

Photoacclimation of *Caulerpa cylindracea*: Light as a limiting factor in the invasion of native Mediterranean seagrass meadows



Jaime Bernardeau-Esteller^{a,b}, Juan Manuel Ruiz^{b,*}, Fiona Tomas^{c,d},
José Miguel Sandoval-Gil^{a,b}, Lazaro Marín-Guirao^b

^a Instituto Español de Oceanografía, Centro Oceanográfico de Murcia, Seagrass Ecology Group, C/Varadero s/n, 30740 San Pedro del Pinatar, Murcia, Spain

^b Departamento de Ciencias del Mar y Biología Aplicada, Universidad de Alicante, P.O. Box 99, 03080 Alicante, Spain

^c Instituto Mediterráneo de Estudios Avanzados (IMEDEA, UIB-CSIC), C/Miquel Marqués 21, 07190 Esporles, Illes Balears, Spain

^d Department of Fisheries and Wildlife, Oregon State University, 104 Nash Hall, Corvallis 97331, OR, USA

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ABSTRACT

Reduction in light availability caused by the canopy of the Mediterranean seagrass *Posidonia oceanica* has been suggested as a critical mechanism to resist the invasion of the exotic macroalga *Caulerpa cylindracea*. We experimentally evaluated the role of light as a limiting factor on the capacity of colonization and spread of this invasive seaweed in *P. oceanica* meadows by assessing photoacclimation responses and productivity and growth capacity of *C. cylindracea* in mesocosm and in situ light manipulation experiments. Despite the high photoacclimative plasticity developed by the alga, the light regime within the seagrass meadow during the study period was close to the minimum light requirements for growth, restricting the development capacity of this species. In addition, while increases in light availability resulting from canopy alteration also enhanced the productive capacity of the invasive seaweed in the field, such increase was not followed by gains in biomass production. Our results thus support the hypothesis that light availability has a major role in the underlying resistance of seagrass meadows to the invasion by *C. cylindracea*, but also indicate that there are additional factors related to the canopy of *P. oceanica* that further hinder the growth and colonization capacity of the alga.

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1. Introduction

A main goal for ecologists is to understand the factors and mechanisms that determine invasive success of introduced species. Phenotypic plasticity has been recognized as an important mechanism related to successful invasion processes. Plasticity enhances ecological niche breadth and allows organisms to express advantageous phenotypes in a broader range of environmental conditions, which contributes to maintain positive population growth and increases the likelihood of invasiveness (Richards et al., 2006). In addition, native communities strongly differ in their resistance to invasions (Lonsdale, 1999). Differences in susceptibility to invasion have been linked, among other processes, to biotic resistance derived from interspecific competition for resources between native and introduced species (Branch and Steffani, 2004; Theoharides and Dukes, 2007).

Abbreviations: Etotal, daily photon flux; Rd, dark respiration rate; P vs. E curve, Photosynthesis–Irradiance response curve; Pmax, maximum rate of net photosynthesis; Ek, saturating irradiance; α, photosynthetic irradiance; Ec, compensation irradiance; gross-Pmax, gross photosynthesis; chl a, chlorophyll a; chl b, chlorophyll b; Hc, daily compensation period; Hk, daily saturation period; FW, fresh weight; DW, dry weight

* Corresponding author. Tel.: +34 968 180500; fax: +34 968 184441.

E-mail address: juanm.ruiz@mu.iao.es (J.M. Ruiz).

Exotic seaweeds are a major threat to coastal marine habitats worldwide as they often have negative effects on the structure and diversity of native communities (Williams and Smith, 2007). Among all the factors influencing macrophyte communities, light is key in regulating productivity, abundance and distribution (Breeman, 1988; Kirk, 1994; Lobban and Harrison, 1997). The minimum light requirement for algal growth is reached when captured light allows organisms to balance loss processes within the tissue (e.g. respiration and exudation, Markager and Sand-Jensen, 1992). If available light in a habitat is below or close to those minimum requirements, development capacity and therefore invasive potential of exotic macrophytes will be hampered. This occurs for example in the case of the tropical red alga *Womersleyella setacea* in the Mediterranean Sea, as light requirements of this species reduce dramatically its invasion capacity at depths greater than 35 m (Cebrian and Rodriguez-Prieto, 2012). A high plasticity in photoacclimation mechanisms allows an exotic alga to develop an efficient photosynthetic response (that determines an efficient use of light) in a wide range of light conditions, that could enhance its competitive capacity and colonizing potential in new habitats (Bernardeau-Esteller et al., 2011; Marín-Guirao et al., In review; Raniello et al., 2006).

Interspecific competition for light is especially relevant in structured communities dominated by large-sized species (canopy formers) since they generate intense changes in the quality and quantity of light

available at the understory layers (Middelboe and Binzer, 2004; Reed and Foster, 1984). Such changes in light availability may be an important mechanism underlying invasion resistance of these communities if shading by the canopy creates light conditions near the minimum light requirements of the introduced species (Arenas et al., 2006). For instance, a decrease in survival of the invasive Japanese seaweed *Sargassum muticum* in nearshore marine communities in the western coast of USA has been linked to shading effects produced by the native canopy species (Britton-Simmons, 2006). The structure of macrophyte assemblages can be modified due to the action of natural (high water movements, herbivory) and human-induced (pollution, fisheries) stressors (Lobban and Harrison, 1997). A reduction in abundance of canopy-forming species following these disturbances will result in an increase in the available light in the lower layers of the community, which can promote growth of species constrained by limited light levels (Reed and Foster, 1984). Therefore, it can be assumed that the disturbance of canopy species could promote success of introduced species whose growth capacity is limited by light conditions.

The Mediterranean Sea, recognized as a hotspot of biodiversity, is one of the seas most affected by species introductions (Coll et al., 2010). To date, 3.3% of total described species in the Mediterranean Sea (more than 900, Zenetos, 2010) are considered exotic, of which 85 are macrophytes. Within this functional group, the green alga *Caulerpa cylindracea* (Sonder) [formerly *Caulerpa racemosa* (Forsskål) J. Agardh var *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque; (hereinafter *C. cylindracea*)] has a strong invasive character, rapidly colonizing most of the Mediterranean Sea (Klein and Verlaque, 2008; Piazzini et al., 2005). Under certain conditions, the alga is able to develop large biomass, which has been linked with significant changes in physico-chemical and community characteristics of receiving habitats (see Klein and Verlaque, 2008 and literature cited therein). The ecological success of this species in invading the Mediterranean Sea has been linked, in addition to other traits (e.g. vegetative and sexual reproductive success, high growth rates) to high morphological and physiological plasticity (Gacia et al., 1996; for a review see Klein and Verlaque, 2008). The alga has been described in a wide range of depths (between 0 and 60 m), suggesting a large capacity of photoacclimation. In this regard, previous studies have reported stable populations of the alga in depths close to 30 m as well as under the leaf canopy of macrophytes, indicating a high tolerance of the species to low light regimes. However, *C. cylindracea* has shown a reduced capacity to colonize healthy meadows of the dominant Mediterranean seagrass *Posidonia oceanica* (Bulleri et al., 2011; Ceccherelli et al., 2014; Katsanevakis et al., 2010), the greater structural complexity of which determines more intense shading conditions (Dalla Via et al., 1998; Enriquez et al., 1992).

Recently, Marín-Guirao et al. (in review) analyzed the photosynthetic and productive characteristics of natural *C. cylindracea* populations growing inside and outside of leaf canopies of *P. oceanica* in a highly invaded area. Results obtained in this study suggest that light availability inside the meadow exceeds the photoacclimation capacity of the alga and seem to be close to the minimum light requirements for growth, suggesting that this factor can play an important role as a mechanism of resistance of *P. oceanica* habitats to invasion. However, the methodological approach used in that study (i.e. non-experimental) precluded isolating the effect of light availability from the influence of other environmental factors that can be related to the development capacity of the algae and that thus may also be altered by canopy structure (e.g. water movement, nutrient availability).

The aim of this study was therefore to experimentally examine the role of light availability in the colonization of the meadows of *P. oceanica* by the alga, testing whether reduced light regimes within this habitat are able per se to explain the resistance phenomena observed. In order to evaluate this hypothesis two complementary experimental approaches were used. We studied photoacclimation responses (through analysis of photosynthetic performance and pigment content)

and productive and growth capacity (by assessment of carbon balance, starch content, apical elongation and variation in stolon biomass) of *C. cylindracea* in a mesocosm and a field experiment in which different light regimes were experimentally manipulated.

2. Material and methods

2.1. Mesocosm experiment

The use of a mesocosm system allowed maintaining controlled environmental conditions (temperature, light, salinity, pH), enabling us to isolate the effect of light from the influence of other factors whose variation could affect the response of the studied variables. The mesocosm system consisted of 24 glass independent aquaria of 100 l capacity. Each aquarium had its own light system (400 W halogen lamp, Aqua Medic aqualight-400), water circulation and filtration system, and contained a plastic tray (22 × 40 cm base and 10 cm high), filled with previously washed coarse sediments.

Eight light treatments (L1 to L8) were established in a range of daily photon flux values (i.e. integrated daily irradiance) comprised between 0 and 13.61 mol quanta m⁻² d⁻¹ (Table 1), which include light regimes of all natural habitats in which the alga is found in the study area (unpublished data). Each light treatment was assigned to 3 randomly selected aquaria. Daily photon flux for each treatment was determined through daily integration of instantaneous irradiance values recorded in the aquarium on a daily cycle of 12:12 h. These values were obtained using a submersible light sensor (PAR spherical quantum sensor MDS MK5, Alec Electronics, Japan) located at the same depth as the tray, with continuous readings every 10 min. The illumination system is not able to simulate the natural, bell-shaped light curve (e.g. Fig. 1). Instead, the daily 'light curve' in aquariums had a rectangular shape with a constant instantaneous irradiance throughout the illumination period (12 h) that was reached in a few minutes once the lamps were switched on and fell down to total darkness immediately after the lamps were switched off. As an example, the artificial light curve of the L6 treatment is represented in Fig. 1. Unavoidably, the difference between the natural and the artificial daily light curves can have consequences in determination of photosynthetic parameters (e.g. compensation and saturation light periods, Hc and Hk, respectively), which should be considered for the interpretation of the results obtained in the mesocosm and in the field. In this same context, differences in light quality between artificial and natural light sources must be also considered.

High quality natural seawater from a nearby, oligotrophic and unpolluted area was employed in the mesocosms. Environmental conditions inside the tanks were similar to those prevailing in the selected areas for of the stolons of *C. cylindracea* (see below) during the time of year in which the experiment took place. For that purpose, a periodic

Table 1

Summary of irradiance measurements in each light treatment in the mesocosm experiment. Data are presented as means ± standard error.

Treatment	Measurements	
	Noon instantaneous irradiance	Integrated daily irradiance
	μmol quanta m ⁻² s ⁻¹	mol quanta m ⁻² d ⁻¹
L1	0.00 ± 0.00	0.00 ± 0.00
L2	6.00 ± 0.20	0.24 ± 0.01
L3	20.97 ± 0.76	0.91 ± 0.11
L4	29.95 ± 0.99	1.29 ± 0.01
L5	43.73 ± 1.09	1.89 ± 0.04
L6	102.50 ± 3.17	4.43 ± 0.05
L7	211.77 ± 4.69	9.15 ± 0.14
L8	314. ± 9.30	13.61 ± 0.52

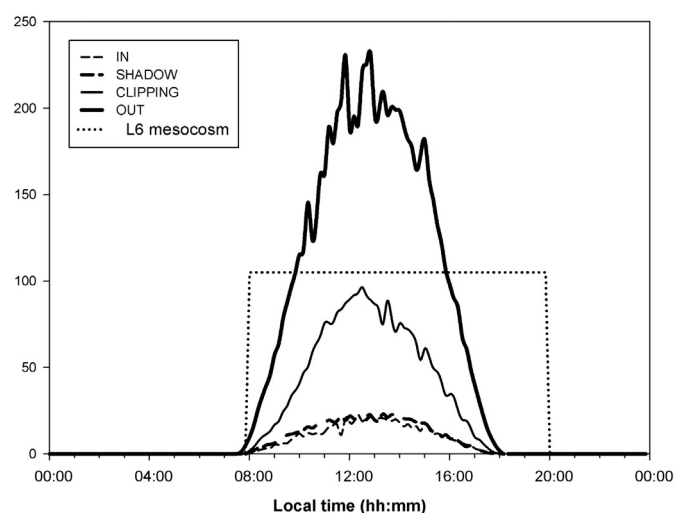


Fig. 1. Daily course of irradiance measured at the sea floor irradiance and summary of irradiance measurements obtained for each field experiment. The dotted line corresponds to the course of the daily irradiance in mesocosm aquaria (only the L6 treatment is represented as an example).

analysis of nutrients by colorimetric test (phosphorus and nitrogen; Merck®), continuous recording of pH with specific electrodes (Aqua Medic AT-Control) and a daily monitoring of the salinity of the water with a conductivimeter WTW (Model Cond. 197i) were carried out in each aquarium. Salinity values were maintained constant (37.5 PSU) by osmosis water addition. Water temperature during the experiment was 19 ± 0.1 °C and was controlled by automatic cooling system (see Marín-Guirao et al., 2011 for more details).

C. cylindracea stolons were collected by hand in a nearby population located on the southwest coast of the Region of Murcia (Isla Grosa, UTM X: 0701991, Y: 4177942, H 30S) at -11 m deep. The collection of stolons was done randomly in a large area (ca 1000 m²) to capture the natural variability. The colonized area is an infralittoral well-illuminated bottom composed by a mosaic of sand and dead *P. oceanica* rhizomes (Ruiz et al., 2011); mean noon irradiance was $195.66 \pm 3.99 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ during the experimental period (i.e. OUT treatment in Fig. 1). Collection was performed in November 2011, a period of high vegetative development (Bernardeau-Esteller, unpublished results). Immediately after collection, the cut end of the stolon was sealed with very small plastic clothes pegs to avoid loss of internal content and was put in a black plastic bag to prevent overexposure to light. Stolons selected were transported in refrigerated containers with seawater to the laboratory. Once here, they were immediately transplanted into the aquaria for acclimatization for 3 days before the start of the experiment. Four stolons were planted in each aquarium, and were of similar characteristics, with an initial length of 25–30 cm, a number of fronds ranging from 10 to 15, and a single apical meristem. During the acclimatization process, experimental units were subjected to daily photon fluxes similar to those recorded in the field (ca $4.43 \pm 0.05 \text{ mol quanta m}^{-2} \text{d}^{-1}$). After the transitional period, light conditions were changed in each aquarium to obtain the experimental light treatments. Algae were exposed to these treatments for 7 days.

2.2. Field experiment

Simultaneously with the mesocosm experiment, a field experiment was conducted in the same area where stolons of *C. cylindracea* were collected from for the mesocosms experiment. This highly colonized area is adjacent to a dense *P. oceanica* meadow, but *C. cylindracea* stolons are not able to penetrate beyond the seagrass meadow edge (Ruiz et al., 2011; Marín-Guirao et al., in review). Four experimental light treatments (two inside the seagrass meadow and two outside) were created: (i) within the meadow (IN), (ii) in areas within the meadow where the height of the leaf stratum was experimentally reduced by clipping (CLIPPING), (iii) outside the meadow (OUT), and (iv) outside the meadow but in areas where light availability was experimentally reduced (SHADED) to be similar to those of the IN treatment. The light regime of each experimental treatment was characterized based on its noon instantaneous irradiance and the integrated daily irradiance obtained from daily light cycles (Fig. 1). To this end, PAR light sensors (spherical quantum sensors; Alec MK5 MDS) were installed on the bottom of all experimental plots. Instantaneous irradiance measurements were recorded every 10 min during the 7 days of the experiment. The integrated daily irradiance (E_{total} , $\text{mol quanta m}^{-2} \text{d}^{-1}$) was obtained by the integration of these instantaneous measurements recorded in each daily cycle. Throughout the experiment, water temperature was recorded in situ by HOBO Pro v2 Water Temperature Data Logger (Onset Computer, EME Systems, Berkeley, CA, USA). The average temperature recorded during the experiment was 19.8 ± 0.1 °C.

Based on the similarities in light regimes obtained in the IN and the SHADED treatments, we would expect that algae under these conditions will present an equivalent photoacclimation response, showing a clear limitation in its production and growth capacity. Moreover, increase in light availability linked to the experimental manipulation of the canopy (CLIPPING treatment) should determine an approximation of the responses of seaweeds under this treatment to those recorded outside of the meadow (OUT treatment).

Light conditions of the SHADED treatment were obtained by using floating structures anchored to the substrate. These structures consisted of a PVC frame with neutral density filters which determined that the light regime beneath them was similar to that recorded within the meadow (IN treatment). A preliminary study showed that this type of structures do not alter the physico-chemical conditions of the bottom, minimizing the effect of any other factor different than light (Bernardeau-Esteller, unpublished results).

In the plots of the CLIPPING treatment the reduction of the leaf layer was carried out manually to obtain a final leaf length of 15 cm (the original leaf length of *P. oceanica* canopy was ca 80 cm). This reduction simulated a high rate of herbivory (e.g. Tomas et al., 2005) and determines light conditions intermediate to those obtained inside and outside the meadow (Fig. 1).

For each experimental treatment, four plots of 2×2 m² were randomly selected. Five stolons with the same characteristics as those used in the mesocosm experiment were transplanted in each plot. Before the start of the experiments, stolons were collected and sealed and then transported in darkness and refrigerated containers to the laboratory where they were marked and initial characterization was conducted (see section below referring to the phenological variables used). Subsequently, stolons were transported back to the study area where they were transplanted in the plots. Stolons were attached to the substrate by a nylon cord anchored by two stainless steel pegs tied at both ends. After seven days, algae were collected and transported back to the laboratory to perform all the measurements.

2.3. Alga analyses

In both experiments (mesocosms and field) the same response variables were considered.

2.3.1. Photosynthesis vs. irradiance curves (P vs. E curves)

Prior to photosynthetic measurements, *C. cylindracea* samples taken from the aquarium system or collection site were held overnight in the dark under controlled temperature in natural seawater.

Photosynthesis and dark respiration rates (Rd) were measured using a polarographic oxygen electrode and a magnetic stirrer (DW3, Hansatech Instruments Ltd) under controlled temperature. Incubation was carried out at the same temperature measured in the mesocosm system and in the field (19 °C). Three replicated apical segments of non-epiphytized *C. cylindracea* fronds of approximately 2 cm height were employed for the measurements. Dark respiration rates were measured by maintaining the fronds in the dark for 15 min. Net oxygen production was then determined at 9 different light intensities (from 1 to 700 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) using a high intensity light source which consists of an array of 36 red LED's (LH36/2R, Hansatech Instruments Ltd). Net photosynthetic rates were plotted against the light intensities (P vs. E curves), and the photosynthetic parameters were calculated as follows: the maximum rate of net photosynthesis (Pmax) was determined by averaging the maximum values above the saturating irradiance (Ek). The photosynthetic efficiency (α , $\mu\text{mol O}_2 \text{ g FW}^{-1} \text{ h}^{-1} / \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) was calculated as the slope of the regression line fitted to the initial linear part of the P vs. E curve, and the compensation irradiance (Ec) as the intercept on the X-axis. Ek was calculated as the ratio Pmax/ α . Gross photosynthesis (gross-Pmax) was calculated as the sum of Pmax and Rd.

2.3.2. Pigment content

Pigment content was determined in the same apical segments of fronds selected for obtaining P vs. E curves ($n = 3$). The analysis was conducted spectrophotometrically after manual extraction of a homogenized suspension using 90% acetone (Dennison, 1990), with MgCO₃ added as a chlorophyll stabilizer. The acetone extracts (10 ml) were stored at 4 °C in the dark for 24 h and centrifuged. Chlorophyll *a* (chl *a*), Chlorophyll *b* (chl *b*) and carotenoid contents were computed using the equations of Lichtenthaler and Wellburn (1983).

2.3.3. Estimated daily irradiance regimes and daily metabolic carbon balances

Mean daily compensation (Hc) and saturation (Hk) periods were calculated by averaging the number of hours per day that irradiance values exceeded the corresponding values of compensation (Ec) and saturation (Ek) irradiances, respectively. The values of Ec and Ek used in these calculations were those obtained from the P vs. E curves.

Daily carbon balance, as a proxy of plant light limitation (Dennison and Alberte, 1985), was calculated according to the Michaelis–Menten function ($P = [\text{gross-Pmax } E / (E + E_k)] + R_d$ (Baly, 1935)) previously applied to *C. cylindracea* (Bernardeau-Esteller et al., 2011; Gatusso and Jaubert, 1985), where P is the net production, gross-Pmax is the maximum gross photosynthetic rate, E is the irradiance measured in the field, Ek is the saturation irradiance, and Rd is the respiration rate. Semicontinuous (i.e. every 10 min) mesocosm and field irradiance measurements were entered into the function to generate estimates of net production, which were integrated across 24 h periods to yield daily net production. If the photosynthetic quotient is assumed to equal 1, and the ratio g C:g O₂ = 0.3 (Matta and Chapman, 1991), then the net production in oxygen units ($\mu\text{mol O}_2 \text{ g FW}^{-1}$) can be multiplied by 0.012 to obtain the equivalent carbon units (mg C g FW^{-1}). This calculation presumes constant dark respiration throughout the day and does not consider other carbon losses (e.g. exudation, grazing) or gains (e.g. light-independent carbon fixation).

2.3.4. Stolon biomass balance and apical growth rate

To calculate stolon biomass balance and apical growth rate in a particular stolon, the following equation was used: $X_x = (X_f - X_i) / t$, where X_x

is the measurement for a variable (biomass or length) expressed in units d^{-1} , X_f and X_i are the observed measurements of the variable at the end and beginning of the experiment, and t , the time duration of the experiment. The average value of all stolons from each tray (mesocosm experiment) or plot (field experiment) constituted each one of the replicates in both experiments ($n = 3$ and $n = 4$, respectively).

Total biomass of a stolon was determined using a precision scale (Mettler-Toledo). Growth rate based on apical stolon length was determined according to the methodology described by Ruitton et al. (2005). At the beginning of the experiment the apical region of the stolon was marked by placing a metal ring on the back of the last frond before the apical meristem and manually measured (cm). This mark was used as a reference for measurement at the end of the experiment. Length measurements included both the main axis of the stolon and the stolon ramifications generated during the experiment.

2.3.5. Starch content

For starch analysis, algae from each tray (mesocosm experiment) and plot (field experiment) were cleaned with distilled water, dried for 48 h at 50 °C and ground to fine powder. Starch content was analyzed following the method described by Yemm and Willis (1954). Ground material (0.150 g) was washed with 80% ethanol to remove all trace of soluble sugars and extracted with 1 N KOH to solubilize starch. Finally, starch was determined spectrophotometrically using an anthrone assay. Starch content was expressed as percentage of the dry weight of the sample. Each of these measurements constituted a replicate of the experiment ($n = 3$ for the mesocosm experiment; $n = 4$ for the field experiment).

2.4. Statistical analysis

2.4.1. Multivariate analysis

To explore the photoacclimative response of *C. cylindracea* to the different treatments in the mesocosm experiment, Principal Component Analysis (PCA) was performed based on the correlation matrix of photoacclimation variables (which includes photosynthetic parameters, dark respiration rate and pigment content). Data were previously transformed to achieve centralization and standardization. PCA was performed with the program CANOCO version 4.5 (Microcomputer Power Ltd).

2.4.2. Univariate analysis

For both the mesocosm and field experiments, differences among treatments for each variable were tested using one-way ANOVAs. Prior to analysis, data were tested for heterogeneity of variance using Cochran's C-test and transformed when necessary. Where variance remained heterogeneous, untransformed data were analyzed, as ANOVA is a robust statistical test and is relative unaffected by the heterogeneity of variances, particularly in balanced experiments (Underwood, 1997). A probability level of 0.05 was regarded as significant except when data transformation was not possible. In such cases the level of significance was reduced to $P < 0.01$ to minimize type I error. The Student–Newman–Keuls (SNK) test was used for a posteriori pairwise comparisons of means. ANOVA analyses were developed with the program GMAV® version 5 for Windows (Underwood and Chapman, 1998).

In addition, for both experiments, relationships between photoacclimation variables and light were explored using simple regressions. These analyses were developed with the program Sigmaplot 10.0 (Systat Software Inc.).

3. Results

3.1. Mesocosm experiment

The first axis of the PCA performed on *C. cylindracea*'s photoacclimative response (Fig. 2A) represents most of the variance

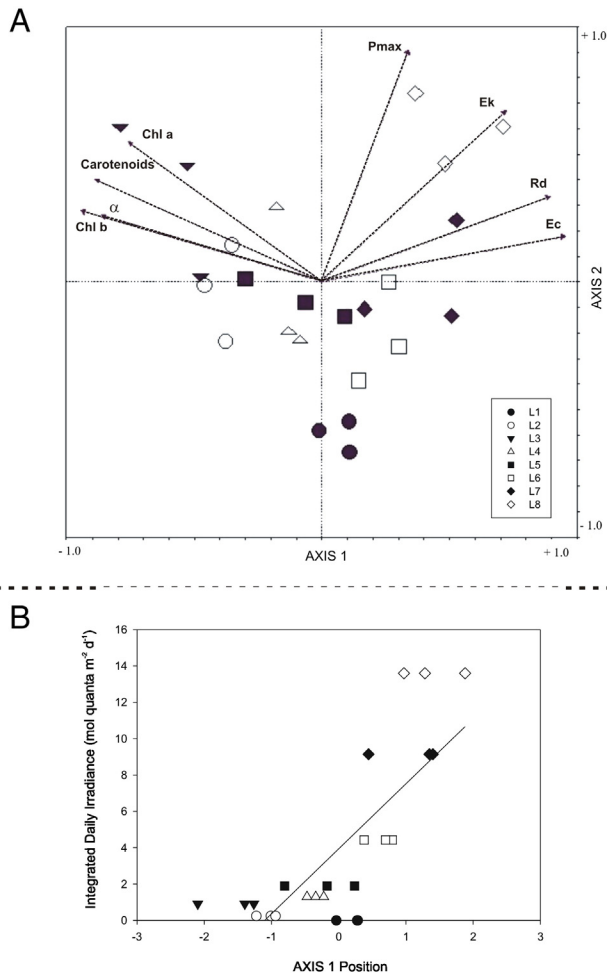


Fig. 2. Multivariate Analysis of photoacclimation responses of *C. cylindracea* in the mesocosm experiment: A. Ordination diagram of the Principal Component Analysis (PCA) with selected photoacclimative variables for mesocosm experiment. B. Relationship between X-axis position and Integrated Daily Irradiance (Table 1).

explained (67%), which had a strong correlation ($r > 0.7$) with all variables except Pmax. This correlation was negative in the case of Ek, R and Ec, while it was positive for pigment content and photosynthetic efficiency (α). The second ordination axis explained 23.8% of the variation and was highly correlated ($r = 0.91$) with Pmax (Fig. 2A).

Distribution of treatments on AXIS 1 can be interpreted in terms of light availability with higher irradiance treatments on the left side of the PCA and lower irradiance treatments in the right side. Indeed, the ordination of treatments along AXIS 1 showed a high and significant correlation with the Integrated daily Irradiance ($R^2 = 0.5833$, $p < 0.0001$, $n = 3$; Fig. 2B).

P vs. E curves allowed the estimation of several photosynthetic parameters, most of which (Pmax, Rd, Ec and Ek) having a positive and significant linear relationship with irradiance (Fig. 3A). The highest values of these parameters were recorded in the treatment of highest irradiance (L8; 13.61 mol quanta m⁻² d⁻¹) and were significantly higher, except for Ec, to the other treatments (SNK, Fig. 3A, Annex Table 1). Minimum values were observed in the low irradiance treatment L3 (0.91 mol quanta m⁻² d⁻¹) in the case of Rd and Ec, and darkness treatment (L1) in the case of Pmax and Ek (Fig. 3A). Values recorded in these treatments represented a reduction of about 70%, in the case of Rd and Ec, and close to 50% for Pmax and Ek compared to treatment L8. Photosynthetic efficiency

(α) decreased with increasing irradiance (Fig. 3A), although significant differences were found only between treatment L3, which yielded the highest value (30% greater than the value recorded in higher irradiance treatments) and the rest of the treatments (SNK, Fig. 3A, Annex Table 1).

Chl a and chl b concentrations and the ratio chl b/a showed a similar pattern to that observed for photosynthetic efficiency, characterized by a negative linear relationship with irradiance (Fig. 3B). Higher values of chlorophyll were generally observed in the low-intermediate irradiance treatments, and were highest in treatment L3 (0.91 mol quanta m⁻² d⁻¹). No clear response for carotenoid content was found regarding different light levels. However, the ratio of these accessory pigments in relation to chlorophyll a and b contents showed a positive linear relationship with irradiance, registering the highest values above 9.15 mol quanta m⁻² d⁻¹ (L7) (Fig. 3B, Annex Table 1).

Daily compensation period (Hc) remained at maximum values (12 h) in all treatments except for L1 and L2, in which values were significantly lower (0 and 10.42 h respectively, Fig. 4). Daily saturation period (Hk) values progressively increased with increasing irradiance, being close to 0 h for darkness treatment L1 and the low light treatment L2 (0.24 mol quanta m⁻² d⁻¹) and with maximum values (12 h) in treatments with more than 5 mol quanta m⁻² d⁻¹ of daily irradiance (L7 and L8) (Fig. 4, Annex Table 1). In accordance with Hc and photoacclimation variables, daily carbon balance also showed a positive response with increasing light. All treatments (except L3 and L4) significantly differed from each other, with carbon balance being negative in treatment L1 and nearly 0 in L2 (0.24 mol quanta m⁻² d⁻¹) (Fig. 4, Annex Table 1). Stolon biomass balance showed negative or very close to 0 values for irradiance levels below 0.3 mol quanta m⁻² d⁻¹ (treatments L1 and L2, Fig. 4). A progressive increase in this variable was identified above these values of irradiance, reaching maximum values from 4 mol quanta m⁻² d⁻¹ (treatment L6 and L7, Fig. 4). Finally, a significant reduction occurred at maximum irradiance (treatment L8, 13.61 mol quanta m⁻² d⁻¹, Fig. 4, Annex Table 1). Apical growth rate had a similar pattern to stolon biomass balance, although values were always positive even in the absence of light (L1), with rates ranging from 0.4 (treatment L1) to 3.4 cm d⁻¹ (treatment L7) (Fig. 4). Starch content ranged from $1.99 \pm 0.04\%$ DW in treatment L1 to $2.57 \pm 0.06\%$ DW in treatment L2, and while there were significant differences among treatments, there was no significant correlation with irradiance.

3.2. Field experiment

Similarly to the mesocosm experiment, parameters derived from P vs. E curves showed a significant linear relationship with irradiance. This relationship was positive in all cases, except for photosynthetic efficiency (α), for which a significant reduction was observed with increasing irradiance (Fig. 5A). SNK test detected differences between treatments for the variables Pmax, Ek and Rd, in which the recorded values were significantly higher in OUT than in the other treatments, while contrarily α values were significantly lower in the OUT treatment than in the IN and SHADED ones (Fig. 5A, Annex Table 2).

With regard to pigment content, no significant differences were observed among treatments. However, the ratio chl b/a showed a positive linear relationship with irradiance, registering significant differences between the conditions of low irradiance (IN-SHADED) and the rest light treatments. On the contrary, ratios between carotenoids and chlorophylls were significantly higher at lower irradiances (IN-SHADED; Fig. 5B). While there were no significant differences in Hc among treatments (always exceeding 7 h), we did detect differences in Hk, which were significantly higher in the OUT and CLIPPING (>5 h) in comparison to SHADED and IN treatments (2 h). Maximum values of carbon balance were observed

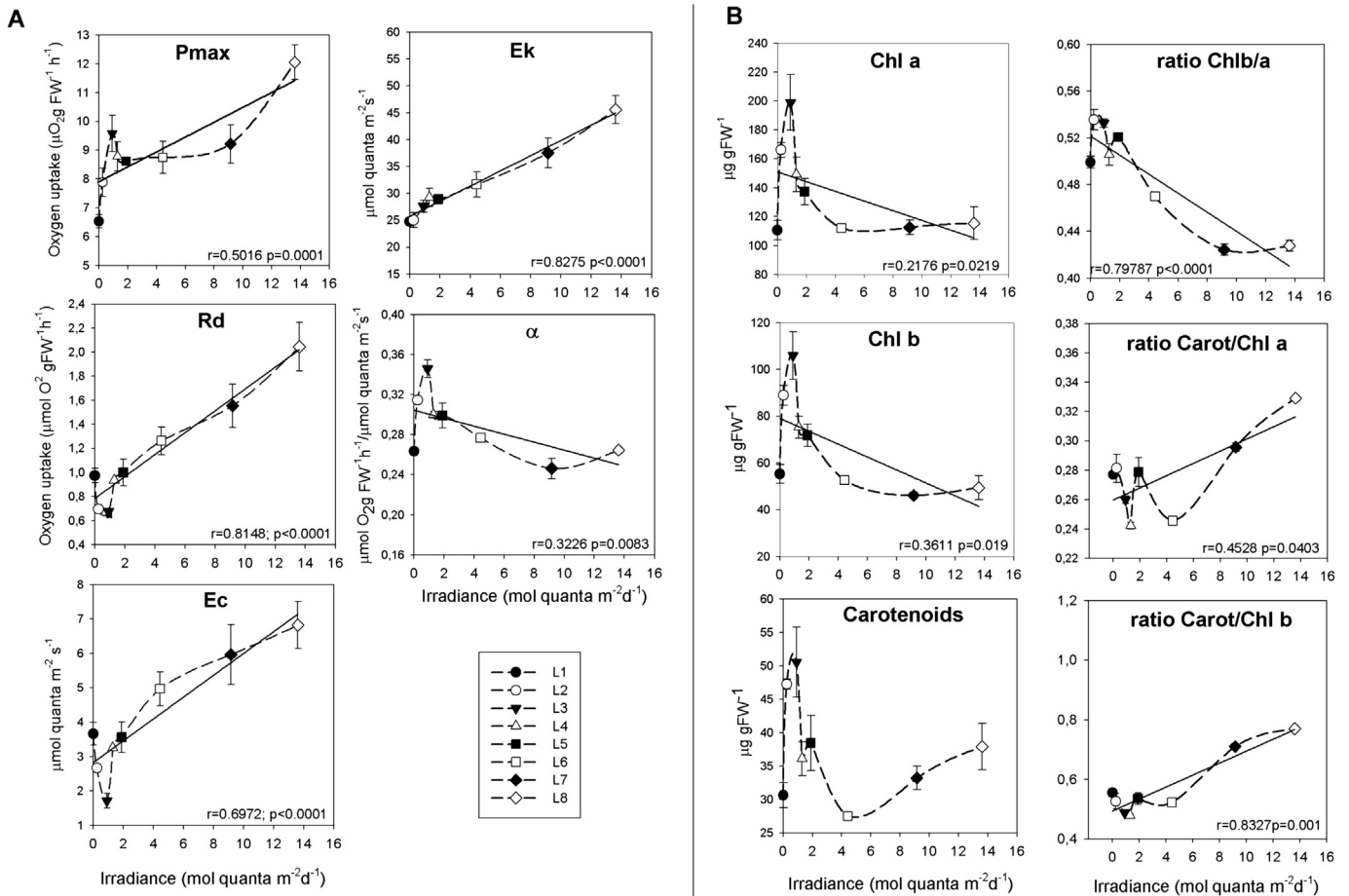


Fig. 3. Photoacclimation response of *C. cylindracea* for mesocosm experiment: A. Photosynthetic parameters derived from P vs. E curves. B. Pigment content. Symbols are mean \pm standard error. Solid lines represent the regression line fitted to data and the smoothed dashed line illustrates the trajectory of the response variable as irradiance increases.

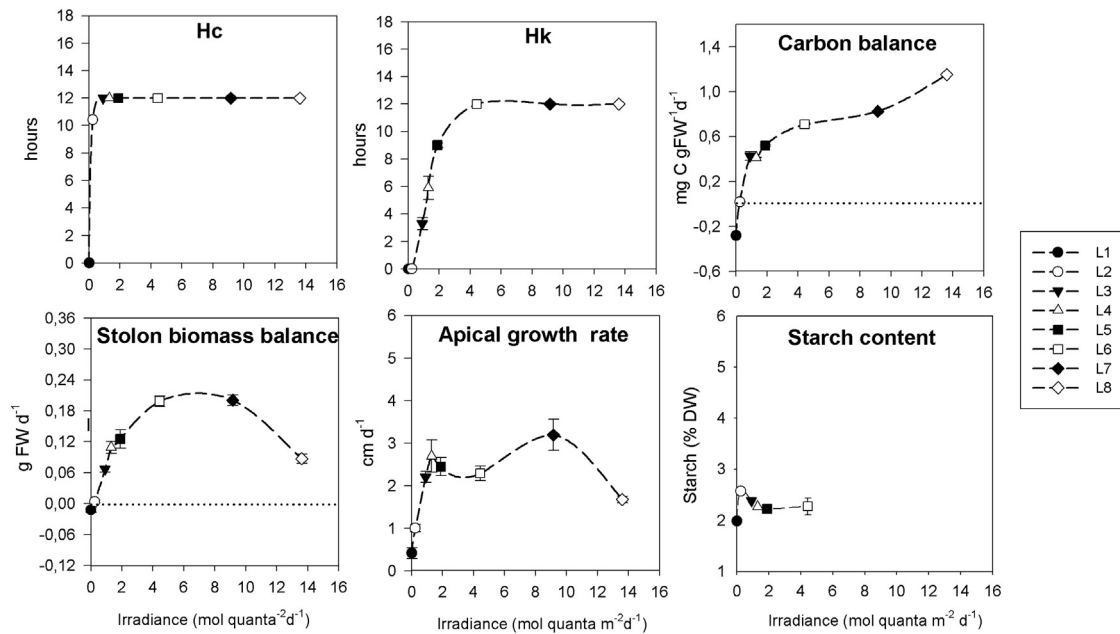


Fig. 4. Productive and growth capacity of *C. cylindracea* in the mesocosm experiment: Light-compensation period (Hc), light-saturation period (Hk), daily carbon balance, stolon biomass balance, apical growth rates and starch content for the mesocosm experiment. Data are mean \pm standard error. The smoothed dashed line illustrates the trajectory of the response variable as irradiance increases.

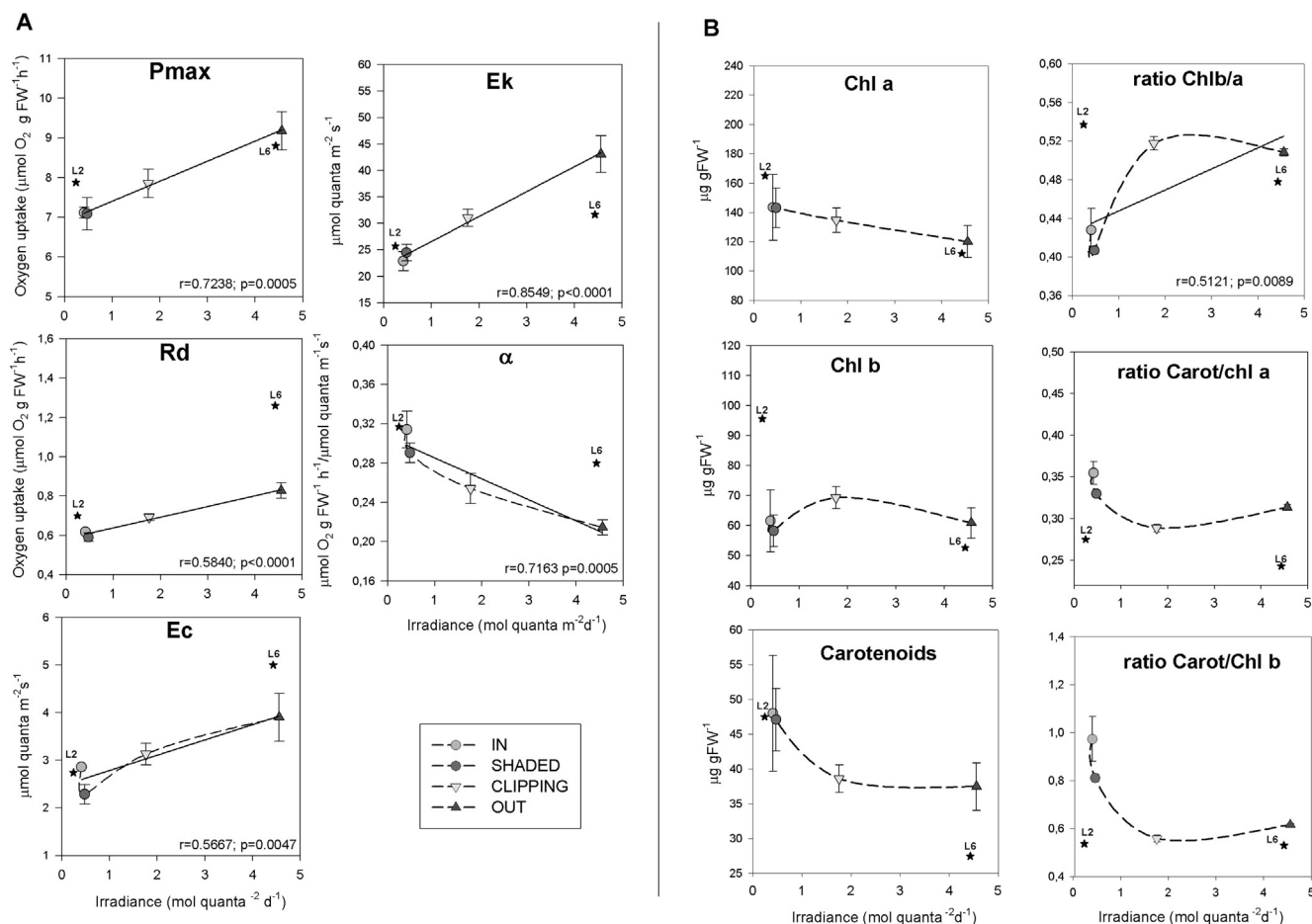


Fig. 5. Photoacclimation response of *C. cylindracea* for the field experiment: A. Photosynthetic parameters derived from P vs. E curves. B. Pigment content. Data are mean \pm standard error. Solid lines represent the regression line fitted to data and the smoothed dashed line illustrates the trajectory of the response variable as irradiance increases. Mean values obtained in treatment L2 and L6 of the mesocosm experiment (asterisks) have been included as a reference.

outside the meadow (OUT; $0.53 \text{ mg C g}^{-1} \text{ FW d}^{-1}$), with a significant reduction of 42% in CLIPPING and of about 80% in lower irradiance conditions (IN and SHADED; Fig. 6, Annex Table 2).

Algae outside the meadow (OUT) exhibited the highest values of stolon biomass balance, apical growth rate and starch reserves. Stolon biomass balance was negative in the rest of treatments,

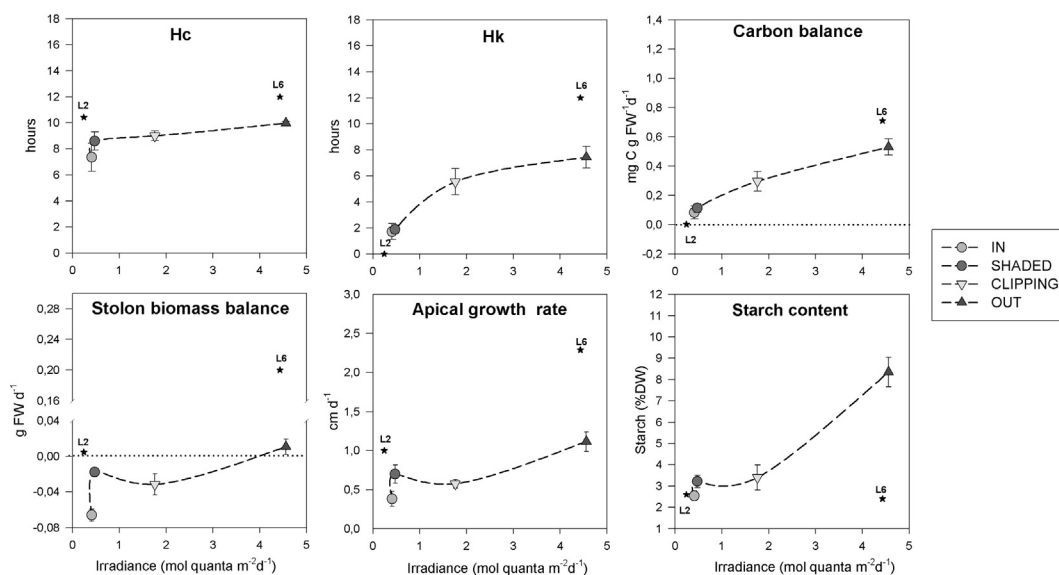


Fig. 6. Productive and growth capacity of *C. cylindracea* in the field experiment: Light-compensation period (Hc), light-saturation period (Hk), daily carbon balance, stolon biomass balance, apical growth rates and starch content for field experiment. Symbols are mean \pm standard error. The smoothed dashed line illustrates the trajectory of the response variable as irradiance increases. Mean values obtained in treatment L2 and L6 of the mesocosm experiment (asterisks) have been included as a reference.

being significantly lower within the meadow (IN) than in SHADED and CLIPPING conditions. In contrast, no significant differences were found between these low light availability treatments in terms of apical growth rate and starch content (Fig. 6, Annex Table 2).

In Figs. 5 and 6 mean values obtained in L2 and L6 treatments of the mesocosm experiments are represented in order to allow some comparative, graphical analysis with those obtained in the field experiment under similar field regimes i.e. the IN and OUT treatments. It can be seen how most of the response variables followed similar patterns in both experimental approaches (except chl *b* and ratio chl *b*:*a*). However, there were appreciable quantitative differences for some of the variables, particularly in the high light treatments (OUT and L6). Major quantitative differences are appreciated in Rd, α , pigment content and composition (Fig. 5), Hk, stolon biomass and growth and starch content (Fig. 6).

4. Discussion

Under the light gradient set in the mesocosm experiment, *C. cylindracea* showed a clear photoacclimation response to reduction in light availability which included (i) a reorganization of the photosynthetic apparatus illustrated by an increase in pigment content (and antenna size) and changes in photosynthetic performance (i.e. reductions in Pmax, Ek, and Ec, and increments in α), as well as (ii) a reduction in respiration rate (Rd). These physiological mechanisms are considered common strategies to overcome low light regimes in marine macrophytes (Falkowski and Raven, 2007; Kirk, 1994; Littler et al., 1986; Lobban and Harrison, 1997; L  nning, 1990) and were consistent with results from previous descriptive field studies that examined photoacclimation capacity of the alga under natural light gradients in the Mediterranean Sea (Bernardeau-Esteller et al., 2011; Marin-Guirao et al., In review; Raniello et al., 2004, 2006). While increase in pigment content and photosynthetic efficiency (α) reflects an improvement in both light harvesting capacity and energy conversion efficiency (Hanelt and L  pez-Figueroa, 2012; Lobban and Harrison, 1997), reduction in Rd reveals a decrease in metabolic demand in order to maximize carbon gains (Bernardeau-Esteller et al., 2011; Markager and Sand-Jensen, 1994; P  rez-Llor  ns et al., 1996). These responses enable the alga to reduce light requirements for growth (illustrated by the decrease in compensation and saturation irradiance [Ec and Ek]) and extend the daily period at which algae photosynthesizes at saturating irradiance (Hk) in order to maintain the photosynthetic and productive capacity under low light regimes (Denisson and Alberte, 1982, 1985; Gantt, 1990; G  mez et al., 1997; Littler et al., 1986; Matta and Chapman, 1991).

Even though there were quantitative differences in some photosynthetic variables (mainly Ek, Rd, α and Chlorophyll *b* in high light treatments), the photoacclimative patterns described above for the mesocosm experiment were very similar to those observed in the field experiment, except for pigment content (Chlorophyll *b*) and composition (Chlorophyll *b* and carotenoid ratios). Such differences could be explained by unavoidable differences in some key factor related with the different nature of both experimental approaches, despite the fact that in the mesocosm system we tried to simulate field conditions as similar as possible. For example, carotenoids (relative to chlorophyll *a* and *b*) differences between mesocosm and field data are likely explained by differences in the spectral composition of light (i.e. change not only in quantity but also in quality of light) related to depth and light capture within the seagrass canopy. As it was already mentioned in the Materials and methods section, we were not able to reproduce exactly the natural light regime in the mesocosm system. In the mesocosm system, light has a greater component of the red part of the spectrum, while these wavelengths are virtually nonexistent in field conditions due to the absorption

by water column and the *P. oceanica* leaf canopy, where there is a predominance of green and blue wavelength. These differences in light quality would promote the development of accessory pigments such as some carotenoids (e.g. siphonaxanthin) that have an enhanced ability to absorb green light (Dalla Via et al., 1998; Kirk, 1994). In fact, increased siphonaxanthin concentrations as a response to light reductions have been previously described in *C. cylindracea* (Raniello et al., 2006; Riechert and Dawes, 1986). The influence of other key factors such as nutrients or temperature in explaining divergences between field and laboratory results is much less probable since these conditions were highly controlled in the mesocosm system. Seawater used in the mesocosm was obtained from the same area where the field experiment was performed and frequently renewed to maintain nutrient levels. Therefore, quality of seawater in both experiments can be considered similar at least in relation to the studied responses. Such differences in pigment composition (or in any other photoacclimative variable) were not as evident between the IN and the SHADED treatment of the field experiment, revealing that while shading structures successfully reproduced the light environment inside the seagrass leaf canopy, they did not modify other factors linked to meadow structure (e.g. hydrodinamism, nutrient availability, etc.) that also appear to have influenced the photoacclimatory responses observed in the field experiment.

In the mesocosm experiment maximum growth rates and biomass production were observed in light levels ranging between 4.43 (L6) and 9.15 (L7) mol quanta $m^{-2} d^{-1}$. Light reductions below these optimum light levels lead to photoacclimatory responses and reductions in the production and growth capacity of the alga. When light levels ranged between 1.89 (L5) and 0.91 (L3) mol quanta $m^{-2} d^{-1}$, photoacclimation mechanisms allowed for an optimization of light capture and use, maintaining positive Hk values and net carbon gains. In fact, while these treatments suffered a reduction in light availability which ranged between 60% and 80% compared to treatment L6 (i.e. optimum light conditions), carbon gains decreased only between 20% and 30% in comparison to L6, and allowed the alga to produce new biomass. In this light range, reduction in growth capacity is not only a consequence of the decrease in carbon production but it is also related to the costs of the process of acclimation. Changes in photosynthetic apparatus determine an increase in maintenance costs of the macrophytes (Copertino et al., 2006; Raven, 1984), while reducing respiratory rates imply a lower use of internal resources for growth (Kirk, 1994; P  rez-Llor  ns et al., 1996).

Under more severe light reductions (i.e. below L3 light levels), no further photoacclimation took place, suggesting that photosynthetic plasticity capacity of the alga was exceeded. This uncoupling between acclimation response and light availability determines an inefficient use of light, as illustrated by the extremely low values of Hk and carbon balance (very close to 0), which in turn determine a limitation in the capacity of the alga to develop new biomass. According to these results, it can be inferred that minimum light requirements for growth under mesocosm conditions are very close to the L2 light regime (0.24 mol quanta $m^{-2} d^{-1}$). In fact, based on light extinction coefficient mean values (Kd) obtained by authors in the same experimental area (Marin-Guirao et al., In review), we estimated that the extremely low light levels measured inside the *P. oceanica* meadow are within those prevailing at the maximum depth range of *C. cylindracea* in the Western Mediterranean Sea (but considering that the spectral composition under seagrass canopies and maximum distributional depths can differ). Positive growth rates based on apical stolon length described in L2 and L1 (i.e. darkness) treatments could be explained as a mechanism of talus expansion resulting from the dilution of internal biomass in response to light-limiting conditions (P  rez-Llor  ns et al., 1996; Sand-Jensen, 1988).

In the field experiment, light availability in treatments that reproduce light regime within a *P. oceanica* meadow (IN and SHADED =

0.41–0.47 mol quanta $\text{m}^{-2} \text{d}^{-1}$) laid between the L2 and L3 mesocosm treatments, suggesting that in these treatments the algae were probably close to the limit of its photoacclimation plasticity and its minimum light requirements for growth. In fact, the very low (although still positive) values of Hk and daily carbon balance registered support the idea that this light regime overcomes the alga's acclimation capacity. Despite the positive values registered for these productivity variables, stolon biomass balance was negative in both treatments, indicating that carbon fixation was not enough to maintain new biomass production and thus that light availability limits the development capacity of the alga. In this field experiment, the growth capacity of the alga was in general considerably lower than that reported in the mesocosm experiment under comparable light levels, suggesting an apparently higher light requirement under field conditions due to, for example, an increase in maintenance costs associated with the impacts of other environmental factors such as grazing and mechanical damage (Markager and Sand-Jensen, 1992). However, once again differences in light regime provided in both experimental approaches could also be involved in such discrepancies in algal productivity. As explained in the Materials and methods section, the illumination system of the mesocosm produced rectangular-shaped daily light 'curves' (Fig. 1), which necessarily resulted in daily Hk periods (i.e. the daily period at which the alga is photosynthesizing at its maximum rate) much larger than those derived from typical, natural bell-shaped light curves. Since Hk is crucial in determining carbon balance and growth (Dennison and Alberte, 1985; Dunton and Shell, 1986; Gómez et al., 1997) it could reasonably account for the higher rates of algal growth and biomass accumulation in the aquariums, likely based on the consumption of internal resources (as indicated by the similar starch content between light treatments). Furthermore, the higher algal productivity of the mesocosm system is consistent with other quantitative differences previously reported for some photosynthetic variables (Ek, Rd and α) between both experimental approaches.

Increases in light availability provided by experimental manipulation of the seagrass leaf canopy (i.e. the CLIPPING treatment) allowed *C. cylindracea* to achieve a carbon balance three times higher than those recorded in the IN and the SHADED treatments, as well as Hk daily periods longer than 5 h. These results reinforce the hypothesis supported by the other results obtained in this study that light availability inside the seagrass canopy limits the photosynthetic performance of the algae, and are consistent with recent experimental studies demonstrating that the removal of *P. oceanica* leaves promotes the establishment and spread of the invasive seaweed (Tamburello et al., 2014). However, despite the efficient use of light demonstrated by the alga, stolons transplanted to the CLIPPING plots presented apical growth rates and biomass losses similar to that recorded in IN and SHADED treatments. Furthermore, these stolons were depleted in starch content relative to those in the OUT plots. These results could be explained if carbon gains and internal reserves are being used to cope with some kind of additional stress instead of for biomass growth and maintenance. In fact, stolons from the CLIPPING plots showed some physical damages at the end of the experimental period; algae stolons from IN plots also displayed these wounds, but not those from SHADED plots, which could explain the significant higher biomass losses measured in stolons from IN treatments. These unexpected results suggest that in addition to light, other stressful factors linked to the meadow structure can be limiting the growth and development of *C. cylindracea* inside the seagrass leaf canopy. Macrophyte canopies may affect the distribution of plant understory species through several differing effects other than shading, such as scouring (Black, 1974; Velimirov and Griffiths, 1979), or exudation of chemical substances (Dayton et al., 1984; Fletcher, 1974). Since wounds appeared in stolons just after a short, stormy event that occurred during the experimental period, a scouring effect caused by seagrass leaves over the bottom could be proposed as a candidate factor. In fact, scouring (Gambi et al.,

1989, 1990) and chemical exudation (Cuny et al., 1995) are mechanisms by which *P. oceanica* can influence the understory assemblages within the meadow. In addition, a shorter seagrass canopy can decrease protection from fish (Farina et al., 2009), some of which being avid consumers of this alga (Tomas et al., 2005).

In summary, our results are consistent with the presumed high photosynthetic plasticity of *C. cylindracea* and its capacity to colonize Mediterranean habitats within a broad range of light regimes (Klein and Verlaque, 2008; Piazzini et al., 2005). However, acclimation mechanisms developed by the alga represent an energy cost which may affect its ability to grow in low light environments, as illustrated by the lower abundances shown by the alga at depths greater than 25–30 m (Bernardeau-Esteller et al., 2011; Katsanevakis et al., 2010; Klein and Verlaque, 2008). Despite the influence of certain experimental factors (mainly light quality and quantity) on algal productivity, results obtained from mesocosm and field experimental approaches consistently showed that light levels inside the *P. oceanica* leaf canopy overcome the phenotypic plasticity capacity of *C. cylindracea*, strongly limiting its photosynthetic performance and leading to carbon balances unable to sustain algal development. A similar conclusion was achieved in a previous field study under winter conditions (Marin-Guirao et al., In review), confirming that the alga is subjected to light regimes under or very close to its minimum light requirements for growth over long periods of its annual life cycle. In fact, the presence of *C. cylindracea* stolons growing at the meadow edge zone can only be explained by net carbon gains obtained by the alga during summer, when light availability allow highly positive carbon balances (Marin-Guirao et al., In review). Thus, results obtained in this and previous studies support the hypothesis that light plays a major role in determining the resistance of *P. oceanica* meadows to *C. cylindracea* bioinvasion in the Mediterranean Sea, but also indicates that other factors linked to the meadow structure could also be involved in the growth and colonization capacity of the alga. Therefore, further experimentation would be necessary in future research to attain a better understanding of the vulnerability of this seagrass habitat to *C. cylindracea* invasions.

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References

- Arenas, F., Sánchez, I., Hawkins, S.L., Jenking, S.R., 2006. The invasibility of marine algal assemblages: role of functional diversity and identity. *Ecology* 87 (11), 2851–2861.
- Baly, E.C.C., 1935. The kinetics of photosynthesis. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* 117, 218–239.
- Bernardeau-Esteller, J., Marin-Guirao, L., Sandoval-Gil, J.M., Ruiz, J.M., 2011. Photosynthesis and daily metabolic carbon balance of the invasive *Caulerpa racemosa* var. *cylindracea* (Chlorophyta: Caulerpales) along a depth gradient. *Sci. Mar.* 75 (4), 803–810.
- Black, R., 1974. Some biological interactions affecting intertidal populations of the kelp *Egregia laevigata*. *Mar. Biol.* 28 (3), 189–198.
- Branch, G.M., Steffani, C.N., 2004. Can we predict the effects of alien species? A case-history of the invasion of South Africa by *Mytilus galloprovincialis* (Lamarck). *J. Exp. Mar. Biol. Ecol.* 300, 189–215.

Annex 1

Table 1

Summary of the one way ANOVA and SNK tests performed to assess the effect of treatment on all *C. cylindracea* variables in the mesocosm experiment; df = degree of freedom, MS = Mean Squares, F = F-statistics, p = p value, SE = standard error; ns = not significant, *p < 0.05, **p < 0.01, ***p < 0.001.

Variable	Mesocosm experiment						SE
	Effect	df	MS	F	p	SNK test	
Pmax	Treatment	7	7.41	7.35	***	L1 = L2 = L5 = L6 = L4 = L7 = L3 < L8	0.58
	Residual	16	1.01				
Rd	Treatment	7	0.65	15.48	***	L3 = L2 = L4 = L1 = L5 = L6 = L7 < L8	0.12
	Residual	16	0.04				
Ec	Treatment	7	8.83	11.73	***	L3 < L2 = L4 = L5 = L1 = L6 = L7 = L8	0.50
	Residual	16	0.75				
Ek	Treatment	7	149.49	12.52	***	L1 = L2 = L3 = L5 = L4 = L6 = L7 < L8	2.00
	Residual	16	11.94				
α	Treatment	7	0.0035	15.25	***	L7 = L1 = L8 = L6 = L5 = L4 = L2 < L3	0.01
	Residual	16	0.0002				
Chl a	Treatment	7	3095.98	9.94	***	L1 = L6 = L7 = L8 = L5 = L4 = L2 < L3	10.19
	Residual	16	311.43				
Chl b	Treatment	7	1357.40	14.09	***	L7 = L8 = L6 = L1 < L5 = L4 = L2 < L3	51.46
	Residual	16	79.43				
Carotenoids	Treatment	7	198.09	8.24	***	L6 = L1 = L7 = L4 = L5 = L8 = L2 = L3	2.83
	Residual	16	24.03				
Chl b/a	Treatment	7	0.0059	64.28	***	L7 = L8 < L6 < L1 = L4 = L5 = L3 = L2	0.01
	Residual	16	0.0001				
Ratio Carotneoids/chl a	Treatment	7	0.0024	28.66	***	L4 = L6 = L3 = L1 = L5 = L2 = L7 < L8	0.00
	Residual	16	0.0001				
Ratio Carotneoids/chl b	Treatment	7	0.0344	65.01	***	L4 = L3 = L6 = L2 = L5 = L1 < 7 < 8	0.00
	Residual	16	0.0005				
Hc	Treatment	7	52.90	6094.9	***	L1 < L2 < L3 = L4 = L5 = L6 = L7 = L8	0.02
	Residual	16	0.0009				
Hk	Treatment	7	81.96	193.08	***	L1 = L2 < L3 < L4 < L5 < L6 = L7 = L8	0.38
	Residual	16	0.42				
Carbon balance	Treatment	7	0.61	3300.79	***	L1 < L2 < L4 = L3 < L5 < L6 < L7 < L8	0.01
	Residual	16	0.0002				
Stolon biomass balance	Treatment	7	0.02	60.38	***	L1 = L2 < L3 = L8 = L4 = L5 < L6 = L7	0.01
	Residual	16	0.0003				
Apical growth rate	Treatment	7	1.53	10.85	***	L1 = L2 < L8 = L3 = L6 = L5 = L4 = L7	0.22
	Residual	16	0.14				
Carbohydrate content	Treatment	5	0.11	5.88	n.s.		
	Residual	12	0.019				

Table 2

Summary of the one way ANOVA and SNK tests performed to assess the effect of treatment on all *C. cylindracea* variables in field experiment; df = degree of freedom, MS = Mean Squares, F = F-statistics, p = p value, SE = standard error; ns = not significant, *p < 0.05, **p < 0.01, ***p < 0.001.

	Field experiment					
Variable	df	MS	F	p	SNK test	SE
Pmax	3	2.89	7.05	*	SHADED = IN = CLIPPING < OUT	0.37
	8	0.41				
Rd	3	0.03	19.02	***	SHADED = IN = CLIPPING < OUT	0.02
	8	0.002				
Ec	3	252.85	16.55	***	SHADED = IN = CLIPPING = OUT	0.30
	8	0.26				
Ek	3	0.006	10.19	**	IN = SHADED = CLIPPING < OUT	22.56
	8	15.28				
α	3	1.35	5.16	*	OUT = CLIPPING = SHADED = IN	0.01
	8	0.001				
Chl <i>a</i>	3	153.40	0.36	n.s.		
	8	423.96				
Chl <i>b</i>	3	65.96	1.00	n.s.		
	8	65.70				
Carotenoids	3	93.50	1.6	n.s.		
	8	58.57				
Chl <i>b/a</i>	3	0.01	14.87	**	SHADED = IN < OUT = CLIPPING	0.01
	8	0.0008				
Ratio Carotneoids/chl <i>a</i>	3	0.0023	12.59	**	CLIPPING = OUT = SHADED = IN	0.00
	8	0.0002				
Ratio Carotneoids/chl <i>b</i>	3	0.11	15.23	**	CLIPPING = OUT < SHADED < IN	0.01
	8	0.001				
Hc	3	8.23	3.34	n.s.		
	24	2.47				
Hk	3	55.50	12.58	***	IN = SHADED < CLIPPING = OUT	0.45

(continued on next page)

Table 2 (continued)

Variable	Field experiment					
	df	MS	F	p	SNK test	SE
Carbon balance	24	4.41	15.83	***	IN = SHADED < CLIPPING = OUT	0.05
	3	0.30				
Stolon biomass balance	24	0.019	15.81	***	IN < CLIPPING = SHADED < OUT	0.01
	3	0.0041				
Apical growth rate	12	0.0003	3.75	*	IN = CLIPPING = SHADED < OUT	0.10
	3	0.12				
	12	0.031				

- Breeman, A.M., 1988. Relative importance of temperature and other factors in determining geographic boundaries of seaweeds: experimental and phenological evidence. *Helgoländer Mar. Res.* 42 (2), 199–241.
- Britton-Simmons, K.H., 2006. Functional group diversity, resource pre-emption and the genesis of invasion resistance in a community of marine algae. *Oikos* 113, 395–401.
- Bulleri, F., Tommaso, A., Ceccherelli, G., Tamburello, L., Pinna, S., Sechi, N., Benedetti-Cecchi, L., 2011. Determinants of *Caulerpa racemosa* distribution in the north-western Mediterranean. *Mar. Ecol. Prog. Ser.* 431, 55–67.
- Cebrian, E., Rodríguez-Prieto, C., 2012. Marine invasion in the Mediterranean Sea: the role of abiotic factors when there is no biological resistance. *PLoS ONE* 7 (2), e31135. <http://dx.doi.org/10.1371/journal.pone.0031135>.
- Ceccherelli, G., Pinna, S., Cusceddu, V., Bulleri, F., 2014. The role of disturbance in promoting the spread of the invasive seaweed *Caulerpa racemosa* in seagrass meadows. *Biol. Invasions*, 16 (12), 2737–2745.
- Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F., 2010. The biodiversity of the Mediterranean sea: estimates, patterns, and threats. *PLoS ONE* 5 (8), e11842. <http://dx.doi.org/10.1371/journal.pone.0011842>.
- Copertino, M.S., Cheshire, A., Watling, J., 2006. Photoinhibition and photoacclimation of turf algal communities on a temperate reef, after in situ transplantation experiments. *J. Phycol.* 42 (3), 580–592.
- Cuny, P., Serve, L., Jupin, H., Boudouresque, C.F., 1995. Water soluble phenolic compounds of the marine phanerogam *Posidonia oceanica* in a Mediterranean area colonised by the introduced chlorophyte *Caulerpa taxifolia*. *Aquat. Bot.* 52, 237–242.
- Dalla Via, J., Strumbauer, C., Schönweger, G., Sötz, 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.
- Dayton, P.K., Currie, V., Gerrodette, T., Keller, B.D., Rosenthal, R., Tresca, D.V., 1984. Patch dynamics and stability of some California kelp communities. *Ecol. Monogr.* 54 (3), 253–289.
- Dennison, W.C., 1990. Chlorophyll content. In: Phillips, R.C., McRoy, C.P. (Eds.), *Seagrass Research Methods*. UNESCO, Paris, France, pp. 83–86.
- 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.
- Dunton, K.H., Shell, D.M., 1986. Seasonal carbon budget and growth of *Laminaria solidungula* in the Alaskan High Arctic. *Mar. Ecol. Prog. Ser.* 31, 57–66.
- Enriquez, S., Agustí, S., Duarte, C.M., 1992. Light absorption by seagrass *Posidonia oceanica* leaves. *Mar. Ecol. Prog. Ser.* 86 (2), 201–204.
- Falkowski, P.G., Raven, J.A., 2007. *Aquatic Photosynthesis*. Blackwell Scientific Publishers, Oxford (512 pp.).
- Farina, S., Tomas, F., Prado, P., Romero, J., Alcoverro, T., 2009. Seagrass meadow structure alters interactions between the sea urchin *Paracentrotus lividus* and its predators. *Mar. Ecol. Prog. Ser.* 377, 131–137.
- Fletcher, R.L., 1974. *Ulva* problem in Kent. *Mar. Pollut. Bull.* 5 (2), 21.
- Gacia, E., Littler, M.M., Littler, D.S., 1996. The relationships between morphology and photosynthetic parameters within the polymorphic genus *Caulerpa*. *J. Exp. Mar. Biol. Ecol.* 204, 209–224.
- Gambi, M.C., Buia, M.C., Casola, E., Scardi, M., 1989. Estimates of water movement in *Posidonia oceanica* beds: a first approach. In: Boudouresque, C.F., Meinesz, A., Fresi, E. (Eds.), *International Workshop on Posidonia Beds*. GIS Posidonie, Marseille, pp. 101–112.
- Gambi, M.C., Nowell, A.R.M., Jumars, P.A., 1990. Flume observations on flow dynamics in *Zostera marina* (eelgrass) beds. *Mar. Ecol. Prog. Ser.* 611, 159–169.
- Gantt, E., 1990. Pigmentation and photoacclimation. In: Kathleen, M.C., Sheat, R.G. (Eds.), *Biology of the Red Algae*. Cambridge University Press, Cambridge, pp. 203–221.
- Gatusso, J.P., Jaubert, J., 1985. Photosynthesis and respiration of *Caulerpa racemosa* (Chlorophyceae, Caulerpales) grown in aquaria: effects of light and temperature. *Bot. Mar.* 28 (8), 327–332.
- Gómez, I., Weykam, G., Klöser, H., Wiencke, C., 1997. Photosynthetic light requirements, metabolic carbon balance and zonate of sublittoral macroalgae from King George Island (Antarctica). *Mar. Ecol. Prog. Ser.* 148, 281–293.
- Hanelt, D., López-Figueroa, F., 2012. Physiological and photomorphogenic effects of light on marine macrophytes. In: Wiencke, C., Bischof, K. (Eds.), *Seaweed Biology: Novel insights into Ecophysiology, Ecology and Utilization*, pp. 3–23.
- Katsanevakis, S., Issaris, Y., Poursanidis, D., Thessalou-Legaki, M., 2010. Vulnerability of marine habitats to the invasive green alga *Caulerpa racemosa* var. *cylindracea* within a marine protected area. *Mar. Environ. Res.* 70, 210–218.
- Kirk, J.T.O., 1994. *Light and Photosynthesis in Aquatic Ecosystems*. Cambridge University Press, Cambridge (528 pp.).
- Klein, J., Verlaque, M., 2008. The *Caulerpa racemosa* invasion: a critical review. *Mar. Pollut. Bull.* 56 (2), 205–225.
- Lichtenthaler, H.K., Wellburn, A.R., 1983. Determination of total carotenoids and chlorophyll a and b of leaf extracts in different solvents. *Biochem. Soc. Trans.* 603, 591–592.
- Littler, M.M., Littler, D.S., Blair, S.M., Norris, J.N., 1986. Deep-water plant communities from an unchartered seamount of San Salvador Island, Bahamas: distribution, abundance and primary productivity. *Deep-Sea Res.* 33, 881–892.
- Lobban, C.S., Harrison, P.J., 1997. *Seaweed Ecology and Physiology*. Cambridge University Press, Cambridge (384 pp.).
- Lonsdale, W.M., 1999. Global patterns of plants invasions and the concept of invisibility. *Ecology* 80 (5), 1522–1536.
- Lünning, K., 1990. *Seaweeds – Their Environment, Biogeography and Ecophysiology*. Wiley-Liss, New York (544 pp.).
- Marín-Guirao, L., Sandoval-Gil, J.M., Ruiz, J.M., Sanchez-Lizaso, J.L., 2011. Photosynthesis, growth and survival of the Mediterranean seagrass *Posidonia oceanica* in response to simulated salinity increases in a laboratory mesocosm system. *Estuar. Coast. Shelf Sci.* 92, 286–296.
- Marín-Guirao, L., Bernardeau-Esteller, J., Ruiz, J.M., Sandoval, J.M., 2014. Resistance of *Posidonia oceanica* seagrass meadows to the spread of the introduced green alga *Caulerpa racemosa*: assessment of the role of light. *Biol. Invasion* (In review).
- Markager, S., Sand-Jensen, K., 1992. Light requirements and depth zonation of marine Macroalgae. *Mar. Ecol. Prog. Ser.* 88, 83–92.
- Markager, S., Sand-Jensen, K., 1994. The physiology and ecology of light-growth relationships in macroalgae. In: Round, F.E., Chapman, D.J. (Eds.), *Progress in Phycological Research* 10. Biopress, Bristol, pp. 209–298.
- 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.
- Middelboe, A.L., Binzer, T., 2004. Importance of canopy structure on photosynthesis in single- and multi-species assemblages of marine macroalgae. *Oikos* 107 (2), 422–432.
- Pérez-Lloréns, J.L., Vergara, J.J., Pino, R.R., Hernández, I., Peralta, G., Niell, F.X., 1996. The effect of photoacclimation on the photosynthetic physiology of *Ulva curvata* and *Ulva rotundata* (Ulvales, Chlorophyta). *Eur. J. Phycol.* 31, 349–359.
- Piazzi, L., Meinesz, A., Verlaque, M., Akçali, B., Antolic, B., Argyrou, M., Balata, D., Ballesteros, E., Calvo, S., Cinelli, F., Cirik, S., Cossu, A., D'archino, R., Djellouli, A.S., Javel, F., Lanfranco, E., Mifsud, C., Pala, D., Panayotidis, P., Peirano, A., Pergent, G., Petrocelli, A., Ruitton, S., Zuljevic, A., Ceccherelli, G., 2005. Invasion of *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) in the Mediterranean Sea: an assessment of the spread. *Cryptogam. Algal.* 26, 189–202.
- Raniello, R., Lorenti, M., Brunet, C., Buia, M.C., 2004. Photosynthetic plasticity of an invasive variety of *Caulerpa racemosa* in a coastal Mediterranean area: light harvesting capacity and seasonal acclimation. *Mar. Ecol. Prog. Ser.* 271, 113–120.
- Raniello, R., Lorenti, M., Brunet, C., Buia, M.C., 2006. Photoacclimation of the invasive alga *Caulerpa racemosa* var. *cylindracea* to depth and daylight patterns and putative new role for siphonaxanthin. *Mar. Ecol.* 27, 20–30.
- Raven, J.A., 1984. *Energetics and Transport in Aquatic Plants* (Vol. 4). AR Liss (587 pp.).
- Reed, D.C., Foster, M.S., 1984. The effects of canopy shadings on algal recruitment and growth in a Giant Kelp Forest. *Ecology* 65, 937–948.
- Richards, C., Bossdorf, O., Muth, N., Gurevitch, J., Pigliucci, M., 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plants invasions. *Ecol. Lett.* 9 (8), 981–993.
- Riechert, R., Dawes, C.J., 1986. Acclimation of the green alga *Caulerpa racemosa* var. *uvifera* to light. *Bot. Mar.* 29 (6), 533–537.
- Ruitton, S., Verlaque, M., Boudouresque, C.F., 2005. Seasonal changes of the introduced *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) at the northwest limit of its Mediterranean range. *Aquat. Bot.* 82, 55–70.
- Ruiz, J.M., Marín-Guirao, L., Bernardeau-Esteller, J., Ramos-Segura, A., García-Muñoz, R., Sandoval-Gil, J.M., 2011. Spread of the invasive alga *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) along the Mediterranean coast of the Murcia region (SE Spain). *Anim. Biodivers. Conserv.* 34 (1), 73–82.
- Sand-Jensen, K., 1988. Minimum light requirements for growth in *Ulva lactuca*. *Mar. Ecol. Prog. Ser.* 50, 187–193.
- Tamburello, L., Bulleri, F., Balata, D., Benedetti-Cecchi, L., 2014. The role of overgrazing and anthropogenic disturbance in shaping spatial patterns of distribution of an invasive seaweed. *J. Appl. Ecol.* <http://dx.doi.org/10.1111/1365-2664.12199>.
- Theorides, K.A., Dukes, J.S., 2007. Plant invasion across space and time: factors affecting nonindigenous species success during four stages of invasion. *New Phytol.* 176 (2), 256–273.

- Tomas, F., Turón, X., Romero, J., 2005. Seasonal and small-scale spatial variability of herbivory pressure on the temperate seagrass *Posidonia oceanica*. Mar. Ecol. Prog. Ser. 301, 95–107.
- Underwood, A.J., 1997. Experiments in Ecology. Cambridge University Press, Cambridge (524 pp.).
- Underwood, A.J., Chapman, M.G., 1998. GMAV 5. Institute of Marine Ecology, University of Sydney, Australia, Sydney, Australia.
- Velimirov, B., Griffiths, C.L., 1979. Wave-induced kelp movement and its importance for community structure. Bot. Mar. 22 (3), 169–172.
- Williams, S.L., Smith, J.E., 2007. A global review of the distribution, taxonomy, and impacts of introduced seaweeds. Annu. Rev. Ecol. Evol. Syst. 38, 327–359.
- Yemm, E.W., Willis, A.J., 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57 (3), 508–514.
- Zenetos, A., 2010. Trend in aliens species in the Mediterranean. An answer to Galil, 2009 «Taking stock: inventory of alien species in the Mediterranean Sea». Biol. Invasions 12 (9), 3379–3381.