



Photo-acclimatory thresholds anticipate sudden shifts in seagrass ecosystem state under reduced light conditions

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ABSTRACT

Seagrass ecosystems usually respond in a nonlinear fashion to increasing pressures and environmental changes. Feedback mechanisms operating at the ecosystem level and involving multiple interactions among the seagrass meadow, its associated community and the physical environment are known to play a major role in such nonlinear responses. Phenotypic plasticity may also be important for buffering these ecological thresholds (i.e., regime shifts) as many physiological processes show nonlinear responses to gradual environmental changes, conferring the appearance of resistance before the effects at the organism and population levels are visible. However, the potential involvement of plant plasticity in driving catastrophic shifts in seagrass ecosystems has not yet been assessed. In this study, we conducted a manipulative 6-month light-gradient experiment in the field to capture nonlinearities of the physiological and population responses of the seagrass *Cymodocea nodosa* to gradual light reduction. The aim was to explore if and how the photo-acclimatory responses of shaded plants are translated to the population level and, hence, to the ecosystem level. Results showed that the seagrass population was rather stable under increasing shading levels through the activation of multilevel photo-acclimative responses, which are initiated with light reduction and modulated in proportion to shading intensity. The activation of photo-physiological and metabolic compensatory responses allowed shaded plants to sustain nearly constant plant productivity (metabolic carbon balance) along a range of shading levels before losing linearity and starting to decline. The species then activated plant- and meadow-scale photo-acclimative responses and drew on its energy reserves (rhizome carbohydrates) to confer additional population resilience. However, when the integration of all these buffering mechanisms failed to counterbalance the effects of extreme light limitation, the population collapsed, giving place to a phase shift from vegetated to bare sediments with catastrophic ecosystem outcomes. Our findings evidence that ecological thresholds in seagrass ecosystems under light limitation can be explained by the role of species' compensatory responses in modulating population-level responses. The thresholds of these plastic responses anticipate the sudden loss of seagrass meadows with the potential to be used as early warning indicators signalling the imminent collapse of the ecosystem, which is of great value for the real-world management of seagrass ecosystems.

1. Introduction

Exposed to increasing environmental stress, ecosystems may undergo precipitous declines with potentially catastrophic consequences for their ecosystem services. While some systems show predictable declines in ecosystem state as conditions deteriorate, others may show remarkable resistance to stressful conditions before declining abruptly

and without much prior warning. Managing these ecosystems presents a special challenge since most standard metrics of ecological health would not detect an approaching transition. This can be especially problematic if ecosystems show alternate stable states, where a series of ecological feedbacks prevent a quick recovery even after environmental conditions have improved. A wide range of terrestrial, freshwater and marine ecosystems respond to gradual environmental changes, showing

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alternative stable state dynamics (Scheffer, 2001). These regime shifts involve a persistent change in the structure and dynamics of the system and usually lead to drastic changes in the provision of ecosystem services (Hilt et al., 2017; Möllmann et al., 2015). Regardless of whether systems show alternate stable states or not, identifying early-warning signals of approaching thresholds has direct application to the real-world management of ecosystems that show non-linear dynamics. An unchanging and seemingly resistant ecosystem state could mask numerous underlying physiological and metabolic changes in the dominant structural species as it does its best to cope with declining environmental conditions. To maintain their ecological state, species may be able to optimise their energetic allocations between maintenance, growth and reproduction to overcome difficult conditions. Species may also have several inherent strategies to acclimate to the new conditions by modifying their physiological, morphological or structural traits. There is, of course a natural limit to these coping mechanisms; beyond a disturbance threshold or if the disturbance persists for too long, species tolerance may no longer be sufficient to maintain ecological states, leading to precipitous ecological declines. Understanding these tolerance mechanisms is the key to be able to anticipate an approaching threshold so that ameliorative action can be taken before an ecological transition occurs.

In ecosystems dominated by a few foundational species, the structure and functioning of the entire system is closely tied in with the responses of these species (Ellison et al., 2005; Thomsen et al., 2010; Connell et al., 2017). How ecosystems respond to increasing stress will depend on the phenotypic plasticity of their dominant species to buffer change (Monaco and Helmuth, 2011; Clements and Ozgul, 2018; Pazzaglia et al., 2021). Many physiological processes show nonlinear responses to gradual environmental changes, conferring the appearance of resistance (i.e., homeostasis) before the effects at the organism and population levels are visible (Laakso et al., 2003; Dorey et al., 2013; Hennon et al., 2019). Large-scale ecosystem shifts are therefore likely to occur if any structural species in the ecosystem is sensitive to an environmental factor that varies around a critical range (Jeppesen et al., 2007). Identifying the underlying physiological parameters of these responses may help assessing the risk of an approaching threshold, making them potentially powerful early warning indicators (Connel and Ghedini, 2015). To date, however, few studies have incorporated species physiological performance and phenotypic plasticity of constituent organisms in predicting and managing ecosystem resilience (Harley et al., 2017; Dakos et al., 2019).

As foundation species, seagrasses have colonised most coastal areas worldwide, where they strongly influence the structure and function of littoral ecosystems. Seagrass meadows are one of the most valuable ecosystems on earth due to their critical contributions to people, including nutrient cycling and coastal water quality control, among others (Costanza et al., 2014; Nordlund et al., 2016). Such meadows are experiencing a global decline as a result of human activities, with eutrophication and climate change as the major causes of seagrass disappearance (Waycott et al., 2009; Orth et al., 2006; Unsworth et al., 2019). Abrupt shifts in seagrass ecosystems following extreme weather events have been documented over recent decades (i.e., marine heat-waves; Seddon et al., 2000; Fraser et al., 2014; Thomson et al., 2015; Shields et al., 2019) as well as with increased coastal inputs of nutrients (Gurbisz et al., 2014; Kemp et al., 2005; Robertson and Savage, 2020). With eutrophication, seagrasses are exposed to increasing light stress. Several studies have identified the feedback among seagrass, sediment and light as a complex interacting mechanism with the potential to induce abrupt shifts in seagrass ecosystems (Adams et al., 2016; Carr et al., 2010; Maxwell et al., 2016). By controlling nutrient levels in the water column and stabilising sediments, seagrasses enhance the availability of light for their growth, creating a self-facilitating environment (van der Heide et al., 2007, 2011). However, when eutrophication persists and nutrient loads continue to increase, seagrasses may suddenly decline as a result of complex ecosystem responses including

increased turbidity, algal blooms and epiphyte loads (Ralph et al., 2007; Cabaço et al., 2013). The severe light limitation that characterises these conditions is a major cause of seagrass decline under eutrophication (e.g., Hauxwell et al., 2003; Burkholder et al., 2007). Climate change is likely to worsen these conditions by increasing the interaction among stressors, further reducing the amount of light seagrass meadows receive (Short and Neckles 1999; Duarte et al., 2013; Pazzaglia et al., 2020). These impacts are likely to be particularly acute in sheltered and semi-enclosed water bodies (Lloret et al., 2008; Le Fur et al., 2019). Climate-driven changes in rainfall patterns, storm activity and coastal currents are enhancing terrestrial runoff, coastal erosion and sediment resuspension, increasing water turbidity and increasing overall light stress on seagrasses. Light limitation is therefore an increasing threat to marine plants, likely to be responsible for triggering abrupt shifts in seagrass ecosystems and furthering their global decline (e.g., Shields et al., 2019; Ralph et al., 2007; Short and Neckles 1999; McMahon et al., 2013).

The photo-physiology of seagrasses dealing with light limitation often follows complex and nonlinear responses (e.g., Hedley et al., 2014; Olesen et al., 2002; Collier et al., 2009; Dattolo et al., 2017). It is likely that plant physiological responses play a role in large-scale ecosystem transitions. Thus far however, little is known of how these physiological parameters drive ecosystem conditions and if these can be used as early-warning indicators of population- and ecosystem-level collapse. In this study, we used a controlled field experiment to explore the photo-physiological responses of seagrass to reducing light. We hypothesise that thresholds of seagrass decline are explained, at least in part, by nonlinearities in how light reduction translates to physiological and plant fitness responses and how these drive subsequent population dynamics that affect ecosystem-level patterns. We used a manipulative light-gradient experiment in the field to determine the physiological and population responses of the seagrass *Cymodocea nodosa* to gradual light reduction (Kreyling et al., 2018) and to explore if and how the physiology of shaded plants translates to changes at the population and the ecosystem level. Population changes (shoot density and plant biomass) were followed for the 6-month duration of the experiment to characterise nonlinear population responses and to determine the level of light reduction inducing abrupt population decline. Morphological, (photo-) physiological and plant fitness responses were also analysed after 2 months of experimental shading to detect and quantify nonlinear trait responses that may underlie subsequent population collapse and abrupt ecological transitions. These responses normally occur in a matter of a few weeks, whereas responses at the meadow-scale (i.e., biomass or shoot density) typically take longer, within a time scale of several months (McMahon et al., 2013). In addition, we use our results to describe potentially useful early warning indicators of rapid change in seagrass systems.

2. Material and methods

2.1. Study species and experimental design

The seagrass *Cymodocea nodosa* is a species characteristic of shallow bays where light regimes are highly dynamic and can also colonise and thrive in deep waters (Borum and Greeve, 2004; Green and Short, 2003). The ability of the species to survive and succeed across a wide range of habitats with contrasting light conditions is likely linked to a suite of photo-acclimative responses acting at different levels of biological organisation (Olesen et al., 2002; Olivé et al., 2013; Schubert et al., 2018; Silva et al., 2013). However, the seagrass has recently suffered sudden losses in the wake of a gradual episode of eutrophication, indicating that it has strong non-linear responses to declining light (Mar Menor coastal lagoon, Torrente et al., 2019).

We conducted a field-based light-gradient experiment in 2018 in a homogenous, monospecific *C. nodosa* meadow (average shoot density \pm SE: 601 ± 45 shoots m^{-2}) growing on sandy sediments at 1 m depth in

the Mar Menor coastal lagoon (SE Spain; 37° 46' 49" N/0° 45' 44" W). We used a gradual light shading experiment with a total of nine conditions to detect potential non-linearities in the plants' photo-acclimative responses to light. We chose a gradient approach to detect ecologically important thresholds and non-linear relationships (Kreyling et al., 2014, 2018). The experiment was conducted from late spring (May) to late autumn (November) to encompass the seasonal growth cycle of the species in this region (Terrados and Ros, 1992). Nine light treatments (L1–L9) were selected to encompass a broad range of light availabilities, from natural light condition (L1 = 0% light reduction) to a highly intense shading treatment (L9 = 94% of light reduction). Neutral shading screens (1.2 × 1.2 m) were randomly allocated within the seagrass bed to reproduce the selected light conditions. Preliminary studies showed that these structures do not alter the physico-chemical conditions of the bottom, minimising the likelihood of experimental artifacts or any factor other than light (Bernardeau-Esteller et al., 2015). Shading screens were periodically cleaned (every 1–2 weeks) to maintain consistent light treatments throughout the experimental period. A 40 × 40-cm plot fixed to the seabed below each shading screen was used to monitor *C. nodosa* responses to imposed light treatments. The vegetation surrounding the plots was removed to avoid clonal integration and resource translocation between experimental plants within the plots and plants growing outside the shaded area (Ruocco et al., 2021; Tuya et al., 2013). The light field in each treatment was continuously recorded using PAR light sensors (spherical quantum sensors, Alec MDS MK5). Sensors were installed at the top of the canopy in experimental plots, and instantaneous irradiance measurements were recorded every 10 min. To calculate the percentage of subsurface irradiance (%E₀) reaching the seagrass canopy in each experimental light treatment, another PAR sensor was also installed just below the seawater surface to characterise subsurface irradiance (E₀) throughout the experiment. Sediment redox potential was determined in each experimental plot at the end of the experiment to characterise treatment-induced changes in the reducing conditions of sediments. Measures were taken in three sediment samples (i.e., upper 10 cm below the sediment-water interface) from each plot, using a Crison Pt electrode connected to a portable pH meter (Crison model 507). The electrode was calibrated with a redox standard solution (Crison 468 mV at 25 °C), and redox measurements were referred to the standard hydrogen electrode (207 mV) as described in APHA (1992).

2.2. Photo-physiological responses

Photo-physiological *C. nodosa* responses were analysed after 2 months of experimental shading to detect and quantify any potential nonlinear trait responses underlying subsequent population collapse and ecological abrupt phase transition. Maximum photosynthetic rates and respiratory rates ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) were measured for five plants from each light treatment, using an incubation chamber with a Clark-type O₂ electrode (Hansatech, UK) and following the methods described in Marín-Guirao et al. (2011). In all incubations, the central part (about 0.6 cm²) of the first mature leaf was used to standardize the measurements. Incubations consisted of a 10-min dark exposure followed by a 7-min exposure to nine increased irradiances (from 5 to 900 $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) and a final 10-min exposure to darkness to determine dark respiration (R_d). Increases in oxygen were measured for each incubation interval and plotted against their respective irradiance values to construct the photosynthesis-irradiance curve (P-E curve). Maximum net photosynthetic rates (net-P_{max}) were determined by averaging the maximum values and gross photosynthesis (gross-P_{max}) was then calculated as the sum of net-P_{max} and R_d. Saturation irradiance (E_k) was calculated as the ratio P_{max}/α; where α (photosynthetic efficiency) was calculated as the slope of the regression line fitted to the initial linear part of the P-E curve and the compensation irradiance (E_c) as the intercept on the X-axis.

Pigment content was analysed in the same five plants employed in photosynthetic incubations. Pigments were extracted by homogenising

leaf tissues in buffered acetone (80%) and maintained overnight at 4 °C to ensure complete tissue disaggregation. After sample centrifugation (10 min at 1000 g), absorbance of supernatants was measured at 470, 646, 663 and 725 nm to calculate chlorophyll *a* and *b*, according to Lichtenthaler and Wellburn (1983), and expressed as per leaf biomass ($\mu\text{g g}^{-1}$).

2.2.1. Net carbon balance

The daily metabolic carbon balance (CB) and the daily period of irradiance-saturated photosynthesis (H_{sat}) were selected as proxies of plant productivity (Denison and Alberte, 1982, 1985; Alcoverro et al., 1999; Lee and Dunton, 1996; Touchette and Bulkholder, 2000). Daily carbon balance was estimated using the function [P = [gross-P_{max} E/(E + E_k)] + R_d (Baly, 1935)], as described in Marín-Guirao et al. (2015), where P is the net photosynthesis, gross-P_{max} is the maximum gross photosynthetic rate, E is the irradiance measured in each experimental light treatment, E_k is the saturation irradiance and R_d is dark respiration. The function integrates the photosynthetic parameters obtained in the incubations (P-E curves) and the continuous recordings of irradiance obtained in light treatments to generate estimates of net production, which were integrated across 24-h periods to yield daily net production values. Finally, net productivity in oxygen units was multiplied by 0.375 to obtain the equivalent carbon units ($\text{mg C g}^{-1} \text{ FW d}^{-1}$) since the photosynthetic quotient is assumed to equal unity, and the ratio g C: g O₂ = 0.3 (Matta and Chapman, 1991). This calculation presumes constant dark respiration through the day and does not consider other carbon losses (exudation, grazing) or gains (light-independent carbon fixation). Mean daily saturation periods (H_{sat}), i.e., the proportion of the day that light intensities saturated photosynthesis (Denison and Alberte, 1985), were calculated for each treatment by averaging the number of hours per day that irradiance values exceeded the corresponding values of saturation (E_k) irradiances. The E_k values used in these calculations were those obtained from the P-E curves.

2.3. Plant fitness and morphological responses

The energy status of plants was characterised by analysing the total carbohydrate content in plant storage tissues (i.e., rhizomes) and used to assess plant fitness (Govers et al., 2015). Analyses were conducted following the anthrone assay as described in Marín-Guirao et al. (2013). Three samples from each shading treatment, each composed of pooled rhizome tissues of three shoots, were analysed. Plant height was measured after 2 months of experimental shading in five plants, randomly selected from each experimental shading treatment, to characterise morphological changes in response to light deprivation.

2.3.1. Population responses

Shoot density was monitored for the 6-month duration of the experiment to characterise nonlinear population responses and the level of light reduction at which the *C. nodosa* population abruptly declined. The number of shoots was recorded by scuba divers in each experimental plot every 1 or 2 weeks for the duration of the experiment. At the end of the experiment, all plant material was harvested and dried to constant weight to determine total plant biomass.

2.4. Statistical analysis

The identification of breakpoints (i.e. thresholds) in the responses of *C. nodosa* to light reduction was carried out by piecewise linear regression using SegReg (<http://www.waterlog.info/segreg.htm>). Segmented/piecewise regression is an effective statistical tool for modeling abrupt thresholds in ecological data (Toms and Lesperance, 2003; Ramsey et al., 2003; Rotvit and Jacobsen, 2013). The method uses an algorithm that tests multiple data fits to locate the best breakpoint that maximizes the statistical coefficient of explanation. The analysis also includes a significance test (ANOVA) to determine whether the

breakpoint gives a significant additional explanation compared to straightforward linear regression.

3. Results

3.1. Experimental light gradient

The nine experimental light treatments produced a consistent light gradient over the course of the experiment, ranging from an averaged (\pm SD) daily photosynthetic flux density of $27.43 (\pm 7.2)$ mol quanta $m^{-2} d^{-1}$ in the L1 treatment (i.e., 0% light reduction) to $1.63 (\pm 1.2)$ mol quanta $m^{-2} d^{-1}$ in the most intense shading treatment L9 (Fig. 1). The percentages of light reduction in each treatment with respect of natural light levels were 0, 18, 43, 56, 64, 66, 78, 87 and 94% from the least to the most intense shading treatments. These values corresponded respectively to 74, 60, 42, 32, 26, 25, 16, 10 and 4% subsurface irradiance (E_0).

At the end of the experiment, the sediment redox potential in experimental plots did not show any clear pattern across the light gradient (Supplementary Table S1). Values ranged between -144.9 ± 51 mV in the L1 treatment to -247.7 ± 38 mV in the L4 treatment, with an average (\pm SD) value of -196.4 ± 33 mV for all nine light treatments.

3.1.1. Patterns of photo-physiological responses to gradual light reduction

The photo-physiological response of plants to light reduction showed a nonlinear pattern for all photosynthetic variables, with breakpoints between 52.6 and 64.9% of light reduction ($p < 0.05$; Table 1; Fig. 2). Gross photosynthetic rates (P_{max}) were almost constant from full light (0% of shading) up to the breakpoint of 52.6% of irradiance (slope: -0.020), after which P_{max} began to drop steeply with a trend that was 56 times greater (slope: -1.128). Respiratory rates of plants also displayed a nonlinear response and a breakpoint at 56.4% of shading intensity ($p = 0.003$; Table 1; Fig. 2b). Respiration showed a biphasic response with average respiratory rates (\pm SE) before and after the breakpoint of 18.5 ± 0.2 and 13.5 ± 0.5 $\mu mol O_2 g^{-1} FW h^{-1}$, respectively. Compensation irradiance (E_c) and saturating irradiance (E_k) both showed a breakpoint once again at 56.4% of light reduction (Table 1; Fig. 2c and d). Averaged E_c values (\pm SE) before and after the cut-off point were 13 ± 0.6 and 7 ± 0.2 $\mu mol quanta m^{-2} s^{-1}$, respectively, whereas the corresponding values for E_k were 64 ± 4 and 41 ± 2 $\mu mol quanta m^{-2} s^{-1}$. The photosynthetic quantum efficiency (α) also responded to gradual shading in a nonlinear response fashion and showed a breakpoint somewhat later, at 64.9% of light reduction (Table 1; Fig. 2e). The efficiency of experimental plants progressively increased before the breakpoint (from 1.3 ± 0.17 to 1.66 ± 0.17 $\mu mol O_2 g^{-1} FW h^{-1} / \mu mol quanta m^{-2} s^{-1}$) and jumped to a higher stable level (1.92 ± 0.04 $\mu mol O_2 g^{-1} FW h^{-1} / \mu mol quanta m^{-2} s^{-1}$) beyond that point.

The analysis also identified a nonlinear response pattern to light

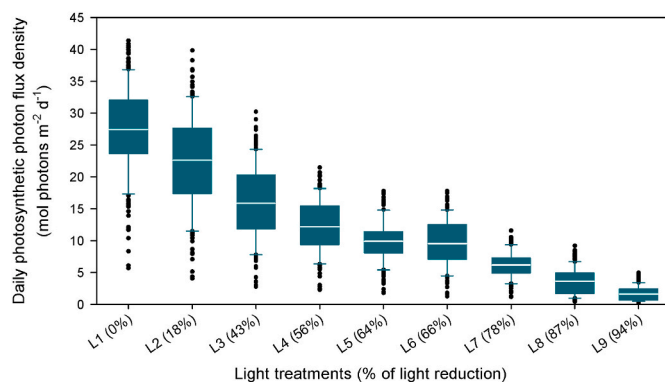


Fig. 1. Integrated daily photosynthetic flux density recorded in the nine experimental light treatments during the course of the experiment.

Table 1

Results from the sequential analysis (Rodionov, 2004) and Chow test for the identification of cut-off points in the multilevel response of *C. nodosa* to gradual light reduction. n.s. non-significant Chow test. * The Chow test cannot be performed when just one treatment is included in one of the two groups identified by the Rodionov sequential analysis.

Response level	Variable	Breakpoint	ANOVA
		(% light reduction)	(p-value)
Physiology	Gross photosynthesis (gross-P)	52.6	0.004
	Respiration (R_d)	56.4	0.003
	Compensation point (E_c)	56.4	0.043
	Saturating point (E_k)	56.4	0.001
	Photosynthetic efficiency (α)	64.9	0.024
	Chlorophyll <i>a</i>	85.5	0.008
Productivity	Chlorophyll <i>b</i>	85.5	0.001
	Daily carbon balance (CB)	72.4	0.001
Fitness	Daily saturation period (H_{sat})	82.7	0.001
	Total non-structural carbohydrates (TNC)	78.0	0.010
Morphology	Plant height	66.8	0.013
Population	Total biomass	65.8	0.008
	Shoot density	86.5	0.024

reduction for chlorophyll *a* and chlorophyll *b* (Supplementary Fig. S1), with a breakpoint at 85.5% of light reduction ($p < 0.01$; Table 1). Photosynthetic pigments linearly increased with shading up to the breakpoint, but further light reductions caused stronger chlorophyll increments for plants from the most intense treatment, showing leaf Chl *a* and Chl *b* concentrations 62% and 81% higher than those of plants under full sunlight, respectively.

3.2. Pattern of net carbon balance to gradual light reduction

The daily metabolic carbon balance (CB) and the daily period of irradiance-saturated photosynthesis (H_{sat}) showed nonlinear responses to increasing shading, with breakpoints at 72.4% and 82.7% light reduction, respectively (Table 1; Fig. 3). The CB of shaded plants decreased slightly with increasing shading before the breakpoint. Beyond that level, however, further shading produced a sharp CB decrease with a slope that was seven times higher than that observed before the breakpoint (Fig. 3). The CB values in the three most intense shading treatments (i.e., 78, 87 and 94% light reduction) were, respectively, 44, 71 and 98% lower than those of plants in the ambient light treatment with no shading. Similarly, H_{sat} was almost unaffected up to the 78% light reduction treatment, with values ranging from 11.4 to 10.1 h. Further shading caused more drastic reductions, and under the most intense shading treatment, H_{sat} was shorter than 5 h.

3.3. Patterns of rhizome carbohydrates and plant morphological responses to gradual light reduction

The energetic status of experimental plants (i.e., rhizome content in total non-structural carbohydrates, TNC) also showed a non-linear response to progressive light reduction, with a breakpoint, identified by piecewise linear regression, at 78% light reduction ($p = 0.01$; Table 1; Fig. 4). The TNC decreased linearly up to the breakpoint (from 21 to 12% of rhizome dry weight), but beyond that point, TNC decreased more steeply, resulting in carbohydrate levels lower than 6% of dry weight.

At the morphological level, the height of *C. nodosa* plants also responded in a non-linear fashion to gradual shading, with a breakpoint identified at 66.8% of light reduction (Supplementary Fig. S2; Table 1). Plants from the most intense shading treatments (i.e., those beyond the breakpoint: 78, 87 and 94% of light reduction) were twice as high as plants under full sunlight and 66% higher than the average of the treatments before the breakpoint (31.8 ± 1.0 vs 19.1 ± 0.7).

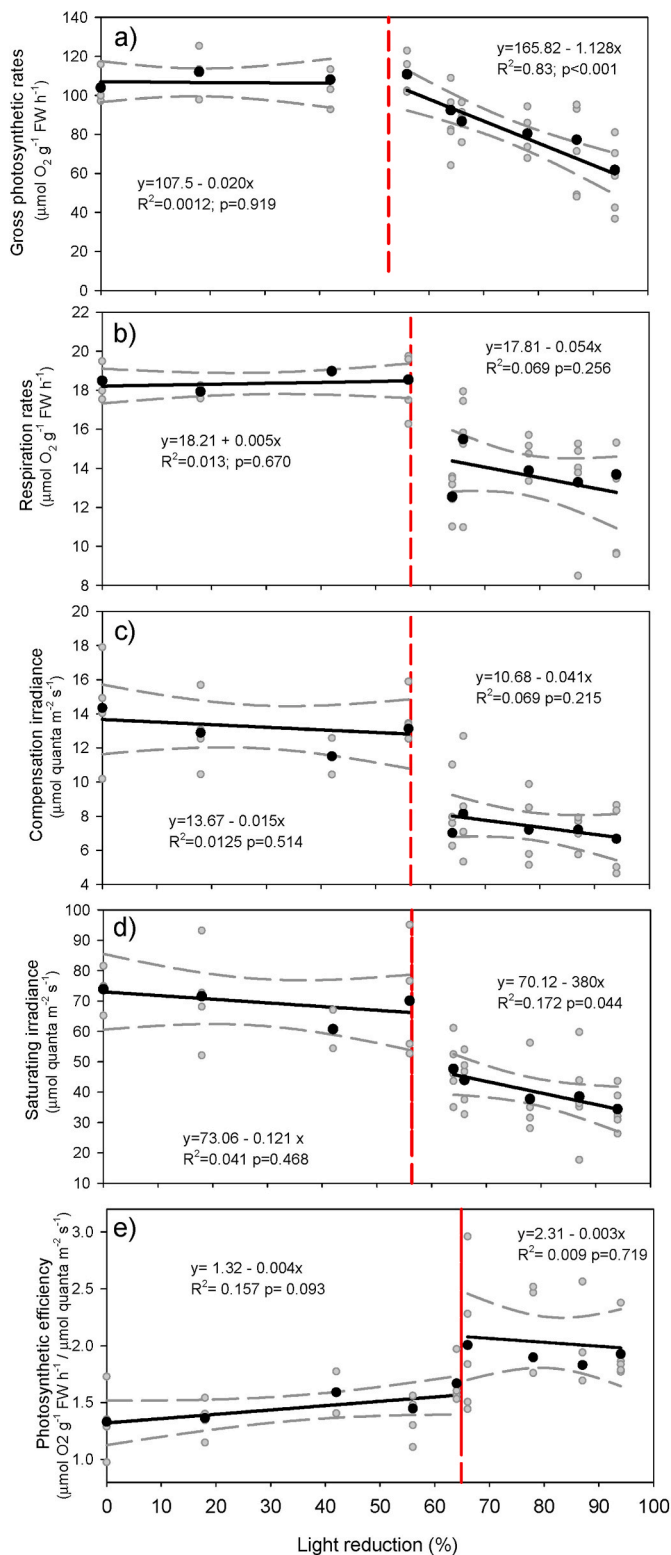


Fig. 2. Gross photosynthetic rates, P_{max} (a), respiratory rates, R_d (b), compensation irradiance, I_c (c), saturating irradiance, E_k (d) and photosynthetic quantum efficiency, α (e) of *C. nodosa* plants after 2 months of experimental shading. Dashed vertical lines in red indicate the breakpoints detected in piecewise linear regressions using all samples (grey dots; $n = 5$). Black dots represent the average value for each light treatment. Solid black lines represent the linear regression of treatments before and after the breakpoint and dashed grey lines the 95% confidence bands; regression equations are shown in the graph.

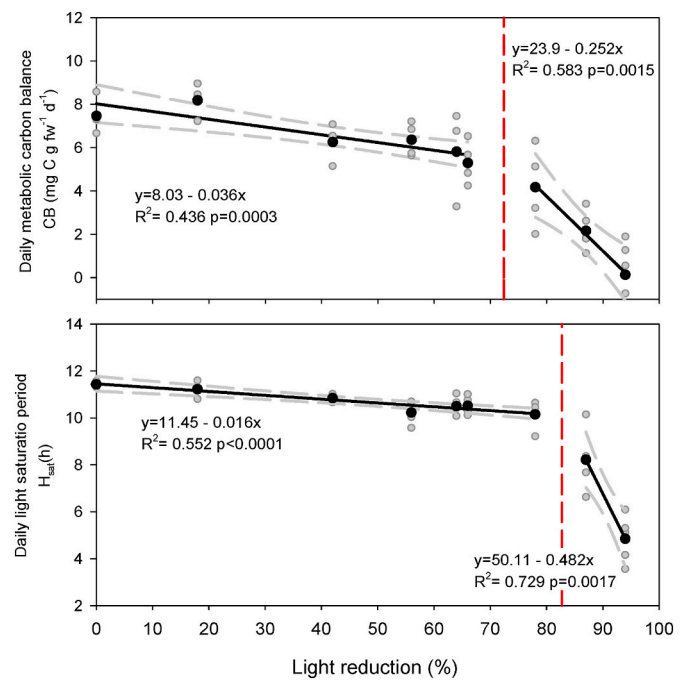


Fig. 3. Daily metabolic carbon balance (CB; upper panel) and daily light saturation period (H_{sat} ; lower panel) of experimental plants along the gradient of light reduction. Values represent the integration of the photo-physiological measurements with the continuous light recordings of the previous two months. Dashed vertical lines in red indicate the breakpoints detected in piecewise linear regressions using all samples (grey dots; $n = 5$). Black dots show the average value for each light treatment. Solid black lines represent the linear regression of treatments before and after the breakpoint and dashed grey lines the 95% confidence bands; regression equations are shown in the graph.

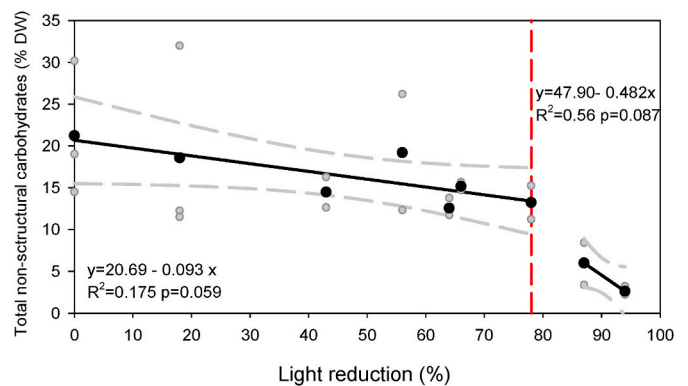


Fig. 4. Total non-structural carbohydrate content in rhizomes of experimental plants along the light reduction gradient. Vertical dashed line in red indicates the breakpoint detected in the piecewise linear regression using all samples (grey dots; $n = 3$). Black dots represent the average value for each light treatment. Solid black lines represent the linear regression of treatments before and after the breakpoint and dashed grey lines the 95% confidence bands; regression equations are shown in the graph.

3.4. Pattern of population responses to gradual light reduction

Total plant biomass responded in a nonlinear fashion to gradual shading and exhibited a breakpoint at 65.8% light reduction at the end of the experimental shading period (Table 1; Fig. 5). Total biomass before the breakpoint ($87.4 \pm 5.5 \text{ g DW m}^{-2}$) was, on average, almost twice as high as the biomass of treatments after the breakpoint ($46.0 \pm 6.2 \text{ g DW m}^{-2}$).

The evolution of net shoot change in light treatments through the

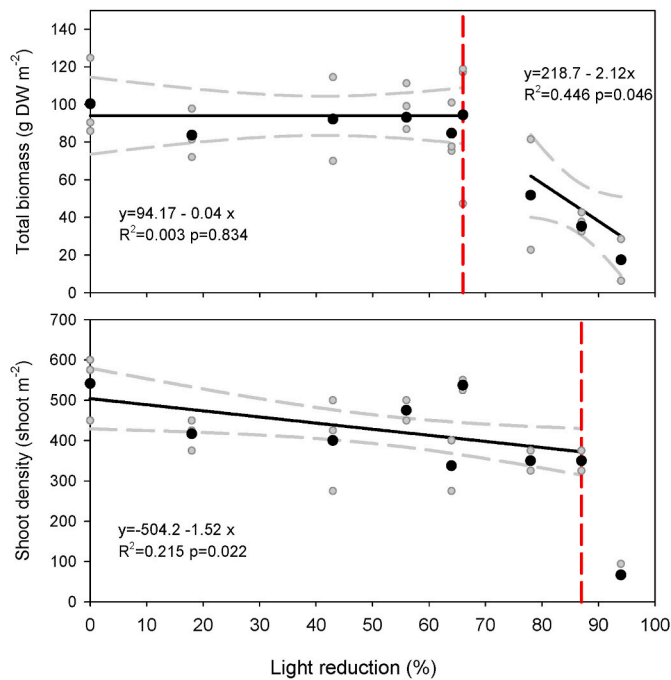


Fig. 5. Total plant biomass (upper panel) and shoot density (lower panel) along the experimental light gradient at the end of the experiment. Dashed vertical lines in red indicate the breakpoints detected in the piecewise linear regressions using all samples (grey dots; $n = 3$). Black dots represent the average value for each light treatment. Solid black lines represent the linear regression of treatments before and after the breakpoint and dashed grey lines the 95% confidence bands; regression equations are shown in the graph.

course of the experiment is shown in [Supplementary Fig. S3](#). In contrast to the most intense shading treatment, in which shoot density decreased dramatically during the experiment, the evolution of net shoot change in the other treatments remained relatively stable. At the end of the 6-month experimental period, shoot density in experimental plots showed a breakpoint at 86.5% light reduction ([Table 1](#), [Fig. 5](#)). Shoot density linearly decreased with increasing shading, suffering 35% reduction from the treatment without shading to the severe treatment of the breakpoint (from 542 to 350 shoots m^{-2}). Beyond that point, however, shoot density drastically dropped by about 90%.

4. Discussion

Several underlying photo-acclimative mechanisms explain the extraordinary resilience of *Cymodocea nodosa* to extreme light limitation, allowing the plant to resist a 80–90% light reduction before an abrupt decline in the population. These underlying mechanisms are manifested long before changes become evident at the population level (i.e., shoot density). As light reduces, it triggers a series of non-linear physiological and morphological feedbacks that serve to maintain a stable carbon balance even when the light reaching the plant had reduced to half. The plant appears to do this by increasing its photosynthetic efficiency, with a simultaneous reduction in respiration. As light decreased even further, plants resorted to expending their energetic reserves to counterbalance carbon limitation, and it is only when those reserves were drastically reduced that abrupt population-level effects were seen. Taken together, these changes in plant performance can be considered as physiological compensatory processes for resisting ecological thresholds (*sensu* [Connell and Ghedini, 2015](#)) and provide a mechanistic explanation for the catastrophic decline *C. nodosa* shows to extreme light reduction. They serve as a series of progressive warnings of impending collapse that may be much more powerful indicators than standard ecosystem- and population-level state variables, whose

remarkable stability may mask dramatic changes in ecosystem health.

Maintaining carbon balance is critical for plant survival, and *C. nodosa* shows considerable physiological and morphological plasticity to ensure it is kept stable under increasing shading levels. The plant employs a series of regulatory feedback mechanisms to maintain plant efficiency before dipping into its energetic stores. Shaded plants were able to maintain their photosynthetic rates at low to moderate light reduction (0–53%; [Fig. 2](#)), with an improved light capture efficiency promoted by increased chlorophyll biosynthesis ([Supplementary Fig. 2S](#); [Mc Mahon et al., 2013](#); [Lee et al., 2007](#)). Further shading, however, progressively reduced plant photosynthesis despite photosynthetic pigments continuing to increase. Beyond a certain point, further pigment production may not confer any additional efficiency because of pigment self-shading – called the package effect, which describes a loss of linearity between light absorption efficiency and pigment concentrations ([Cummings and Zimmerman, 2003](#); [Enríquez, 2005](#)). As photosynthesis began to decline beyond 53% light reduction, the respiratory activity of shaded plants declined sharply (ca. 30%) as a likely compensatory metabolic response to light deprivation ([Noguchi, 2005](#); [Touchette and Burkholder, 2000](#)). This response, together with the increase in pigments, favoured the enhancement of the photosynthetic quantum efficiency (α) of shaded plants and the reduction of their light compensation (E_c) and light saturating (E_k) points ([Falkowski and Raven, 2007](#)). Thanks to the integration of these photo-physiological and metabolic adjustments, overall plant productivity (i.e., the proxies CB and H_{sat}) remained relatively unchanged even when light was reduced to one third ([Dennison and Alberte 1982, 1985](#); [Zimmerman et al., 1995](#)). It was only at further shading levels (i.e., 73–83% light reduction) that the plant carbon balance declined abruptly.

Coinciding with the reduction in the plant carbon balance (i.e., CB), the population showed a substantial reduction in standing biomass at the same time as plants became increasingly taller as shoots grew towards the water surface to find a more favourable light environment for photosynthesis ([Ralph et al., 2007](#)). The reduction in seagrass biomass, promoted mainly by belowground biomass losses, is a fundamental structural response at the meadow level to cope with decreasing irradiances since it facilitates functional adjustments in whole-plant carbon balances ([Collier et al., 2007](#); [Enríquez et al., 2019](#); [Olesen et al., 2002](#)). In addition, the population also shows a gradual decline in shoot density, which is another key long-term response to light reduction at the meadow scale potentially serving to reduce leaf self-shading and facilitating light penetration within the seagrass canopy ([Collier et al., 2007](#); [Dalla Via et al., 1998](#); [Enríquez et al., 2019](#); [Enríquez and Pantoja-Reyes, 2005](#); [Mackey et al., 2007](#); [Olesen et al., 2002](#)). These modifications at the level of plant architecture and meadow structure would help to partially offset the impact of severe low-light stress and the maintenance of relatively dense populations.

Because all these photo-acclimative responses are not completely effective under such intense shading conditions, *C. nodosa* also falls back on its energy reserves to make up for the abrupt decline in carbon gains. The carbohydrate content in plant rhizomes decreased gradually with shading, and it is only beyond that intense shading level (i.e., 78% light reduction), that the plant registers a sharp drop, signalling a collapse in its energy stores. Dipping into rhizome reserves is costly since this has direct consequences for plant fitness and survival ([Touchette and Burkholder, 2000](#)). It is clearly a mechanism of the last resort but enables *C. nodosa* to maintain its shoot density in much more light-limited conditions than multilevel photo-acclimative feedback mechanisms alone would allow. Beyond this point, *C. nodosa* suffers from starvation and shoot density eventually collapses. Remarkably, this occurs when light is practically absent – with a 95% reduction of ambient light levels. Taken together, this series of resistance mechanisms may explain the extraordinary success of *C. nodosa* in the different light regimes in which it is found, from shallow highly lit waters to deep, light-limited environments and in estuaries where light conditions can change dramatically (e.g., [Belando et al., 2021](#); [Pérez and Romero, 1994](#); [Silva et al.,](#)

2013).

Despite this resistance, the decline of the *C. nodosa* ecosystem, when it occurs, is abrupt and unpredictable. The recent ecosystem-wide collapse of *C. nodosa* meadows in the Mar Menor is a classic case in point, where several months of severe light limitation led to the widespread loss of seagrass meadows (Pérez-Ruzafa et al., 2019; Torrente et al., 2019) which had remained stable during previous decades under increasing eutrophication pressure (Belando et al., 2021). Predicting such imminent ecosystem collapses has been a challenge for ecology and ecosystem management. When complex systems show abrupt transitions, the consequences of state changes can be far-ranging and sometimes permanent. The last two decades have seen a growing interest in identifying reliable signals of an impending collapse, linked to subtle changes observed in key state variables including increased variance, flickering, etc. (Dakos et al., 2013). Importantly, most of these critical transition indicators are phenomenological rather than mechanistic. In the case of *C. nodosa*, its photo-acclimative responses to light reduction represents a clear set of compensatory mechanisms and plant responses that, together, lead to a nonlinear transition in ecosystem state. The progressive photo-acclimative changes and fitness responses represent a series of step changes in overall plant performance that eventually translate into dramatic shifts in the population. The abrupt change in the population seen at extreme low light levels is a function of the abrupt changes observed in the carbon balance. This mechanism may also have been at work in the nutrient-enrichment experiments by Connel et al. (2017), which showed similar thresholds in the seagrass *Amphibolis antarctica*, most likely as a result of shading caused by epiphyte overgrowth.

Having identified mechanisms to explain abrupt transitions, it is possible to anticipate their eventual decline and to plan ameliorative actions. For seagrass managers, these could represent progressive thresholds that presage an ecosystem collapse (Fig. 6). With a linear decline in light conditions, *C. nodosa* shows staged responses, each defined by marked thresholds. Starting with physiological thresholds (photosynthesis and respiration), the species then responds with a threshold in plant fitness (carbohydrate reserves in rhizomes) and in the standing biomass of the meadow, followed by a population threshold (shoot density). Beyond this point, the population and the ecosystem itself experiences a dramatic collapse. While it was beyond the scale of this experimental study, recent observations in the Mar Menor coastal lagoon indicate that seagrass loss can result in a transition from a clear, macrophyte-dominated state to a turbid, phytoplankton-dominated state. Identifying similar thresholds for other macrophyte species may considerably advance our ability to manage these systems against eutrophication, sediment input and other light-limiting stressors. Integrating these thresholds into the real-world management of seagrass systems will give managers a significant advantage over traditional seagrass monitoring strategies that are based on the assumption of linear relationships between human pressures and habitat structure and function. These thresholds have the potential to be used as early warning indicators that signal a system approaching collapse (Ceccherelli et al., 2018). Some of these traits have already proven to be useful indicators of ecosystem condition. For example, the total non-structural carbohydrate content in storage tissues is a recognised plant-fitness trait and a robust bioindicator of light limitation in seagrasses (Mc Mahon et al., 2013; Soissons et al., 2016). This fitness-related trait can potentially help to predict sudden seagrass population declines under increasing shading conditions and likely under other environmental changes (Baruah et al., 2019). In addition, these results could inform process-based models to develop ecological forecasting tools for seagrass ecosystems and to predict ecosystem resilience (Adams et al., 2020). Mechanistic models could also be useful to forecast ecological responses to cumulative stressors and to test how present and future scenarios of climate change modify the resilience and thresholds of ecosystem collapse in seagrass meadows. Armed with this information, local managers could work to improve water quality with upstream management, coastal protection

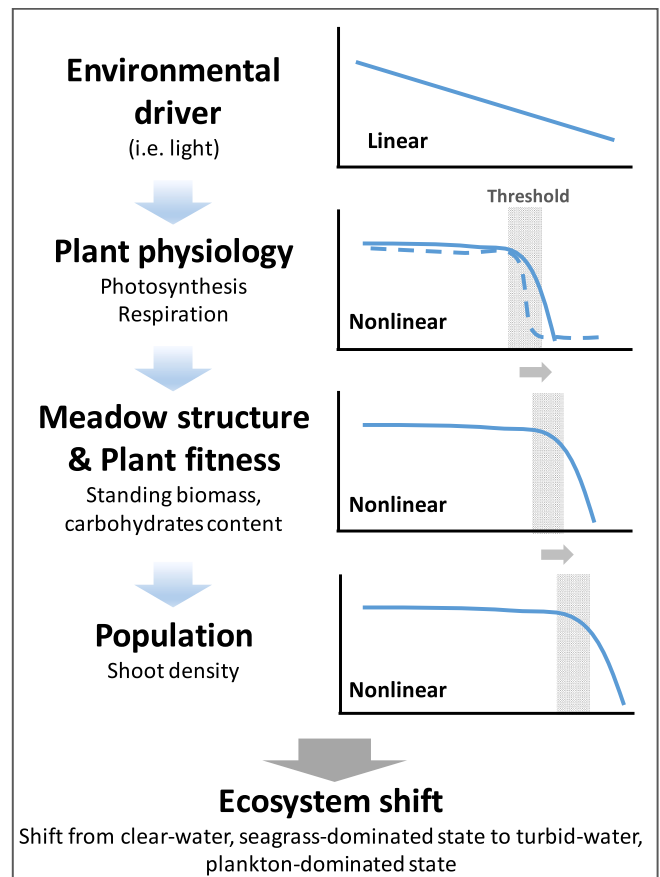


Fig. 6. Schematic representation of the results from this study. *C. nodosa* responded non-linearly to a gradual reduction in light availability. The species first showed physiological thresholds (photosynthesis and respiration) at moderate light reduction, followed by a threshold in plant fitness and meadow biomass under strong light reduction. Finally, when light reduction is extreme, a threshold occurred at the population level (shoot density), after which the population collapses dramatically. The sudden loss of seagrass meadows produces an ecosystem regime shift from clear-water, macrophyte-dominated state to turbid waters dominated by phytoplankton.

or other water quality measures long before ecosystems decline.

Author contributions

MGL, BEJ, AT and RJM conceived and designed the experiment. BEJ, BMD, GMR, RSA, MFM and RJM performed the experiment and analysed plant responses. MGL and BEJ analysed the results and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2022.105636>.

References

- Adams, M.P., Hovey, R.K., Hipsey, M.R., Bruce, L.C., Ghisalberti, M., Lowe, R.J., Gruber, R.K., Ruiz-Montoya, L., Maxwell, P.S., Callaghan, D.P., Kendrick, G.A., O'Brien, K.R., 2016. Feedback between sediment and light for seagrass: where is it important? *Limnol. Oceanogr.* 61, 1937–1955. <https://doi.org/10.1002/lno.10319>.
- Adams, M.P., Koh, E.J.Y., Vilas, M.P., Collier, C.J., Lambert, V.M., Sisson, S.A., Quiroz, M., McDonald-Madden, E., McKenzie, L.J., O'Brien, K.R., 2020. Predicting seagrass decline due to cumulative stressors. *Environ. Model. Software* 130, 104717.
- Alcoverro, T., Zimmerman, R.C., Kohrs, D.G., Alberte, R.S., 1999. Resource allocation and sucrose mobilization in light-limited eelgrass *Zostera marina*. *Mar. Ecol. Prog. Ser.* 187, 121–131.
- Apha, 1992. *Standard Methods for the Examination of Water and Wastewater*, eighteenth ed. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Pollution Control Federation (WPCF), Washington DC.
- Baruah, G., Clements, C.F., Guillaume, F., Ozgul, A., 2019. When do shifts in trait dynamics precede population declines? *Am. Nat.* 193, 633–644.
- Belando, M.D., Bernardeau-Esteller, J., Paradinas, I., Ramos-Segura, A., García-Muñoz, R., García-Moreno, P., Marín-Guirao, L., Ruiz, J.M., 2021. Long-term coexistence between the macroalga *Caulerpa prolifera* and the seagrass *Cymodocea nodosa* in a Mediterranean lagoon. *Aquat. Bot.* 173, 103415. <https://doi.org/10.1016/j.aquabot.2021.103415>.
- Bernardeau-Esteller, J., Ruiz, J.M., Tomas, F., Sandoval-Gil, J.M., Marín-Guirao, L., 2015. Photoacclimation of *Caulerpa cylindracea*: light as a limiting factor in the invasion of native Mediterranean seagrass meadows. *J. Exp. Mar. Biol. Ecol.* 465, 130–141.
- Borum, J., Greve, T.M., 2004. The four European seagrass species. In: Borum, J., Duarte, C.M., Krause-Jensen, D., Greve, T.M. (Eds.), *European Seagrasses: an Introduction to Monitoring and Management*, EU Project Monitoring and Managing of European Seagrasses (M&MS), vol. 88. <http://www.seagrasses.org/>.
- Burkholder, J.M., Tomasko, D.A., Touchette, B.W., 2007. Seagrasses and eutrophication. *J. Exp. Mar. Biol. Ecol.* 350, 46–72.
- Cabaço, S., Apostolaki, E.T., García-Marín, P., Gruber, R., Hernández, I., Martínez-Crego, B., Mascará, O., Pérez, M., Prathep, A., Robinson, C., Romero, J., Schmidt, A. L., Short, F.T., van Tussenbroek, B.I., Santos, R., 2013. Effects of nutrient enrichment on seagrass population dynamics: evidence and synthesis from the biomass–density relationships. *J. Ecol.* 101, 1552–1562. <https://doi.org/10.1111/1365-2745.12134>.
- Carr, J., D'Odorico, P., McGlathery, K., Wiberg, P., 2010. Stability and bistability of seagrass ecosystems in shallow coastal lagoons: role of feedbacks with sediment resuspension and light attenuation. *J. Geophys. Res.* 115, G03011. <https://doi.org/10.1029/2009JG001103>.
- Ceccherelli, G., Oliva, S., Pinna, S., Piazzini, L., Procaccini, G., Marín-Guirao, L., Bulleri, F., 2018. Seagrass collapse due to synergistic stressors is not anticipated by phenological changes. *Oecologia* 186, 1137–1152. <https://doi.org/10.1007/s00442-018-4075-9>.
- Clements, C.F., Ozgul, A., 2018. Indicators of transitions in biological systems. *Ecol. Lett.* 21, 905–919.
- Collier, C.J., Lavery, P.S., Masini, R.J., Ralph, P.J., 2007. Morphological, growth and meadow characteristics of the seagrass *Posidonia sinuosa* along a depth-related gradient of light availability. *Mar. Ecol. Prog. Ser.* 337, 103–115.
- Collier, C.J., Lavery, P.S., Ralph, P.J., Masini, R.J., 2009. Shade-induced response and recovery of the seagrass *Posidonia sinuosa*. *J. Exp. Mar. Biol. Ecol.* 370, 89–103.
- Connell, S.D., Fernandes, M., Burnell, O.W., Doubleday, Z.A., Griffin, K.J., Irving, A.D., Leung, J.Y.S., Owen, S., Russell, B.D., Falkenberg, L.J., 2017. Testing for thresholds of ecosystem collapse in seagrass meadows. *Conserv. Biol.* 31, 1196–1201.
- Connell, S.D., Ghedini, G., 2015. Resisting regime-shifts: the stabilising effect of compensatory processes. *Trends Ecol. Evol.* 30, 513–515.
- Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S.J., Kubiszewski, I., et al., 2014. Changes in the global value of ecosystem services. *Global Environ. Change* 26, 152–158. <https://doi.org/10.1016/j.gloenvcha.2014.04.002>.
- Cummings, M.E., Zimmerman, R.C., 2003. Light harvesting and the package effect in the seagrasses *Thalassia testudinum* Banks ex König and *Zostera marina* L.: optical constraints on photoacclimation. *Aquat. Bot.* 75, 261–274.
- Dakos, V., Matthews, B., Hendry, A.P., Levine, J., Loeuille, N., Norberg, J., Nosil, P., Scheffer, M., De Meester, L., 2019. Ecosystem tipping points in an evolving world. *Nature Ecology & Evolution* 3, 355–362.
- Dakos, V., van Nes, E.H., Scheffer, M., 2013. Flickering as an early warning signal. *Theor. Ecol.* 6, 309–317. <https://doi.org/10.1007/s12080-013-0186-4>.
- Dalla Via, J., Strumbauer, C., Schonweger, G., Sotz, E., Mathekowitsch, S., Stifter, M., Rieger, R., 1998. Light gradients and meadow structure in *Posidonia oceanica*: ecomorphological and functional correlates. *Mar. Ecol. Prog. Ser.* 163, 267–278.
- Dattolo, E., Marín-Guirao, L., Ruiz, J.M., Procaccini, G., 2017. Long-term acclimation to reciprocal light conditions suggests depth-related selection in the marine foundation species *Posidonia oceanica*. *Ecol. Evol.* 7 (4), 1148–1164.
- Dennison, W.C., Alberte, R.S., 1982. Photosynthetic responses of *Zostera marina* L. (eelgrass) to in situ manipulations of light intensity. *Oecologia* 55, 137–144.
- Dennison, W.C., Alberte, R.S., 1985. Role of daily light period in the depth distribution of *Zostera marina* (eelgrass). *Mar. Ecol. Prog. Ser.* 25, 51–61.
- Dorey, N., Lançon, P., Thorndyke, M., Dupont, S., 2013 Nov. Assessing physiological tipping point of sea urchin larvae exposed to a broad range of pH. *Global Change Biol.* 19 (11), 3355–3367. <https://doi.org/10.1111/gcb.12276>. Epub 2013 Jul 29. PMID: 23744556.
- Duarte, C.M., Losada, I.J., Hendriks, I.E., Mazarrasa, I., Marbà, N., 2013. The role of coastal plant communities for climate change mitigation and adaptation. *Nat. Clim. Change* 3, 961–968. <https://doi.org/10.1038/nclimate1970>.
- Ellison, A.M., Bank, M.S., Clinton, B.D., Colburn, E.A., Elliott, K., Ford, C.R., Foster, D.R., Kloepfel, B.D., Knoepp, J.D., Lovett, G.M., Mohan, J., Orwig, D.A., Rodenhouse, N. L., Sobczak, W.V., Stinson, K.A., Stone, J.K., Swan, C.M., Thompson, J., Von Holle, B., Webster, J.R., 2005. Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Front. Ecol. Environ.* 3, 479–486.
- Enríquez, S., 2005. Light absorption efficiency and the package effect in the leaves of the seagrass *Thalassia testudinum*. *Mar. Ecol. Prog. Ser.* 289, 141–150.
- Enríquez, S., Olivé, I., Cayabyab, N., et al., 2019. Structural complexity governs seagrass acclimatization to depth with relevant consequences for meadow production, macrophyte diversity and habitat carbon storage capacity. *Sci. Rep.* 9, 14657. <https://doi.org/10.1038/s41598-019-51248-z>.
- Enríquez, S., Pantoja-Reyes, N.I., 2005. Form-function analysis of the effect of canopy morphology on leaf self-shading in the seagrass *Thalassia testudinum*. *Oecologia* 145, 234–242. <https://doi.org/10.1007/s00442-005-0111-7>.
- Falkowski, P.G., Raven, J.A., 2007. *Aquatic Photosynthesis*. Blackwell Scientific Publishers, Oxford.
- Fraser, M.W., Kendrick, G.A., Statton, J., Hovey, R.K., Zavala-Perez, A., Walker, D.I., 2014. Extreme climate events lower resilience of foundation seagrass at edge of biogeographical range. *J. Ecol.* 102, 1528–1536.
- Govers, L.L., Suykerbuyk, W., Hoppenreijns, J.H.T., Giesen, K., Bouma, T.J., van Katwijk, M.M., 2015. Rhizome starch as indicator for temperate seagrass winter survival. *Ecol. Indic.* 49, 53–60.
- Green, E.P., Short, F.T., 2003. *World Atlas of Seagrasses*. University of California Press.
- Gurbisz, Cassie, Kemp, W. Michael, 2014. Unexpected resurgence of a large submersed plant bed in Chesapeake Bay: analysis of time series data. *Limnol. Oceanogr.* 59 <https://doi.org/10.4319/lno.2014.59.2.0482>.
- Harley, C.D.G., Connell, S.D., Doubleday, Z.A., Kelaher, B., Russell, B.D., Sarà, G., Helmuth, B., 2017. Conceptualizing ecosystem tipping points within a physiological framework. *Ecol. Evol.* 7, 6035–6045.
- Hauxwell, J., Cebrian, J., Valiela, I., 2003. Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. *Mar. Ecol. Prog. Ser.* 247, 59–73.
- Hedley, J.D., McMahon, K., Fearn, P., 2014. Seagrass canopy photosynthetic response is a function of canopy density and light environment: a model for *Amphibolis griffithii*. *PLoS One* 9 (10), e111454. <https://doi.org/10.1371/journal.pone.0111454>.
- Hennon, G.M.M., Williamson, O.M., Hernández Limón, M.D., Haley, S.T., Dyhrman, S.T., 2019 Feb. Non-linear physiology and gene expression responses of harmful alga heterosigma akashiwo to rising CO₂. *Protist* 170 (1), 38–51. <https://doi.org/10.1016/j.protis.2018.10.002>. Epub 2018 Oct 24. PMID: 30576874.
- Hilt, S., Brothers, S., Jeppesen, E., Veraart, A.J., Kosten, S., 2017. Translating regime shifts in shallow lakes into changes in ecosystem functions and services. *Bioscience* 67, 928–936. <https://doi.org/10.1093/biosci/bix106>.
- Jeppesen, E., Sondergaard, M., Pedersen, A.R., Jørgensen, K., Strzelczak, A., Lauridsen, T. L., Johansson, K.S., 2007. Salinity induced regime shift in shallow brackish lagoons. *Ecosystems* 10, 47–57.
- Kemp, W.M., Boynton, W.R., Adolf, J.E., Boesch, D.F., Boicourt, W.C., Brush, G., et al., 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. *Mar. Ecol. Prog. Ser.* 303, 1–29.
- Kreyling, J., Jentsch, A., Beier, C., 2014. Beyond realism in climate change experiments: gradient approaches identify thresholds and tipping points. *Ecol. Lett.* 17 (1), 125–e1. <https://doi.org/10.1111/ele.12193>.
- Kreyling, J., Schweiger, A.H., Bahn, M., Ineson, P., Migliavacca, M., Morel-Journel, T., Christiansen, J.R., Schtickzelle, N., Larsen, K.S., 2018. To replicate, or not to replicate – that is the question: how to tackle nonlinear responses in ecological experiments. *Ecol. Lett.* 21, 1629–1638. <https://doi.org/10.1111/ele.13134>.
- Laakso, J., Kaitala, V., Ranta, E., 2003. Non-linear biological responses to disturbance: consequences on population dynamics. *Ecol. Model.* 162, 247–258. [https://doi.org/10.1016/S0304-3800\(02\)00385-X](https://doi.org/10.1016/S0304-3800(02)00385-X).
- Lee, K.-S., Duntun, K.H., 1996. Production and carbon reserve dynamics of the seagrass *Thalassia testudinum* in Corpus Christi Bay, Texas, USA. *Mar. Ecol. Prog. Ser.* 143, 201–210.
- Lee, K.-S., Park, S.R., Kim, Y.K., 2007. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: a review. *J. Exp. Mar. Biol. Ecol.* 350, 144–175. <https://doi.org/10.1016/j.jembe.2007.06.016>.

- Le Fur, I., De Wit, R., Plus, M., Oheix, J., Derolez, V., Simier, M., Malet, N., Ouisse, V., 2019. Re-oligotrophication trajectories of macrophyte assemblages in Mediterranean coastal lagoons based on 17-year time-series. *Mar. Ecol. Prog. Ser.* 608, 13–32. <https://doi.org/10.3354/meps12814>.
- Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* 11, 591–592.
- Lloret, J., Marín, A., Marín-Guirao, L., 2008. Is coastal lagoon eutrophication likely to be aggravated by global climate change? *Estuar. Coast Shelf Sci.* 78, 403–412.
- Mackey, P., Collier, C.J., Lavery, P.S., 2007. Effects of experimental reduction of light availability on the seagrass *Amphibolis griffithii*: Effects of experimental reduction of light availability on the seagrass. *Amphibolis griffithii*. *Marine Ecology Progress Series* 342, 117–126. <https://doi.org/10.3354/meps342117>.
- Marín-Guirao, L., Bernardeau-Esteller, J., Ruiz, J.M., Sandoval-Gil, J.M., 2015. Resistance of *Posidonia oceanica* seagrass meadows to the spread of the introduced green alga *Caulerpa cylindracea*: assessment of the role of light. *Biol. Invasions* 17, 1989–2009.
- Marín-Guirao, L., Sandoval-Gil, J.M., Bernardeau-Esteller, J., Ruiz, J.M., Sánchez-Lizaso, J.L., 2013. Responses of the Mediterranean seagrass *Posidonia oceanica* to hypersaline stress duration and recovery. *Mar. Environ. Res.* 84, 60–75.
- Marín-Guirao, L., Sandoval-Gil, J.M., Ruiz, J.M., Sánchez-Lizaso, J.L., 2011. Photosynthesis, growth and survival of the Mediterranean seagrass *Posidonia oceanica* in response to simulated salinity increase in a laboratory mesocosm system. *Estuar. Coast Shelf Sci.* 92, 286–296.
- Matta, J.L., Chapman, D.J., 1991. Photosynthetic responses and daily carbon balance of *Colpomenia peregrina*: seasonal variations and differences between intertidal and subtidal populations. *Mar. Biol.* 108, 303–313.
- Maxwell, P.S., Eklöf, J.S., van Katwijk, M.M., O'Brien, K.R., de la Torre-Castro, M., Boström, C., Bouma, T.J., Krause-Jensen, D., Unsworth, R.K.F., van Tussenbroek, B. I., van der Heide, T., 2017. The fundamental role of ecological feedback mechanisms for the adaptive management of seagrass ecosystems – a review. *Biol. Rev.* 92, 1521–1538. <https://doi.org/10.1111/brv.12294>.
- McMahon, K., Collier, C., Lavery, P.S., 2013. Identifying robust bioindicators of light stress in seagrasses: a meta-analysis. *Ecol. Indic.* 30, 7–15. <https://doi.org/10.1016/j.ecolind.2013.01.030>.
- Möllmann, C., Folke, C., Edwards, M., Conversi, A., 2015. Marine regime shifts around the globe: theory, drivers and impacts. *Phil. Trans. R. Soc. B* 370, 20130260. <https://doi.org/10.1098/rstb.2013.0260>.
- Monaco, C.J., Helmuth, B., 2011. Chapter three - tipping points, thresholds and the keystone role of physiology in marine climate change Research. In: Lesser, M. (Ed.), *Advances in Marine Biology*. Academic Press, pp. 123–160.
- Noguchi, K., 2005. Effects of light intensity and carbohydrates status on leaf and root respiration. In: Lambers, H., Ribas-Carbo, M. (Eds.), *Plant Respiration. From Cell to Ecosystem*. Springer, Dordrecht, ISBN 1-4020-3588-8. <https://doi.org/10.1007/1-4020-3589-6>.
- Nordlund, L.M., Koch, E.W., Barbier, E.B., Creed, J.C., 2016. Seagrass ecosystem services and their variability across genera and geographical regions. *PLoS One* 11, e0163091. <https://doi.org/10.1371/journal.pone.0169942>.
- Olesen, B., Enriquez, S., Duarte, C.M., 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.
- Olivé, I., Vergara, J.J., Pérez-Lloréns, J.L., 2013. Photosynthetic and morphological photoacclimation of the seagrass *Cymodocea nodosa* to season, depth and leaf position. *Mar. Biol.* 160, 285–297.
- Orth, R.J., Carruthers, T.J.B., Dennison, W.C., Duarte, C.M., Fourqurean, J.W., Heck, K. L., Hughes, A.R., Kendrick, G.A., Kenworthy, W.J., Olyarnik, S., Short, F.T., Waycott, M., Williams, S.L., 2006. A global crisis for seagrass ecosystems. *Bioscience* 56, 987–996.
- Pazzaglia, J., Reusch, T.B.H., Terlizzi, A., Marín-Guirao, L., Procaccini, G., 2021. Phenotypic plasticity under rapid global changes: the intrinsic force for future seagrasses survival. *Evol Appl* 14, 1181–1201. <https://doi.org/10.1111/eva.13212>.
- Pazzaglia, J., Santillán-Sarmiento, A., Helber, S.B., Ruocco, M., Terlizzi, A., Marín-Guirao, L., Procaccini, G., 2020. Does warming enhance the effects of eutrophication in the seagrass *Posidonia oceanica*? *Front. Mar. Sci.* 7, 564805. <https://doi.org/10.3389/fmars.2020.564805>.
- Pérez, M., Romero, J., 1994. Growth dynamics, production, and nutrient status of the seagrass *Cymodocea nodosa* in a mediterranean semi-estuarine environment. *Mar. Ecol.* 15, 51–64. <https://doi.org/10.1111/j.1439-0485.1994.tb00041.x>.
- Pérez-Ruzafa, A., Campillo, S., Fernández-Palacios, J.M., García-Lacunza, A., García-Oliva, M., Ibañez, H., Navarro-Martínez, P.C., et al., 2019. Long-term dynamic in nutrients, chlorophyll a, and water quality parameters in a coastal lagoon during a process of eutrophication for decades, a sudden break and a relatively rapid recovery. *Front. Mar. Sci.* 6, 1–24. <https://doi.org/10.3389/fmars.2019.00026>.
- Ralph, P.J., Durako, M.J., Enriquez, S., Collier, C.J., Doblin, M.A., 2007. Impact of light limitation on seagrasses. *J. Exp. Mar. Biol. Ecol.* 350, 176–193.
- Ramsey, P.W., Rillig, M.C., Feris, K.P., Gordon, N.S., Moore, J.N., Holben, W.E., Gannon, J.E., 2005. Relationship between communities and processes; new insights from a field study of a contaminated ecosystem. *Ecol. Lett.* 8 (11), 1201–1210.
- Robertson, B.P., Savage, C., 2020. Thresholds in catchment nitrogen load for shifts from seagrass to nuisance macroalgae in shallow intertidal estuaries. *Limnol. Oceanogr.* 66, 1353–1366. <https://doi.org/10.1002/lno.11689>.
- Rotvit, L., Jacobsen, D., 2013. Temperature increase and respiratory performance of macroinvertebrates with different tolerances to organic pollution. *Limnologia* 43 (6), 510–515.
- Ruocco, M., Entrambasaguas, L., Dattolo, E., Milito, A., Marín-Guirao, L., Procaccini, G., 2021. A king and vassals' tale: molecular signatures of clonal integration in *Posidonia oceanica* under chronic light shortage. *J. Ecol.* 109, 294–312. <https://doi.org/10.1111/1365-2745.13479>.
- Scheffer, M., Carpenter, S., Foley, J.A., Folke, C., Walker, B., 2001. Catastrophic shifts in ecosystems. *Nature* 413, 591–596.
- Schubert, N., Freitas, C., Silva, A., Costa, M.M., Barrote, I., Horta, P.A., Rodrigues, A.C., Santos, R., Silva, J., 2018. Photoacclimation strategies in northeastern Atlantic seagrasses: integrating responses across plant organizational levels. *Sci. Rep.* 8, 14825.
- Seddon, S., Connolly, R.M., Edyvane, K.S., 2000. Large-scale seagrass dieback in northern spencer gulf, south Australia. *Aquat. Bot.* 66, 297–310.
- Shields, E.C., Parrish, D., Moore, K., 2019. Short-term temperature stress results in seagrass community shift in a temperate estuary. *Estuar. Coast* 42, 755–764. <https://doi.org/10.1007/s12237-019-00517-1>.
- Short, F.T., Neckles, H.A., 1999. The effects of global climate change on seagrasses. *Aquat. Bot.* 63, 169–196. [https://doi.org/10.1016/S03043770\(98\)00117-X](https://doi.org/10.1016/S03043770(98)00117-X).
- Silva, J., Barrote, I., Costa, M.M., Albano, S., Santos, R., 2013. Physiological responses of *Zostera marina* and *Cymodocea nodosa* to light-limitation stress. *PLoS One* 8, e81058.
- Soissons, L.M., Li, B., Han, Q., van Katwijk, M.M., Ysebaert, T., Herman, P.M.J., et al., 2016. Understanding seagrass resilience in temperate systems: the importance of timing of the disturbance. *Ecol. Indic.* 66, 190–198. <https://doi.org/10.1016/j.ecolind.2016.01.030>.
- Terrados, J., Ros, J.D., 1992. Growth and primary production of *Cymodocea nodosa* (ucuria) ascherson in a mediterranean coastal lagoon: the mar menor (SE Spain). *Aquat. Bot.* 43, 63–74.
- Thomsen, M.S., Wernberg, T., Altieri, A., Tuya, F., Gulbransen, D., McGlathery, K.J., Holmer, M., Silliman, B.R., 2010. Habitat cascades: the conceptual context and global relevance of facilitation cascades via habitat formation and modification. *Integr. Comp. Biol.* 50, 158–175.
- Thomson, J.A., Burkholder, D.A., Heithaus, M.R., Fourqurean, J.W., Fraser, M.W., Statton, J., Kendrick, G.A., 2015. Extreme temperatures, foundation species, and abrupt ecosystem change: an example from an iconic seagrass ecosystem. *Global Change Biol.* 21, 1463–1474.
- Toms, J.D., Lesperance, M.L., 2003. Piecewise regression: a tool for identifying ecological thresholds. *Ecology* 84 (8), 2034–2041.
- Torrente, M.B., Ruiz, J., Muñoz, R.G., Segura, A.R., Esteller, J.B., Casero, J.J., Guirao, L. M., Moreno, P.G., Navarro, I.F., Nuez, E.F., Mercado, J.M., 2019. Collapse of macrophytic communities in a eutrophicated coastal lagoon. In: *Front. Mar. Sci. Conference Abstract: XX Iberian Symposium on Marine Biology Studies*. SIEBM XX. <https://doi.org/10.3389/conf.fmars.2019.08.00192>.
- Touchette, B.W., Burkholder, J.M., 2000. Overview of the physiological ecology of carbon metabolism in seagrasses. *J. Exp. Mar. Biol. Ecol.* 250, 169–205.
- Tuya, F., Espino, F., Terrados, J., 2013. Preservation of seagrass clonal integration buffers against burial stress. *J. Exp. Mar. Biol. Ecol.* 439, 42–46.
- Unsworth, R.K.F., McKenzie, L.J., Collier, C.J., Cullen-Unsworth, L.C., Duarte, C.M., Eklöf, J.S., Jarvis, J.C., Jones, B.L., Nordlund, L.M., 2019. Global challenges for seagrass conservation. *Ambio* 48, 801–815.
- van der Heide, T., van Nes, E.H., Geerling, G.W., et al., 2007. Positive feedbacks in seagrass ecosystems: implications for success in conservation and restoration. *Ecosystems* 10, 1311–1322. <https://doi.org/10.1007/s10021-007-9099-7>.
- van der Heide, T., van Nes, E.H., van Katwijk, M.M., Olf, H., Smolders, A.J.P., 2011. Positive feedbacks in seagrass ecosystems – evidence from large-scale empirical data. *PLoS One* 6, e16504.
- Waycott, M., Duarte, C.M., Carruthers, T.J., Orth, R.J., Dennison, W.C., Olyarnik, S., Calladine, A., Fourqurean, J.W., Heck Jr., 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., Reguzzoni, J.L., Alberte, R.S., 1995. Eelgrass (*Zostera marina* L.) transplants in San Francisco Bay: role of light availability on metabolism, growth and survival. *Aquat. Bot.* 51, 67–86.