increase in leaf abundance. Taken together, these observations suggest that the decreased pH associated with the elevated [CO₂] is somehow favourable for *C. nodosa*, most likely through enhancement of photosynthetic activity (Invers et al. 2001) that could, in turn, translate into elevated levels of carbon fixation and, consequently, more resources to support plant growth.

Mechanical traits are important in determining how plants resist abiotic and biotic physical forces (Read & Stokes 2006), so an alteration of the biomechanics of seagrass leaves may have important ecological implications over extended periods of time. Here, we found that *C. nodosa* leaves may become slightly more tolerant of physical forces when growing in a moderately CO₂-enriched medium. Hence, changes in its leaf mechanical properties might offset, either wholly or in part, the loss of habitat and

persistence under various environmental futures, such as novel hydrodynamical forces associated with moderate levels of climate change (Young et al. 2011), or high nutrient regimes (La Nafie et al. 2012). On the other hand, the strengthening of leaf abscission could make it difficult for leaf shedding, leading to negative consequences such as overgrowth of epiphytes and resource demand by non-functional leaves. Variation in the breaking force of leaves is determined by morphological adjustments in the leaf cross-section area, changes in the material strength or both (Niklas 1992). Since we did not find any adjustment in the morphological attributes of *C. nodosa* leaves, we conclude that the observed changes in the leaf-breaking force are due to differences in the material strength of the tissues, in agreement with previous studies for intra-specific variation in breaking force of seagrass leaves (de los Santos et al. 2016a). Breaking force of seagrass leaves is often associated with structural reinforcement of the leaves, such as the proportion of fibre content (de los Santos et al. 2016a). We contend here that the higher leaf-breaking force of seagrass *C. nodosa* in a short-term projected [CO₂] scenario is most likely to be associated with a greater allocation of resources (structural carbohydrates) to the supporting tissues of the leaves. Accordingly, Kopp (1999) found a correlation between breaking force and leaf growth of seagrasses, such that seagrass leaves withstand higher forces during the active growing season than they do during lower periods of growth in winter. Clearly, insights from longer exposures, or from seagrass communities growing within the plumes of CO₂ vents (e.g. Hall-Spencer et al. 2008) will be insightful, particularly with respect to determining the full extent and mechanistic basis for any response and to determine the generality, or otherwise, of our findings.

Biomechanical acclimation of *C. nodosa* leaves to CO₂ enrichment is also likely to affect leaf palatability, as leaf fracture properties are intimately associated with the fibre content and C:N ratio in seagrasses (de los Santos et al. 2012). For instance, Tomas et al. (2015) hypothesised that the alteration of structural traits of *C. nodosa* in an enriched CO₂ medium could influence the feeding patterns of some herbivores. Biomechanical acclimation to CO₂ needs to be investigated in concert with other traits involved in the leaf palatability, especially because other studies report that elevated CO₂ leads to losses in protective phenolic compounds that commonly act as protection against grazing (Arnold et al. 2012) and a reduction in epiphytic infestation (Martin et al. 2008).

Our study emphasises the need to account for the interdependencies between environmental conditions and variations in multiple aspects of the structure and functioning of seagrass communities when considering the likely consequences of climate change. Elucidating the relative importance of the multitude of seagrass responses to ocean acidification, and the form of the interplay between them, will be challenging because ecological responses may be species-specific (Koch et al. 2013, Ow et al. 2015), mechanical traits are context dependent (interaction with multiple stressors, La Nafie et al. 2012, 2013; seasonal and developmental timing, de los Santos et al. 2012, 2016b), and both of these are complicated further by acclimation/adaptive capacity (Stillman & Paganini 2015).

Acknowledgements. C.B.S. was supported, at the time of the experiment performance, by the Mobility Fellowships Programme of the EuroMarine Consortium (European Commission Seventh Framework Programme, FP7-ENV-2010.2.2.1-3), and presently by the Foundation of Science and Technology of Portugal (SFRH/BPD/119344/2016 and PTDC/MAR-EST/3223/2014). Additional support was received from the Natural Environment Research Council (NERC) through the UK Ocean Acidification Research Programme (UKOARP, grant reference NE/H017445/1, awarded to M.S. and J.A.G.). We thank G. Taylor and H. K. Smith (School of Biological Sciences, University of Southampton) for use of the Instron testing machine, M. Ribas-Ribas (Carl von Ossietzky Universität Oldenburg, Germany), C. Sturdy, A. Currie, R. Hale and M. Stinchcombe (Ocean and Earth Science, University of Southampton) for technical assistance and support. We appreciate the constructive criticism given by the anonymous reviewers.

LITERATURE CITED

- ✦ Alexandre A, Silva J, Buapet P, Björk M, Santos R (2012) Effects of CO₂ enrichment on photosynthesis, growth, and nitrogen metabolism of the seagrass *Zostera noltii*. *Ecol Evol* 2:2625–2635
- ✦ Arnold T, Mealey C, Leahey H, Miller AW, Hall-Spencer JM, Milazzo M, Maers K (2012) Ocean acidification and the loss of phenolic substances in marine plants. *PLOS ONE* 7:e35107
- ✦ Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67:1–48
- ✦ Buapet P, Gullström M, Björk M (2013) Photosynthetic activity of seagrasses and macroalgae in temperate shallow waters can alter seawater pH and total inorganic carbon content at the scale of a coastal embayment. *Mar Freshw Res* 64:1040–1048
- ✦ Campbell JE, Fourqurean JW (2013) Effects of *in situ* CO₂ enrichment on the structural and chemical characteristics of the seagrass *Thalassia testudinum*. *Mar Biol* 160: 1465–1475
- ✦ Collier CJ, Waycott M, Ospina AG (2012) Responses of four Indo-West Pacific seagrass species to shading. *Mar Pollut Bull* 65:342–354

- de los Santos CB, Brun FG, Bouma TJ, Vergara JJ, Pérez-Lloréns JL (2010) Acclimation of seagrass *Zostera noltii* to co-occurring hydrodynamic and light stresses. *Mar Ecol Prog Ser* 398:127–135
- de los Santos CB, Brun FG, Onoda Y, Cambridge ML, Bouma TJ, Vergara JJ, Pérez-Lloréns JL (2012) Leaf-fracture properties correlated with nutritional traits in nine Australian seagrass species: implications for susceptibility to herbivory. *Mar Ecol Prog Ser* 458:89–102
- de los Santos CB, Brun FG, Vergara JJ, Pérez-Lloréns JL (2013) New aspect in seagrass acclimation: Leaf mechanical properties vary spatially and seasonally in the temperate species *Cymodocea nodosa* Ucria (Ascherson). *Mar Biol* 160:1083–1093
- de los Santos CB, Onoda Y, Vergara JJ, Pérez-Lloréns JL and others (2016a) A comprehensive analysis of mechanical and morphological traits in temperate and tropical seagrass species. *Mar Ecol Prog Ser* 551:81–94
- de los Santos CB, Vicencio-Rammsy B, Lepoint G, Remy F, Bouma TJ, Gobert S (2016b) Ontogenic variation and effect of collection procedure on leaf biomechanical properties of Mediterranean seagrass *Posidonia oceanica* (L.) Delile. *Mar Ecol* 37:750–759
- Duarte CM (1991) Allometric scaling of seagrass form and productivity. *Mar Ecol Prog Ser* 77:289–300
- Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES J Mar Sci* 65:414–432
- Form A, Riebesell U (2012) Acclimation to ocean acidification during long-term CO₂ exposure in the cold-water coral *Lophelia pertusa*. *Glob Change Biol* 18:843–853
- Garrard S, Gambi MC, Scipione MB, Patti FP and others (2014) Indirect effects may buffer negative responses of seagrass invertebrate communities to ocean acidification. *J Exp Mar Biol Ecol* 461:31–38
- Godbold JA, Solan M (2013) Long-term effects of warming and ocean acidification are modified by seasonal variation in species responses and environmental conditions. *Phil Trans R Soc Lond B Biol Sci* 368:20130186
- Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Buia MC (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* 454:96–99
- Hendriks IE, Olsen YS, Ramajo L, Basso L and others (2014) Photosynthetic activity buffers ocean acidification in seagrass meadows. *Biogeosciences* 11:333–346
- Invers O, Romero J, Pérez M (1997) Effects of pH on seagrass photosynthesis: a laboratory and field assessment. *Aquat Bot* 59:185–194
- Invers O, Zimmerman RC, Alberte RS, Pérez M, Romero J (2001) Inorganic carbon sources for seagrass photosynthesis: an experimental evaluation of bicarbonate use in species inhabiting temperate waters. *J Exp Mar Biol Ecol* 265:203–217
- Jiang ZJ, Huang XP, Zhang JP (2010) Effects of CO₂ enrichment on photosynthesis, growth, and biochemical composition of seagrass *Thalassia hemprichii* (Ehrenb.) Aschers. *J Integr Plant Biol* 52:904–913
- Koch M, Bowes G, Ross C, Zhang XH (2013) Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Glob Change Biol* 19:103–132
- Kopp BS (1999) Effects of nitrate fertilization and shading on physiological and biomechanical properties of eelgrass (*Zostera marina* L.). PhD dissertation, University of Rhode Island, Kingston, RI
- Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol Lett* 13:1419–1434
- Kroeker KJ, Kordas RL, Crim R, Hendriks IE and others (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob Change Biol* 19:1884–1896
- La Nafie YA, de los Santos CB, Brun FG, van Katwijk MM, Bouma TJ (2012) Waves and high nutrient loads jointly decrease survival and separately affect morphological and biomechanical properties in the seagrass *Zostera noltii*. *Limnol Oceanogr* 57:1664–1672
- La Nafie YA, de los Santos CB, Brun FG, Mashoreng S, van Katwijk MM, Bouma TJ (2013) Biomechanical response of two fast-growing tropical seagrass species subjected to in situ shading and sediment fertilization. *J Exp Mar Biol Ecol* 446:186–193
- Malta EJ, Brun FG, Vergara JJ, Hernández I, Pérez-Lloréns JL (2006) Recovery of *Cymodocea nodosa* (Ucria) Ascherson photosynthesis after a four-month dark period. *Sci Mar* 70:413–422
- Martin S, Rodolfo-Metalpa R, Ransome E, Rowley S, Buia MC, Gattuso JP, Hall-Spencer J (2008) Effects of naturally acidified seawater on seagrass calcareous epibionts. *Biol Lett* 4:689–692
- Morris EP, Peralta G, Benavente J, Freitas R and others (2009) *Caulerpa prolifera* stable isotope ratios reveal anthropogenic nutrients within a tidal lagoon. *Mar Ecol Prog Ser* 390:117–128
- Newcomb LA, Milazzo M, Hall-Spencer JM, Carrington E (2015) Ocean acidification bends the mermaid's wineglass. *Biol Lett* 11:20141075
- Niklas KJ (1992) Plant biomechanics: an engineering approach to plant form and function. University of Chicago Press, Chicago, IL
- Olivé I, Vergara JJ, Pérez-Lloréns JL (2013) Photosynthetic and morphological photoacclimation of the seagrass *Cymodocea nodosa* to season, depth and leaf position. *Mar Biol* 160:285–297
- Ow YX, Collier CJ, Uthicke S (2015) Responses of three tropical seagrass species to CO₂ enrichment. *Mar Biol* 162:1005–1017
- Pagès JF, Pérez M, Romero J (2010) Sensitivity of the seagrass *Cymodocea nodosa* to hypersaline conditions: a microcosm approach. *J Exp Mar Biol Ecol* 386:34–38
- Palacios SL, Zimmerman RC (2007) Response of eelgrass *Zostera marina* to CO₂ enrichment: possible impacts of climate change and potential for remediation of coastal habitats. *Mar Ecol Prog Ser* 344:1–13
- Peralta G, Pérez-Lloréns JL, Hernández I, Brun FG and others (2000) Morphological and physiological differences between two morphotypes of *Zostera noltii* Hornem. from the south-western Iberian Peninsula. *Helgol Mar Res* 54:80–86
- Peralta G, Brun FG, Hernández I, Vergara JJ, Pérez-Lloréns JL (2005) Morphometric variations as acclimation mechanisms in *Zostera noltii* beds. *Estuar Coast Shelf Sci* 64:347–356
- Pérez M, Romero J, Duarte CM, Sand-Jensen K (1991) Phosphorus limitation of *Cymodocea nodosa* growth. *Mar Biol* 109:129–133
- R Development Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

- ✦ Ragazzola F, Foster LC, Form A, Anderson PS, Hansteen TH, Fietzke J (2012) Ocean acidification weakens the structural integrity of coralline algae. *Glob Change Biol* 18:2804–2812
- ✦ Read J, Stokes A (2006) Plant biomechanics in an ecological context. *Am J Bot* 93:1546–1565
- Riebesell U, Fabry VJ, Hansson L, Gattuso JP (2010) Guide to best practices for ocean acidification research and data reporting. Publications Office of the European Union, Luxembourg
- ✦ Russell BD, Connell SD, Uthicke S, Muehllehner N, Fabricius KE, Hall-Spencer JM (2013) Future seagrass beds: Can increased productivity lead to increased carbon storage? *Mar Pollut Bull* 73:463–469
- ✦ Sharma N, Sinha PG, Bhatnagar AK (2014) Effect of elevated [CO₂] on cell structure and function in seed plants. *Clim Chang Environ Sustain* 2:69–104
- ✦ Stillman JH, Paganini AW (2015) Biochemical adaptation to ocean acidification. *J Exp Biol* 218:1946–1955
- Stocker TF, Qin D, Plattner GK, Tignor M and others (eds) (2013) *Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge
- ✦ Thom RM (1996) CO₂-enrichment effects on eelgrass (*Zostera marina* L.) and bull kelp (*Nereocystis luetkeana* (Mert.) P & R.). *Water Air Soil Pollut* 88: 383–391
- ✦ Tomas F, Martínez Crego B, Hernán G, Santos R (2015) Responses of seagrass to anthropogenic and natural disturbances do not equally translate to its consumers. *Glob Change Biol* 21:4021–4030
- ✦ Unsworth RKF, Collier CJ, Henderson GM, McKenzie LJ (2012) Tropical seagrass meadows modify seawater carbon chemistry implications for coral reefs impacted by ocean acidification. *Environ Res Lett* 7:024026
- ✦ Vergara JJ, García-Sánchez MP, Olivé I, García-Marín P, Brun FG, Pérez-Lloréns JL, Hernández I (2012) Seasonal functioning and dynamics of *Caulerpa prolifera* meadows in shallow areas: an integrated approach in Cadiz Bay Natural Park. *Estuar Coast Shelf Sci* 112:255–264
- ✦ Vergés A, Becerro MA, Alcoverro T, Romero J (2007) Variation in multiple traits of vegetative and reproductive seagrass tissues influences plant-herbivore interactions. *Oecologia* 151:675–686
- ✦ Young IR, Zieger S, Babanin AV (2011) Global trends in wind speed and wave height. *Science* 332:451–455

*Editorial responsibility: William Kemp,
Cambridge, Maryland, USA*

*Submitted: April 28, 2016; Accepted: April 12, 2017
Proofs received from author(s): May 22, 2017*