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Short-term ecophysiological and biochemical responses of *Cystoseira tamariscifolia* and *Ellisolandia elongata* to environmental changes

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ABSTRACT: Short-term ecophysiological and biochemical responses of *Cystoseira tamariscifolia* and *Ellisolandia elongata* to changes in solar irradiance and nutrient levels were analyzed *in situ* in oligotrophic coastal waters by transferring macroalgae collected at 0.5 and 2.0 m depth and exposing them to 2 irradiance levels (100 and 70% of surface irradiance) and nutrient conditions (nutrient-enriched and non-enriched). Both species were affected by changes in irradiance and nutrient levels. Few interactive effects between these 2 physical stressors were found, suggesting major additive effects on both species. *C. tamariscifolia* collected at 0.5 m and exposed to 70% irradiance had the highest maximal electron transport rate (ETR_{max}), saturated irradiance (Ek_{ETR}) and chl *a* content and the lowest antioxidant activity. Under the same conditions, *E. elongata* had increased Ek_{ETR}, antheraxanthin and β-carotene content. At 100% irradiance, *C. tamariscifolia* collected at 2.0 m had higher maximal quantum yield (F_v/F_m), photosynthetic efficiency (α_{ETR}), ETR_{max}, maximal non-photochemical quenching (NPQ_{max}), saturation irradiance for NPQ (Ek_{NPQ}), and antheraxanthin and polyphenol content increased, whereas in *E. elongata* only α_{ETR} increased. In nutrient-enriched conditions, phenolic compounds, several carotenoids and N content increased in *C. tamariscifolia* at both depths. *E. elongata* from 2.0 m depth at 100% irradiance and nutrient-enriched conditions showed increased N content and total mycosporine-like amino acids (MAAs). Our results show rapid photophysiological responses of *C. tamariscifolia* to variations in *in situ* irradiance and nutrient conditions, suggesting efficient photoacclimation to environmental changes. In *E. elongata*, F_v/F_m and ETR_{max} did not change in the transplant experiment; in contrast, N content, pigment and MAAs (biochemical variables) changed. The responses of these macroalgae to nutrient enrichment indicate oligotrophic conditions at the study site and environmental stress.

KEY WORDS: *Cystoseira tamariscifolia* · *Ellisolandia elongata* · Antioxidant activity · Carotenoids · Irradiance · Nutrient · Polyphenols · Photoprotection

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INTRODUCTION

Environmental stressors can interact and have synergistic or antagonistic effects on physiological responses (Bischof et al. 2006). When multiple stressors act synergistically, there can be unpredictable effects on organisms (Xenopoulos et al. 2002). In contrast, when stressors operate in an additive way, species' responses are easier to predict (Martínez et al. 2012). It is important to understand the mechanisms of combined environmental stressors in order to predict an organism's responses to future climate scenarios. Experimental transplants can provide a better understanding of such effects (Marzinelli et al. 2009, 2011).

Benthic intertidal organisms are subjected to major changes during the tidal cycle (Davison & Pearson 1996). The responses of intertidal and benthic organisms to stressors can be very rapid, and involve adjustments in their photosynthetic and respiratory activities (Southward et al. 1995, Hoegh-Guldberg & Bruno 2010, Sorte et al. 2010). Temperate intertidal rocky communities can be dominated by habitat-forming macroalgae that drive the biodiversity and functioning of these ecosystems. The algae provide food and shelter, and also reduce environmental stress (Davison & Pearson 1996, Jones 1997, Helmuth et al. 2002, 2006). However, the increasing environmental stresses associated with climatic changes and anthropogenic impacts (e.g. coastal eutrophication, increase in UV light) can affect macroalgal communities at the biochemical, ecophysiological, morphological and population levels (Figueroa & Gómez 2001, Bischof et al. 2006).

Light availability is a key factor affecting marine environments (Huovinen & Gómez 2011). Light promotes photosynthetic activity, but can inhibit many biological processes if radiation becomes excessive (Hanelt & Figueroa 2012). Macroalgae have several photoprotective mechanisms such as energy dissipation by specific pigments (e.g. carotenoids) through the xanthophyll cycle (Goss & Jakob 2010); dynamic photoinhibition, i.e. reversible changes in photosynthetic efficiency and capacity, accumulation of ultraviolet screen compounds and increase of antioxidant activity (Gómez et al. 2011). For instance, brown algae accumulate UV screen compounds (polyphenols) with a strong antioxidant activity under high photosynthetically active radiation (PAR) and UVR (Pavia et al. 1997, Connan et al. 2004, Cruces et al. 2012), whereas the tolerance of most red algae to excessive light, including UV, is driven by the accumulation of mycosporine-like amino acids (MAAs) (de la Coba et al. 2009).

Nutrient availability is another environmental factor limiting macrophyte growth in temperate and oligotrophic habitats (Hanisak 1979, Conolly & Drew 1985). Nitrogen limitation affects many processes in macroalgae including photosynthetic capacity (Pérez-Lloréns et al. 1996), protein content (Vergara et al. 1995, Martínez & Rico 2002) and photoprotection mechanisms (Korbee-Peinado et al. 2004, Korbee et al. 2005b, Huovinen et al. 2006). Under moderate to highly desiccated conditions, some intertidal macroalgae increase their nitrogen and carbon uptake (Lobban & Harrison 1994, Flores-Moya et al. 1998, Nygard & Dring 2008). In terms of nutrient metabolism and nutrition, macroalgae vary according to their growth strategies (Lobban & Harrison 1994, Pedersen & Borum 1997). On one side, slow-growing perennial macroalgae, adapted to stable or seasonally variable N conditions, can develop large N and P storage pools (Martínez et al. 2012). At the another extreme, fast-growing opportunistic algae are unable to store large amounts, but show remarkably high N- and P-uptake rates to profit from unstable N-supply conditions (Teichberg et al. 2008). Finally, nutrient enrichment increases the photoprotection capacity of seaweeds due to the increase in protein content, MAAs (Korbee-Peinado et al. 2004, Huovinen et al. 2006, Figueroa et al. 2012) or polyphenols (Arnold & Targett 2002).

Cystoseira tamariscifolia Papenfuss (Phaeophyceae, Fucales) and *Ellisolandia elongata* (Ellis & Solander) Hind & Saunders (Florideophyceae, Corallinales) are 2 important species on Mediterranean rocky shores. *Cystoseira* spp. are indicators of high quality coastal waters (Arévalo et al. 2007, Ballesteros et al. 2007, Bermejo et al. 2013), according to the criteria of the Water Framework Directive of the European Union (WFD, 2000/60/EC). *E. elongata* is a stress-tolerant, calcareous species dominating zones subjected to disturbance.

In this study, the physiological and biochemical responses of *C. tamariscifolia* and *E. elongata*, collected from 2 different depths, were investigated in relation to the independent and/or interactive effects of ambient radiation and nutrient availability. Based on previous research on the additive effects of physical stressors on fucoid algae (Martínez et al. 2012), we hypothesized that changes in light and nitrogen will have an additive effect on *C. tamariscifolia* and *E. elongata.* Algae collected from 0.5 m depth and under nutrient enriched conditions were expected to be less vulnerable under the transplant conditions.

MATERIALS AND METHODS

Studied species

Cystoseira tamariscifolia is a habitat-forming species that dominates intertidal and shallow-subtidal Mediterranean communities in pristine sites and oligotrophic waters. Although this is a perennial species, receptacles are most developed in spring and summer (Gómez-Garreta et al. 2001). *Ellisolandia elongata* is an articulated calcareous species that dominates benthic intertidal communities replaced by ulvacean algae at intermediate levels of nutrient enrichment (Arévalo et al. 2007). Resembling a small bush and up to 20 cm in height (Braga et al. 2009), it is a perennial species and can occupy both well-lit and shaded habitats (Algarra & Niell 1987, Häder et al. 1997, Figueroa & Gómez 2001). It has been recorded to be in the fertile tetrasporophyte phase throughout the year (Rodríguez & Polo 1986).

Experimental design

The experiment was performed from September 19 to 21, 2012. *C. tamariscifolia* and *E. elongata* were randomly collected from 2 different depths (0.5 and 2.0 m) (Fig. 1a) at the 'Cabo de Gata-Níjar' Natural Park (36° 51′ 0″ N; 2° 6′ 0″ W; southwestern Mediterranean Sea, Spain). Immediately after collection, macroalgal samples (5 g fresh weight [FW]) were placed into mesh cylinders (15 cm long × 5 cm in diameter) and suspended in the water column (at a depth of 0.2 m) by a floating longline system anchored to the bottom and parallel to the coast (Fig. 1b). This system comprised 4 lines of 12 m length. Each line contained 12 cylinders (separated by 1 m). Two lines were placed at one site for the enriched nitrogen treatment and the other 2 lines were placed at another site for the non-enrichment treatment (Fig. 1b). Both sites were separated by 50 m with a small artificial breakwater between them. Each cylinder contained specimens of one unique species and collection depth (in triplicate) was fixed along each line (Fig. 1c). Two light levels were assigned within each treatment, i.e. 70 and 100% of surface irradiance defined as PAB irradiance (PAR + UVR) under nutrient-enriched and non-enriched conditions (Fig. 1c). With regard to the irradiance treatment, a neutral screen was used which attenuates 30% of the incident light. Half of the cylinders (containing algae from both depths) were covered with mesh (1 mm^2) to attain 70% incoming irradiance (simulating conditions at a depth of 2.0 m, thereafter 70 $\%_{PAB}$), and the remaining cylinders were without the screen to attain 100% incoming irradiance

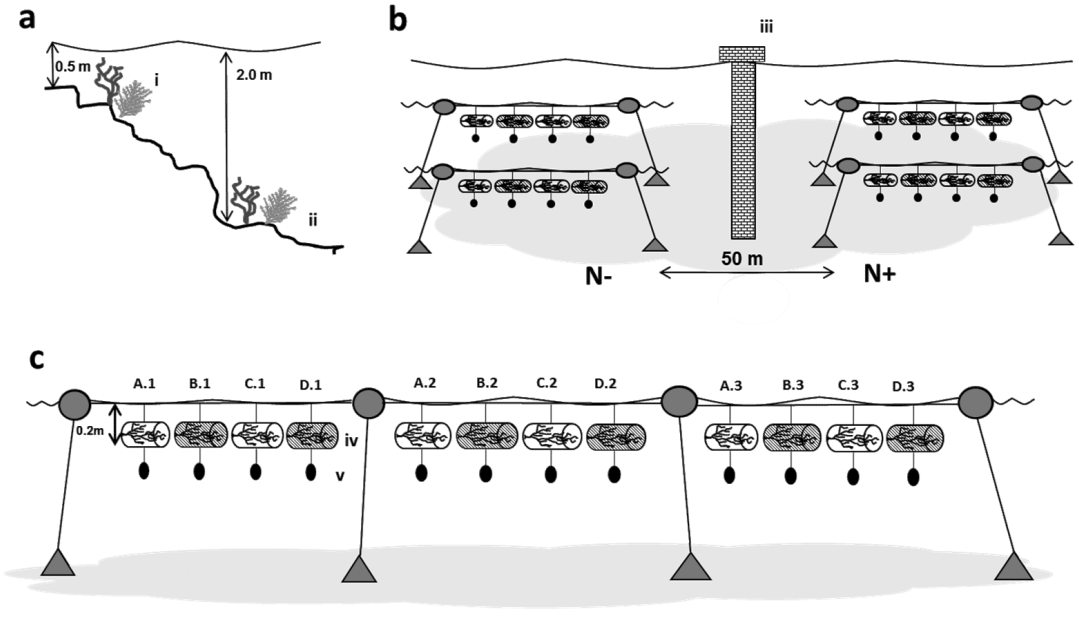

Fig. 1. (a) Depths of origin (i: 0.5 m; ii: 2.0 m) of both collected species *Cystoseira tamariscifolia* and *Ellisolandia elongata*. (b) Schematic layout of the floating lines system separated by a physical barrier (iii: breakwater) comprising 4 longline systems 50 m apart for each treatment. N+ and N– indicate nutrient-enriched and non-enriched treatments, respectively. (c) Schematic layout of one floating line system for each macroalgae with 12 cylinders (iv: cylinder; v: bag with fertilizer or sand). White cylinders (A.1, A.2, A.3, C.1, C.2 and C.3) indicate 100 $\%_{PAB}$ treatment with all replicates, and grey cylinders (B.1, B.2, B.3, D.1, D.2 and D.3) indicate 70 $\%_{PAB}$ with all replicates for both depths

(simulating a depth of 0.5 m, thereafter 100 %$_{PAB}$). Thereby, algae collected at 0.5 m depth (shallow waters) were exposed to 70 %$_{PAB}$ (as a transplant treatment) and 100 %$_{PAB}$ (as a control of natural conditions at 0.5 m depth). On the other hand, those algae collected at 2.0 m depth were exposed to 100 %$_{PAB}$ (as a transplant treatment) and 70 %$_{PAB}$ (as a control of natural conditions at 2.0 m depth) (Fig. 1b). For the nutrient-enriched treatments, mesh bags containing 100 g of a slow-release resin-coated fertilizer (Multicote®, Haifa Chemicals) (modified from Martínez et al. 2012) and fixed below each cylinder was used to simulate nutrient enrichment. Fertilizer composition was 17 % N (NH_4^+ and NO_3^-), 17 % P (P_2O_5) and 17 % K. For non-enriched treatments, a neutral bag with 100 g of sand was used as a control of the effect of the fertilizer bag and the modifying buoyancy (Fig. 1b).

Three replicate cylinders were used for each combination of treatment level, species and depth (2 species × 2 depths × 2 irradiance levels × 2 nutrient levels), resulting in a total of 48 cylinders with macroalgal samples (Fig. 1b). Several physiological variables were obtained from the algae within each cylinder after the *in situ* experiment. These variables were also measured in *C. tamariscifolia* and *E. elongata* from natural populations (at 0.5 and 2.0 m depth) in order to know the initial values. Additionally, water nutrient concentrations, irradiance (PAR and UVR) and underwater temperature were measured during the experiment.

Environmental conditions

Nutrient enrichment (N and P) through fertilizer was assessed by taking triplicate seawater samples at both enriched and non-enriched sites. Seawater was filtered *in situ* using portable GF/F filters (Whatman), transported to the laboratory inside an isotherm bag (4°C, in darkness) and kept at –20°C (Martínez et al. 2012). Nitrate (NO_3^-), ammonium (NH_4^+) and orthophosphate (HPO_4^{3-}) were determined using an automated wet chemistry analyzer (SanPlus^{++} System, SKALAR) applying standard colorimetric procedures (Koroleff 1983).

Irradiance of solar radiation was continuously measured in the air at 3 wavelength bands (UVB = 280–315 nm, UVA = 315–400 nm and PAR = 400–700 nm) using 2 hyperspectral irradiance sensors for UV and PAR (Ramses, TrioS). Attenuation coefficients in water (Kd_{PAR} and Kd_{UVA}) were measured using PAR (QSO-SUN 2.5V) and UV-R (USB-SU 100, Onset Computer) sensors sealed within a waterproof polycarbonate box (OtterBox3000). Kd_{UVB} was not measured due to the high absorption of the polycarbonate box in the UVB spectral band (Quintano et al. 2013).

Underwater temperature was continuously measured using a HOBO U22 Water Temp Pro v2 logger (Onset Computer).

Physiological and biochemical variables

Carbon and nitrogen contents on a dry weight (DW) basis were determined using an element analyzer CNHS-932 (LECO).

In vivo chlorophyll *a* (chl *a*) fluorescence associated with Photosystem II (PSII) was determined by using a portable pulse amplitude modulated fluorometer Diving-PAM (Walz). Algal pieces were collected from natural populations (initial time) and after 60 h of incubation (for each cylinder) and were placed in 10 ml incubation chambers in order to conduct rapid light curves, one for each cylinder. F_o and F_m were determined after 15 min in darkness to obtain the maximum quantum yield (F_v/F_m), where $F_v = F_m - F_o$, F_o is the basal fluorescence of dark-adapted thalli after 15 min and F_m is the maximal fluorescence after a saturation light pulse of >4000 µmol m^{-2} s^{-1} (Schreiber et al. 1995, Figueroa et al. 2009). The electron transport rate (ETR, µmol electrons m^{-2} s^{-1}) as rapid light curves (RLC) was determined after a 20 s exposure period in 8 increasing irradiances (E1 = 9.3, E2 = 33.8, E3 = 76, E4 = 145, E5 = 217, E6 = 301, E7 = 452, E8 = 629, E9 = 947 µmol m^{-2} s^{-1}) of white light (halogen lamp provided by the Diving-PAM). ETR was calculated according to Schreiber et al. (1995) as follows:

$$ETR = \Delta F/F_m' \times E \times A \times F_{II} \quad (1)$$

where $\Delta F/F_m'$ is the effective quantum yield, $\Delta F = F_m' - F_t$ (F_t is the intrinsic fluorescence of alga incubated in light and F_m' is the maximal fluorescence reached after a saturation pulse of algae incubated in light), E is the incident PAR irradiance expressed in µmol photons m^{-2} s^{-1}, A is the thallus absorptance as the fraction of incident irradiance that is absorbed by the algae (see Figueroa et al. 2003) and F_{II} is the fraction of chlorophyll related to PSII (400–700 nm), being 0.8 in brown and 0.15 in red macroalgae (Grzymski et al. 1997, Figueroa et al. 2003). Maximum ETR (ETR_{max}) and the initial slope of ETR versus irradiance function (α_{ETR}), as an estimator of photosynthetic efficiency, were obtained from the tangential function reported by Eilers & Peeters (1988). Finally, the saturation irradiance for ETR (Ek_{ETR}) was calculated from the intercept between ETR_{max} and α_{ETR}.

Non-photochemical quenching (NPQ) was calculated according to Schreiber et al. (1995) as:

$$NPQ = (F_m - F_m')/F_m' \quad (2)$$

Maximal NPQ (NPQ_{max}) and the initial slope of NPQ versus irradiance function (α_{NPQ}) were obtained from the tangential function of NPQ versus irradiance function according to Eilers & Peeters (1988). Finally, the saturation irradiance for NPQ (Ek_{NPQ}) was calculated from the intercept between NPQ_{max} and α_{NPQ}.

Chl *a* and carotenoid pigments were determined in both species, whereas chlorophyll *c* (chl *c*) only in *C. tamariscifolia* and phycobiliproteins only in *E. elongata*.

Chl *a* was determined spectrophotometrically, whilst chl *c* was identified and quantified using HPLC. Both chlorophyll analyses were made by extracting pigments from thalli (25 mg FW) using 1 ml of N,N-dimethylformamide (DMF) and maintained in darkness at 4°C for 12 h. After centrifugation at 5000 × *g* for 10 min (Labofuge 400R, Heraeus, Kendro Laboratory Products), each supernatant was used to measure chlorophyll spectrophotometrically. In the case of chl *c*, the extracts were filtered (0.2 µM) before analyzing with HPLC. The chlorophyll concentrations were calculated using equations by Wellburn (1994). Carotenoid composition was determined by HPLC according to García-Sánchez et al. (2012), using commercial standards (DHI LAB Products).

Phycobiliproteins of *E. elongata* were extracted in 0.1 M phosphate buffer (pH 6.5), centrifuged at 2253 × *g* for 30 min at 4°C. Phycoerythrin (PE) and phycocyanin (PC) concentrations were calculated following Sampath-Wiley & Neefus (2007) equations.

Total phenolic compounds (polyphenols) were determined only in *C. tamariscifolia* using 0.25 g FW. Samples were pulverized in a mortar and pestle with sea sand using 2.5 ml of 80% methanol. After keeping the samples overnight, the mixture was centrifuged at 2253 × *g* for 30 min at 4°C, and then the supernatant was collected. Total phenolic compounds were determined colorimetrically using Folin-Ciocalteu reagent (Folin & Ciocalteu 1927) and phloroglucinol (1,3,5-trihydroxybenzene, Sigma P-3502) as standard. Finally, the absorbance was determined at 760 nm using a Shimadzu UVMini-1240 spectrophotometer. Phenolic concentration was expressed as mg g^{-1} DW after determining the fresh to dry weight ratio in the tissue (4.3 and 1.5 for *C. tamariscifolia* and *E. elongata*, respectively). The results are expressed as mean ± SE from 3 replicates of each treatment.

Antioxidant activity, determined by the 2,2-diphenyl-1-picrylhydrazyil (DPPH) method, was measured on the polyphenol compound extracts according to Blois (1958). Each extract had 150 µl of DPPH, prepared in 90% methanol, added. The reaction was complete after 30 min in darkness at ambient temperature (~20°), and the absorbance was read at 517 nm in a spectrophotometer UVmini-1240 (Shimadzu). The calibration curve made from DPPH was used to calculate the remaining concentration of DPPH in the reaction mixture after incubation. Values of DPPH concentration (mM) were plotted against plant extract concentration (mg DW ml^{-1}) in order to obtain the EC_{50} value (oxidation index), which represents the concentration of the extract (mg ml^{-1}) required to scavenge 50% of the DPPH in the reaction mixture. Ascorbic acid was used as a positive control (Connan et al. 2006).

Total MAA content was determined only in *E. elongata* using HPLC (Waters 600) as described by Korbee-Peinado et al. (2004). Results were expressed as mg g^{-1} DW after determining the fresh to dry weight ratio in the tissue (1.5 for *E. elongata*).

Statistical analysis

The effects of the *in situ* treatments on the ecophysiological response variables of *C. tamariscifolia* and *E. elongata* were assessed using ANOVA (Underwood 1997). For that purpose, 2 factors were considered: Nutrient (fixed with 2 levels) and Irradiance (fixed with 2 levels). This design allows the testing of interactive and additive effects of the variables on the ecophysiological responses. Data used in the analyses were those obtained at the end of the experimental period (after 60 h of photoacclimation). Student-Newman-Keuls tests (SNK) were performed after significant ANOVA interactions (Underwood 1997). Homogeneity of variance was tested using Cochran tests and by visual inspection of the residuals. Analyses were performed by using SPSS v.21 (IBM).

RESULTS

Environmental conditions

Nitrate (NO_3^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) concentrations at the non-enriched site were 1.34 ± 0.31 µM, 1.17 ± 0.35 µM and 0.09 ± 0.01 µM, respectively. In contrast, concentrations at the nutrient-enriched site were 107.51 ± 9.67 µM, 163.31

± 6.10 µM and 24.52 ± 1.51 µM, respectively (mean ± SE, n = 6). Hence, on average, the nutrient-enriched treatment increased nitrate, ammonium and phosphate concentrations in the water column by 80, 139 and 272 times, respectively. The average daily integrated surface irradiance for the experimental period (September 20 and 21, 2012) was 5842 KJ m^{-2} for PAR, 673.3 KJ m^{-2} for UVA and 27.3 KJ m^{-2} for UVB. The attenuation coefficients for PAR (Kd_{PAR}) and UVA (Kd_{UVA}) were 0.076 m^{-1} and 0.137 m^{-1}, respectively. The average seawater temperature at 0.2 m (mean ± SE, n = 1440) ranged between 24.42 ± 0.42°C (during the day) and 23.8 ± 0.19°C (at night).

Physiological response variables

Internal N content was higher in *Cystoseira tamariscifolia* than in *Ellisolandia elongata* (Table 1, Fig. 2). ANOVA results showed that both species from 0.5 m depth presented significantly higher N content and a lower C:N ratio under the nutrient-enriched treatment (Table 1, Figs. 2 & 3). However, the N content from 2.0 m depth samples was different for both species (Table 1, Fig. 2). *C. tamariscifolia* specimens collected from 2.0 m showed similar N content to those from 0.5 m and the C:N ratio increased under the non-enriched treatments (Figs. 2a & 3a). In contrast, *E. elongata* showed a significant interaction between nutrients and irradiance (Table 1). N content in the nutrient-enriched treatment was lower under the 100 $\%_{PAB}$ treatments and the C:N ratio was higher under the same conditions (Figs. 2b & 3b).

F_v/F_m in *C. tamariscifolia* showed a significant interaction with nutrients and irradiance in algae collected at 2.0 m depth (Table 2). Specimens of *C. tamariscifolia* transplanted to 100 $\%_{PAB}$ presented higher F_v/F_m under non-enriched treatments (Table 3). Neither of the species collected at 0.5 m

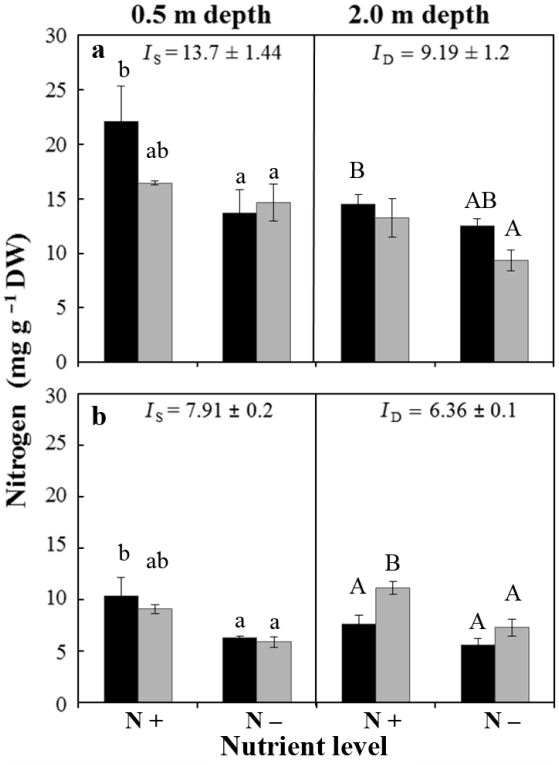

Fig. 2. Total internal N content (mean ± SE, n = 3) of (a) *Cystoseira tamariscifolia* and (b) *Ellisolandia elongata* from 0.5 and 2.0 m depth under irradiance and nutrient treatments. Black bars indicate 100 $\%_{PAB}$, and grey bars indicate 70 $\%_{PAB}$. N+ and N– indicate nutrient-enriched and non-enriched treatments, respectively. Upper values in each box indicate initial values (I_S: 0.5 m depth; I_D: 2.0 m depth). Lowercase letters denote significant differences after SNK test for 0.5 m and capital letters for 2.0 m algae

Table 1. ANOVA results after *in situ* experiment testing for the effect of irradiance and nutrients on C and N contents and C:N ratios of *Cystoseira tamariscifolia* and *Ellisolandia elongata* collected at 2 different depths. We used a significance level of α = 0.05, shown in **bold**

		df	*Cystoseira tamariscifolia*						*Ellisolandia elongata*					
			0.5 m depth			2.0 m depth			0.5 m depth			2.0 m depth		
			MS	*F*	p	MS	*F*	p	MS	*F*	p	MS	*F*	p
C	Nutrients (*N*)	1	75.5	0.276	0.613	753.7	1.366	0.276	**561.7**	**5.805**	**0.043**	149.1	1.400	0.271
	Irradiance (*E*)	1	27.9	0.102	0.758	2578.4	4.672	0.063	25.5	0.264	0.621	231.4	2.173	0.179
	N × *E*	1	144.9	0.530	0.487	108.6	0.197	0.669	2.2	0.022	0.885	**744.2**	**6.987**	**0.030**
	Residual	8	273.4			551.8			96.8			106.5		
N	Nutrients (*N*)	1	**77.6**	**5.625**	**0.045**	**25.8**	**6.639**	**0.033**	**39.9**	**14.145**	**0.006**	**25.6**	**15.540**	**0.004**
	Irradiance (*E*)	1	16.1	1.163	0.312	14.9	3.836	0.086	2.1	0.753	0.411	**20.3**	**12.321**	**0.008**
	N × *E*	1	32.9	2.382	0.161	2.7	0.695	0.429	0.5	0.189	0.675	2.5	1.543	0.249
	Residual	8	13.8			3.9			2.8			1.6		
C:N	Nutrients (*N*)	1	103.6	5.098	0.054	**154.7**	**5.962**	**0.040**	**132.0**	**23.959**	**0.001**	**182.8**	**11.883**	**0.009**
	Irradiance (*E*)	1	0.5	0.023	0.884	27.2	1.047	0.336	10.1	1.830	0.213	**149.6**	**9.723**	**0.014**
	N × *E*	1	39.9	1.963	0.199	18.5	0.712	0.423	0.0	0.006	0.941	10.3	0.668	0.437
	Residual	8	20.3			26.0			5.5			15.4		

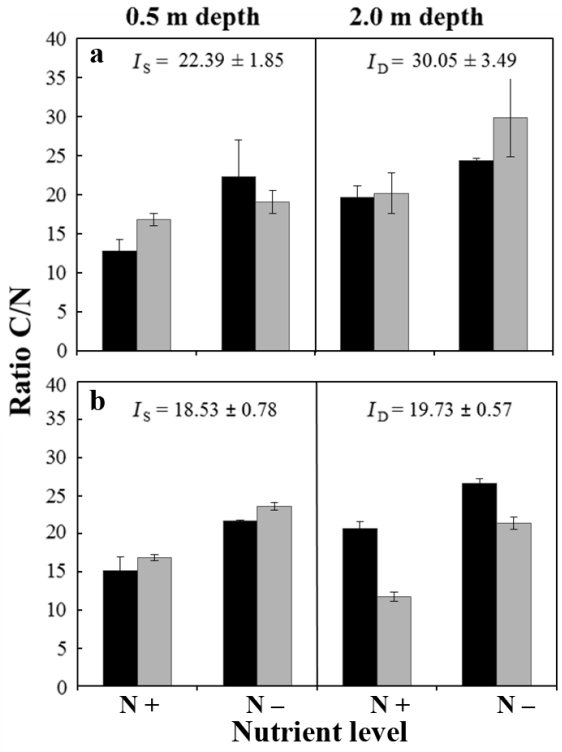

Fig. 3. C:N ratio (mean ± SE, n = 3) of (a) *Cystoseira tamariscifolia* and (b) *Ellisolandia elongata* from 0.5 and 2.0 m depth under irradiance and nutrient treatments. Black bars indicate 100 %$_{PAB}$, and grey bars indicate 70 %$_{PAB}$. N+ and N– indicate nutrient-enriched and non-enriched treatments, respectively. Upper values in each box indicate initial values (I_S: 0.5 m depth; I_D: 2.0 m depth)

nor *E. elongata* at 2.0 m showed significant differences (Table 2). In contrast, ETR_{max} of *C. tamariscifolia* showed significant differences among irradiance treatments (70 %$_{PAB}$ and 100 %$_{PAB}$) at 0.5 m depth (Table 2). This value was higher when they were transplanted to 70 %$_{PAB}$ (Table 3). Conversely, specimens of both species collected at 2.0 m depth did not show any significant differences for either depth. α_{ETR} in *C. tamariscifolia* showed a significant interaction with nutrients and irradiances at both depths (Table 2). This value was lower at 70 %$_{PAB}$ (transplant treatment) and non-nutrient enriched conditions. In both cases, α_{ETR} equaled initial observations from its natural habitat after incubation in the cylinders. (Table 3). To compare, *E. elongata* α_{ETR} values showed 2 different significant results depending on the depth. α_{ETR} in algae collected from 0.5 m depth showed a significant increase at the nutrient-enriched site and in the 70 %$_{PAB}$ treatment (Tables 2 & 3). In contrast, algae collected from 2.0 m had higher α_{ETR} values under the non-enriched treatment (Tables 2 & 3).

In *C. tamariscifolia* collected from 0.5 m depth, Ek_{ETR} showed a significant interaction with nutrients and irradiance. In algae collected at 0.5 m depth under 70 %$_{PAB}$ in the non-enriched treatment, Ek_{ETR} was higher than in the other 3 combinations of treatments (Table 3). However, in algae collected from 2.0 m depth, Ek_{ETR} did not show any significant differences (Table 2). On the other hand, in *E. elongata*, Ek_{ETR} at both depths showed significant differences with the nutrients (Table 2). Ek_{ETR} values for algae collected from 0.5 m depth were higher in non-enriched treatments, whereas in algae from 2.0 m depth, the values were higher in nutrient-enriched treatments (Table 2).

NPQ_{max} in *C. tamariscifolia* showed significant differences due to nutrient treatments in algae collected from 0.5 m depth, and a significant interaction was observed with nutrients and irradiance in algae collected from 2.0 m depth (Table 2). In algae from both depths, NPQ_{max} was higher in non-enriched treatments, whereas the NPQ_{max} increased under 100 %$_{PAB}$ conditions in algae collected from 2.0 m depth (Table 3). NPQ_{max} did not show any significant differences among treatments in *E. elongata* (Table 2), in contrast to *C. tamariscifolia* which showed significant differences due to nutrients at both depths (Table 2). Ek_{NPQ} values in algae collected from 0.5 m were higher in enriched treatments, whereas values were higher under non-enriched treatments in algae from 2.0 m (Table 3). Finally, Ek_{NPQ} showed no significant differences among treatments in *E. elongata* (Table 2).

Pigment content

Chl *a* in *C. tamariscifolia* increased significantly when algae from 0.5 m depth were exposed to lower irradiance levels (70 %$_{PAB}$ treatment). Similar results were found for chl *c* in algae collected from 2.0 m (Tables 4 & 5). Chl *c* content in *C. tamariscifolia* collected from 0.5 m was significantly higher in the nutrient-enriched treatment than in the non-enriched one (Tables 4 & 5). Chl *a* and *c* contents were initially higher in algae collected from 0.5 m (Table 5). Chl *a* in *E. elongata* did not present any significant differences among treatments (Tables 4 & 5).

PC content was significantly higher in the nutrient-enriched treatment in *E. elongata* collected from 0.5 m depth. In contrast, PE content did not show any differences after the experiment (Tables 4 & 5).

The carotenoids fucoxanthin and violaxanthin in *C. tamariscifolia* showed a significant increase under nutrient-enriched treatment in algae from 0.5 m depth

Table 2. ANOVA results after *in situ* experiment testing for the effect of irradiance and nutrients on photosynthetic parameters of *Cystoseira tamariscifolia* and *Ellisolandia elongata* collected at 2 different depths. We used a significance level of $\alpha = 0.05$, shown in **bold**. F_v/F_m: maximal quantum yield, α_{ETR}: photosynthetic efficiency, ETR_{max}: maximal electron transport rate, Ek_{ETR}: saturated irradiance of ETR, NPQ_{max}: maximal non-photochemical quenching, Ek_{NPQ}: saturated irradiance of NPQ

	df	*Cystoseira tamariscifolia*						*Ellisolandia elongata*					
		0.5 m depth			2.0 m depth			0.5 m depth			2.0 m depth		
		MS	*F*	p	MS	*F*	p	MS	*F*	p	MS	*F*	p
F_v/F_m													
Nutrients (*N*)	1	0.001	0.214	0.656	0.000	0.047	0.834	0.001	0.202	0.665	0.012	5.293	0.050
Irradiance (*E*)	1	0.005	1.228	0.300	0.002	0.702	0.426	0.000	0.156	0.703	0.005	2.129	0.183
N × *E*	1	0.013	3.408	0.102	**0.019**	**5.925**	**0.041**	0.000	0.036	0.854	0.007	3.153	0.114
Residual	8	0.004			0.003			0.003			0.002		
α_{ETR}													
Nutrients (*N*)	1	**0.025**	**29.197**	**0.001**	0.001	0.927	0.364	**0.026**	**16.605**	**0.004**	**0.029**	**19.660**	**0.002**
Irradiance (*E*)	1	0.002	2.948	0.124	0.001	1.076	0.330	**0.009**	**5.491**	**0.047**	0.006	4.160	0.076
N × *E*	1	**0.008**	**9.009**	**0.017**	**0.007**	**6.695**	**0.032**	0.007	4.680	0.062	0.000	0.010	0.921
Residual	8	0.001			0.001			0.002			0.001		
ETR_{max}													
Nutrients (*N*)	1	2468.2	4.427	0.069	2320.8	4.728	0.061	0.122	0.294	0.602	0.009	0.019	0.895
Irradiance (*E*)	1	**3773.9**	**6.769**	**0.032**	2139.0	4.358	0.070	0.093	0.224	0.648	0.000	0.001	0.978
N × *E*	1	345.6	0.620	0.454	710.5	1.448	0.263	0.110	0.264	0.621	0.017	0.036	0.854
Residual	8	557.5			490.8			0.416			0.470		
Ek_{ETR}													
Nutrients (*N*)	1	**102164.0**	**20.450**	**0.002**	27666.1	2.289	0.169	**101.4**	**26.275**	**<0.001**	**68.4**	**14.259**	**0.005**
Irradiance (*E*)	1	**82554.8**	**16.525**	**0.004**	47574.2	3.937	0.083	8.4	2.188	0.177	18.2	3.796	0.087
N × *E*	1	**36962.9**	**7.399**	**0.026**	288.0	0.024	0.881	3.9	1.019	0.342	8.7	1.819	0.214
Residual	8	4995.8			12085.2			3.9			4.8		
NPQ_{max}													
Nutrients (*N*)	1	**1.186**	**9.827**	**0.014**	**12.065**	**71.55**	**<0.001**	0.002	0.060	0.813	0.000	0.001	0.979
Irradiance (*E*)	1	0.000	0.002	0.969	0.883	5.234	0.051	0.000	0.004	0.951	0.001	0.066	0.803
N × E	1	0.020	0.169	0.692	**0.946**	**5.608**	**0.045**	0.002	0.069	0.799	0.001	0.066	0.803
Residual	8	0.121			0.169			0.033			0.021		
Ek_{NPQ}													
Nutrients (*N*)	1	**558682.0**	**13.364**	**0.006**	**11110334**	**94.365**	**<0.001**	113.6	0.578	0.469	79.1	0.154	0.705
Irradiance (*E*)	1	48629.6	1.163	0.312	609492.6	5.177	0.052	37.1	0.189	0.676	18.8	0.036	0.853
N × *E*	1	9645.9	0.231	0.644	216.7	0.002	0.967	37.5	0.191	0.674	18.8	0.036	0.853
Residual	8	41803.7			117737.9			196.5			515.2		

(Tables 4 & 5). In contrast, carotenoid content in algae collected from 2.0 m depth was significantly higher the under 70 $\%_{PAB}$ treatment (Tables 4 & 5). Additionally, antheraxanthin and β-carotene in *C. tamariscifolia* collected at the same depth had a significant interaction between nutrients and irradiance. Both compounds increased significantly at 70 $\%_{PAB}$ in the non-enriched treatment site (Tables 4 & 5). In *E. elongata*, fucoxanthin, antheraxanthin and β-carotene contents in algae collected from 0.5 m depth showed a significant increase in the 70 $\%_{PAB}$ irradiance treatment (Tables 4 & 5). Additionally, fucoxanthin content increased significantly in algae cultured under nutrient-enrichment conditions (Tables 4 & 5). Zeaxanthin content did not show any differences after the *in situ* experiment (Tables 4 & 5) for either species.

Total phenolic compounds. Total phenolic compounds in *C. tamariscifolia* were significantly different among nutrient treatments in algae from both 0.5 and 2.0 m depths (Table 6). Additionally, algae collected from 2.0 m showed significant differences in both irradiance treatments (Table 6). In algae collected from 0.5 m depth, the total phenolic compounds were higher in the nutrient-enriched treatment (Fig. 4a). In *C. tamariscifolia* from 2.0 m depth, the increase of phenolic compounds was higher under 100 $\%_{PAB}$ than under 70 $\%_{PAB}$, whereas this increase was higher under non-enrichment than that under the enrichment treatment (Fig. 4a).

Antioxidant activity (EC_{50}). EC_{50} in *C. tamariscifolia* collected at 0.5 m depth showed a significant interaction between nutrients and irradiance

Table 3. Maximal quantum yield (F_v/F_m), photosynthetic efficiency (α_{ETR}), maximal electron transport rate (ETR_{max}, µmol m^{-2} s^{-1}), saturated irradiance of ETR (Ek_{ETR}, µmol m^{-2} s^{-1}), maximal non-photochemical quenching (NPQ_{max}) and saturated irradiance of NPQ (Ek_{NPQ}, µmol m^{-2} s^{-1}) (mean ± SE, n = 3) of *Cystoseira tamariscifolia* and *Ellisolandia elongata* in relation to irradiance (70 %$_{PAB}$ and 100 %$_{PAB}$) and nutrient (Nutrients+ and Nutrients–) treatments. Initial values (I_S: 0.5 m depth; I_D: 2.0 m depth) are shown in the first column and in **bold** for each depth. Lowercase letters denote significant differences after SNK test for algae collected at 0.5 m and capital letters for algae collected at 2.0 m

	I_S	0.5 m depth				I_D	2.0 m depth			
		Nutrients+		Nutrients–			Nutrients+		Nutrients–	
		100 %$_{PAB}$	70 %$_{PAB}$	100 %$_{PAB}$	70 %$_{PAB}$		100 %$_{PAB}$	70 %$_{PAB}$	100 %$_{PAB}$	70 %$_{PAB}$
Cystoseira tamariscifolia										
F_v/F_m	**0.72 ± 0.01**	0.69 ± 0.03	0.71 ± 0.01	0.74 ± 0.02	0.63 ± 0.05	**0.61 ± 0.01**	0.67 ± 0.02	0.72 ± 0.01	0.74 ± 0.03	0.64 ± 0.01
α_{ETR}	**0.33 ± 0.01**	0.36 ± 0.02^{bc}	0.38 ± 0.01^{b}	0.32 ± 0.01^{b}	0.24 ± 0.01^{a}	**0.28 ± 0.01**	0.28 ± 0.01^{A}	0.31 ± 0.01^{AB}	0.34 ± 0.01^{B}	0.28 ± 0.03^{A}
ETR_{max}	**67.98 ± 8.91**	67.23 ± 11.51^{a}	91.97 ± 9.13^{ab}	85.18 ± 21.98^{ab}	131.38 ± 6.64^{b}	**63.51 ± 8.66**	57.01 ± 7.76	99.09 ± 2.26	100.21 ± 12.25	111.51 ± 20.95
Ek_{ETR}	**204.18 ± 36.74**	182.77 ± 25.51^{a}	237.66 ± 28.43^{a}	256.31 ± 59.35^{a}	533.20 ± 40.98^{b}	**228.67 ± 34.52**	201.92 ± 29.18	318.05 ± 10.38	288.16 ± 39.04	423.88 ± 116.744
NPQ_{max}	**1.11 ± 0.14**	0.34 ± 0.08^{a}	0.41 ± 0.01^{ab}	1.05 ± 0.04^{b}	0.96 ± 0.38^{ab}	**1.05 ± 0.04**	0.44 ± 0.01^{A}	0.46 ± 0.14^{A}	3.01 ± 0.28^{C}	1.91 ± 0.34^{B}
Ek_{NPQ}	**602.73 ± 39.93**	697.69 ± 39.93^{ab}	881.71 ± 219.08^{b}	322.85 ± 4.11^{a}	393.47 ± 78.28^{a}	**312.47 ± 11.16**	1302.26 ± 91.89^{A}	860.03 ± 165.71^{A}	3235.21 ± 61.18^{B}	2775.96 ± 326.32^{B}
Ellisolandia elongata										
F_v/F_m	**0.53 ± 0.06**	0.501 ± 0.037	0.519 ± 0.011	0.521 ± 0.043	0.527 ± 0.016	**0.49 ± 0.03**	0.465 ± 0.031	0.456 ± 0.023	0.480 ± 0.034	0.570 ± 0.019
α_{ETR}	**0.017 ± 0.001**	0.013 ± 0.003^{a}	0.024 ± 0.001^{b}	0.009 ± 0.002^{a}	0.009 ± 0.002^{a}	**0.011 ± 0.001**	0.016 ± 0.001^{AB}	0.012 ± 0.004^{A}	0.026 ± 0.001^{C}	0.022 ± 0.002^{BC}
ETR_{max}	**1.65 ± 0.19**	1.41 ± 0.43	1.77 ± 0.05	1.4 ± 0.42	1.38 ± 0.42	**1.36 ± 0.39**	1.42 ± 0.38	1.51 ± 0.51	1.44 ± 0.34	1.38 ± 0.30
Ek_{ETR}	**95.27 ± 5.46**	103.63 ± 10.21^{a}	75.41 ± 3.96^{a}	146.66 ± 11.87^{b}	148.63 ± 16.08^{b}	**115.71 ± 19.44**	85.35 ± 18.51^{A}	127.04 ± 11.87^{B}	54.65 ± 10.94^{A}	62.24 ± 9.35^{A}
NPQ_{max}	**0.51 ± 0.18**	0.45 ± 0.09	0.42 ± 0.14	0.45 ± 0.09	0.47 ± 0.08	**0.36 ± 0.11**	0.446 ± 0.09	0.446 ± 0.9	0.42 ± 0.07	0.46 ± 0.05
Ek_{NPQ}	**78.72 ± 28.94**	72.05 ± 3.86	64.99 ± 12.04	74.66 ± 7.78	74.78 ± 6.43	**47.41 ± 9.11**	71.57 ± 10.16	71.57 ± 10.16	68.93 ± 14.24	63.93 ± 16.65

(Table 6). In the non-enriched treatment, EC_{50} was higher (lower antioxidant activity) than in the other treatment combinations (Fig. 4b). In algae collected at 2.0 m depth, significant differences were only found in nutrient-enriched treatments (Table 6), i.e. EC_{50} was higher (lower antioxidant activity) in the nutrient-enriched treatment (Fig. 4b) than in the non-enriched treatment.

Total MAA content. Total MAA content in *E. elongata* was higher in algae collected at 0.5 m depth than in those collected at 2.0 m (Fig. 5a). MAA content in algae from 0.5 m depth showed a significant increase under 100 %$_{PAB}$ in nutrient-enriched treatments (Table 7, Fig. 5a). In contrast, total MAA content in algae collected from 2.0 m depth was significantly higher at 100 %$_{PAB}$ for both enriched and non-enriched nutrient treatments (Table 7, Fig. 5a). The most abundant MAAs detected in this species were shinorine (50 to 60 %) and palythine (approx. 40 %), other MAAs such as asterina-330 were present in trace amounts. After the *in situ* experiment, algae collected from 2.0 m depth showed significantly higher palythine content under nutrient-enriched treatments, and shinorine increased in non-enriched treatments (Table 7, Fig. 5b,c). In contrast, algae collected from 0.5 m did not show any differences (Table 7).

DISCUSSION

We found high photoacclimation in *Cystoseira tamariscifolia* and *Ellisolandia elongata*, with photosynthetic parameters and biochemical composition changing in response to the short-term irradiance and nutrient treatments (60 h). The algae collected from 0.5 m depth had a higher production (ETR) and efficiency (α_{ETR}) than those from 2.0 m depth. These differences can be explained by the high transparency in the coastal waters of Cabo de Gata-Níjar Natural Park, allowing high penetration of both PAR and UVR, which can produce negative biological effects such as photoinhibition or DNA damage. In our study, the attenuation coefficients for PAR (Kd_{PAR}) and UVA (Kd_{UVA}) were 0.076 m^{-1} and 0.137 m^{-1}, respectively. Figueroa & Gómez (2001) described these coefficients with similar results for PAR (Kd_{PAR}) and UVA (Kd_{UVA}), 0.070 m^{-1} and 0.100 m^{-1}, respectively, and a Kd_{UVB} value of 0.22 m^{-1} in the same coastal area.

Table 4. ANOVA results after *in situ* experiment testing for the effect of irradiance and nutrients on the photosynthetic pigment content of *Cystoseira tamariscifolia* and *Ellisolandia elongata* collected at 2 different depths. We used a significance level of $\alpha = 0.05$, shown in **bold**; nd: no data

	df	*Cystoseira tamariscifolia*						*Corallina elongata*					
		0.5 m depth			2.0 m depth			0.5 m depth			2.0 m depth		
		MS	*F*	p	MS	*F*	p	MS	*F*	p	MS	*F*	p
Chl *a*													
Nutrients (*N*)	1	0.177	1.085	0.328	0.624	3.579	0.095	0.034	3.971	0.081		nd	
Irradiance (*E*)	1	**2.152**	**13.170**	**0.007**	0.040	0.231	0.644	0.040	4.694	0.062			
N × *E*	1	0.038	0.233	0.642	0.770	4.416	0.069	0.005	0.635	0.448			
Residual	8	0.163			0.174			0.009					
Chl *c*													
Nutrients (*N*)	1	**0.015**	**7.653**	**0.024**	0.000	0.162	0.698		nd			nd	
Irradiance (*E*)	1	0.000	0.064	0.807	**0.012**	**6.201**	**0.038**						
N × *E*	1	0.000	0.002	0.963	0.001	0.318	0.588						
Residual	8	0.002			0.002								
Phycoerythrin													
Nutrients (*N*)	1		nd			nd		0.860	3.418	0.102		nd	
Irradiance (*E*)	1							0.421	1.672	0.232			
N × *E*	1							0.017	0.066	0.803			
Residual	8							0.252					
Phycocyanin													
Nutrients (*N*)	1		nd			nd		**0.067**	**5.903**	**0.041**		nd	
Irradiance (*E*)	1							0.06	5.31	0.05			
N × *E*	1							0.001	0.113	0.745			
Residual	8							0.011					
Fucoxanthin													
Nutrients (*N*)	1	**184106.8**	**9.560**	**0.015**	4812.6	0.229	0.645	**36.41**	**6.890**	**0.030**		nd	
Irradiance (*E*)	1	2085.6	0.108	0.751	**132991.1**	**6.328**	**0.036**	**78.77**	**14.904**	**0.005**			
N × *E*	1	256.3	0.013	0.911	4799.9	0.228	0.646	5.92	1.120	0.321			
Residual	8	19257.5			21016.5			5.29					
Violoxanthin													
Nutrients (*N*)	1	**3327.25**	**13.924**	**0.006**	6.55	0.032	0.863	0.102	1.326	0.283		nd	
Irradiance (*E*)	1	1.74	0.007	0.934	**2108.12**	**10.235**	**0.013**	0.200	2.590	0.146			
N × *E*	1	18.83	0.079	0.786	107.35	0.521	0.491	0.150	1.950	0.200			
Residual	8	238.96			205.98			0.077					
Anteraxanthin													
Nutrients (*N*)	1	0.08	0.004	0.953	**43.88**	**28.707**	**0.001**	89.32	4.422	0.069		nd	
Irradiance (*E*)	1	31.07	1.460	0.261	2.62	1.713	0.227	**204.13**	**10.106**	**0.013**			
N × *E*	1	7.77	0.365	0.562	**9.00**	**5.885**	**0.041**	3.59	0.178	0.684			
Residual	8	21.28			1.53			20.20					
Zeaxanthin													
Nutrients (*N*)	1	54.69	1.455	0.262	2.02	0.066	0.804	3.58	1.727	0.225		nd	
Irradiance (*E*)	1	0.06	0.002	0.969	0.55	0.018	0.896	4.67	2.257	0.171			
N × *E*	1	85.64	2.278	0.170	9.10	0.297	0.601	0.41	0.199	0.667			
Residual	8	37.59			30.63			2.07					
β-carotene													
Nutrients (*N*)	1	**623.87**	**6.230**	**0.037**	**328.83**	**7.710**	**0.024**	13.12	4.432	0.068		nd	
Irradiance (*E*)	1	97.13	0.970	0.354	58.13	1.363	0.277	**45.76**	**15.465**	**0.004**			
N × *E*	1	457.29	4.566	0.065	**1016.02**	**23.821**	**0.001**	2.32	0.785	0.401			
Residual	8	100.14			42.65			2.96					

Table 5. Pigment contents (mean values ± SE, n = 3) of *Cystoseira tamariscifolia* and *Ellisolandia elongata* collected at 2 different depths (0.5 m and 2.0 m) in relation to irradiance (70 $\%_{PAB}$ and 100 $\%_{PAB}$) and nutrient (Nutrients+ and Nutrients–) treatments. Chl *a*, chl *c*, phycoerythrin and phycocyanin contents are expressed in mg g^{-1} DW. Fuxocanthin, violaxanthin, antheraxanthin, zeaxanthin and β-carotene contents are expressed in µg g^{-1} DW. Initial values (I_S: 0.5 m depth; I_D: 2.0 m depth) are shown in the first column and in **bold** for each depth. Uppercase letters denote significant differences after SNK test in algae collected at 2.0 m depth, nd: no data

	I_S	0.5 m depth				I_D	2.0 m depth			
		Nutrients+		Nutrients–			Nutrients+		Nutrients–	
		100 $\%_{PAB}$	70 $\%_{PAB}$	100 $\%_{PAB}$	70 $\%_{PAB}$		100 $\%_{PAB}$	70 $\%_{PAB}$	100 $\%_{PAB}$	70 $\%_{PAB}$
Cystoseira tamariscifolia										
Chl *a*	**4.87 ± 0.27**	1.51 ± 0.07	2.24 ± 0.44	1.15 ± 0.11	2.11 ± 0.02	**2.11 ± 0.29**	1.73 ± 0.3	1.34 ± 0.23	0.77 ± 0.26	1.39 ± 0.1
Chl *c*	**0.17 ± 0.01**	0.18 ± 0.01	0.18 ± 0.03	0.11 ± 0.01	0.11 ± 0.02	**0.08 ± 0.01**	0.08 ± 0.02	0.16 ± 0.02	0.09 ± 0.02	0.13 ± 0.02
Fucoxanthin	**557.6 ± 1.5**	611.9 ± 28.3	629. ± 119.5	354.9 ± 38.2	390.5 ± 95.6	**265.8 ± 50.4**	292.5 ± 68.4	543.1 ± 88.5	292.5 ± 76.4	463 ± 98.3
Violaxanthin	**60.47 ± 2.56**	82.88 ± 3.23	86.15 ± 12.64	52.09 ± 6.13	50.34 ± 10.51	**29.76 ± 5.79**	38.38 ± 9.56	70.87 ± 7.9	42.88 ± 7.49	63.41 ± 8.02
Antheraxanthin	**13.89 ± 0.59**	14.27 ± 2.54	9.44 ± 2.65	12.51 ± 1.91	10.89 ± 3.34	**9.06 ± 1.51**	6.51 ± 0.37^{AB}	5.71 ± 1.04^{A}	8.61 ± 0.84^{B}	11.27 ± 0.28^{C}
Zeaxanthin	**13.58 ± 1.91**	8.03 ± 1.17	2.83 ± 0.70	6.95 ± 2.03	12.44 ± 6.52	**12.91 ± 1.81**	12.36 ± 3.47	10.19 ± 2.64	9.81 ± 2.00	11.11 ± 4.21
β-carotene	**71.15 ± 5.57**	20.50 ± 11.08	7.39 ± 2.46	22.57 ± 1.63	29.23 ± 1.38	**33.99 ± 2.29**	20.76 ± 0.93^{B}	6.76 ± 0.9^{A}	12.83 ± 2.98^{AB}	35.64 ± 6.8^{C}
Ellisolandia elongata										
Chl *a*	**0.43 ± 0.04**	0.35 ± 0.05	0.51 ± 0.01	0.29 ± 0.08	0.36 ± 0.00	**0.21 ± 0.01**	0.24 ± 0.03	0.39 ± 0.06	nd	0.38 ± 0.01
Phycoerythrin	**1.54 ± 0.05**	2.61 ± 0.28	2.16 ± 0.47	2.00 ± 0.13	1.7 ± 0.10	**0.86 ± 0.15**	0.74 ± 0.05	0.69 ± 0.12	nd	0.45 ± 0.08
Phycocyanin	**0.16 ± 0.01**	0.59 ± 0.06	0.42 ± 0.09	0.42 ± 0.03	0.29 ± 0.01	**0.27 ± 0.03**	0.18 ± 0.00	0.16 ± 0.02	nd	0.1 ± 0.02
Fucoxanthin	**7.76 ± 2.42**	4.12 ± 0.20	10.64 ± 2.42	2.04 ± 0.18	5.76 ± 1.03	**3.72 ± 0.38**	1.91 ± 0.38	3.33 ± 0.52	nd	4.25 ± 0.66
Violaxanthin	**0.49 ± 0.09**	0.67 ± 0.27	0.71 ± 0.06	0.26 ± 0.02	0.75 ± 0.15	**0.37 ± 0.01**	0.32 ± 0.02	0.43 ± 0.07	nd	0.35 ± 0.01
Antheraxanthin	**21.31 ± 1.73**	14.84 ± 4.31	22.00 ± 0.85	8.29 ± 0.91	17.63 ± 2.61	**7.34 ± 0.77**	10.00 ± 1.57	19.41 ± 1.54	nd	17.70 ± 1.06
Zeaxanthin	**3.15 ± 0.12**	3.67 ± 0.31	2.79 ± 0.06	5.13 ± 1.48	3.51 ± 0.66	**5.98 ± 1.92**	2.66 ± 0.51	6.09 ± 1.66	nd	8.01 ± 0.28
β-carotene	**11.95 ± 0.52**	6.77 ± 1.62	9.8 ± 0.45	3.81 ± 0.66	8.59 ± 0.8	**4.03 ± 0.83**	5.08 ± 1.02	10.63 ± 0.62	nd	5.05 ± 2.57

The C:N ratio was more favorable physiologically (<23) in *C. tamariscifolia* from 0.5 m than in algae from 2.0 m (>30). On the other hand, the elevated NPQ_{max} indicated high photoprotection capacity. The sun-type photosynthetic pattern of the species analyzed is shown by the high Ek_{ETR} values (200 to 220 µmol photons m^{-2} s^{-1}) in algae collected at both 0.5 and 2.0 m (initial conditions). These values were lower than those reported by Celis-Plá (2011) and Figueroa et al. (2014, this Theme Section) in *C. tamariscifolia* growing in a nearby coastal area of the Mediterranean Sea but subjected to emersion conditions, in contrast to the subtidal species of Cabo de Gata-Níjar, i.e. higher nutrient and irradiance levels than those found in this study.

According to the physiological status, algae grown at 0.5 m will be less vulnerable to higher irradiance conditions (100 $\%_{PAB}$) than algae grown at 2.0 m. At the initial natural conditions, the phenolic compounds (photoprotectors) in *C. tamariscifolia* are expected to be higher in algae grown at 0.5 m than at 2.0 m. However, in algae collected at 0.5 m depth, the phenolic compounds were lower than algae collected at 2.0 m, during the initial period. This can be explained as a consequence of the high irradiance found at 0.5 m, since phenolic compounds could be released under high solar irradiance, preventing the photodamage as a photoprotection strategy (Abdala-Díaz et al. 2006). Photoacclimation responses were also affected by nitrate supply in general; nitrate enrichment increased the photosynthetic rate and the accumulation of photoprotectors. This indicates that the algae are nutrient-limited in this oligotrophic system (Figueroa & Gómez 2001).

C. tamariscifolia collected from 0.5 m depth maintained ETR values 60 h after transferring to 100 $\%_{PAB}$ in both nutrient conditions, but phenolic compounds and internal N content increased only in nutrient-enriched conditions. The transplantation to 70 $\%_{PAB}$ provoked an increase in ETR_{max}, indicating that algae at 0.5 m depth were photoinhibited under initial conditions. The increase of ETR_{max} at 70 $\%_{PAB}$ is related to a decrease in NPQ_{max}, indicating less energy dissipation as a consequence of decreased

Table 6. ANOVA results after *in situ* experiment testing for the effect of irradiance and nutrients on the phenolic compounds and antioxidant activity (EC_{50}) of *Cystoseira tamariscifolia* collected at 2 different depths. We used a significance level of α = 0.05, shown in **bold**

	df	0.5 m depth			2.0 m depth		
		MS	*F*	p	MS	*F*	p
Phenolic compounds							
Nutrients (*N*)	1	**262.2**	**7.956**	**0.022**	**107.7**	**5.955**	**0.041**
Irradiance (*E*)	1	30.1	0.912	0.367	**331.7**	**18.346**	**0.003**
N × *E*	1	36.1	1.094	0.326	0.3	0.014	0.908
Residual	8	33.0			18.1		
EC_{50}							
Nutrients (*N*)	1	0.014	1.417	0.268	**0.094**	**10.86**	**0.011**
Irradiance (*E*)	1	0.032	3.273	0.108	0.001	0.078	0.787
N × *E*	1	**0.068**	**6.918**	**0.030**	0.034	3.919	0.083
Residual	8	0.010			0.009		

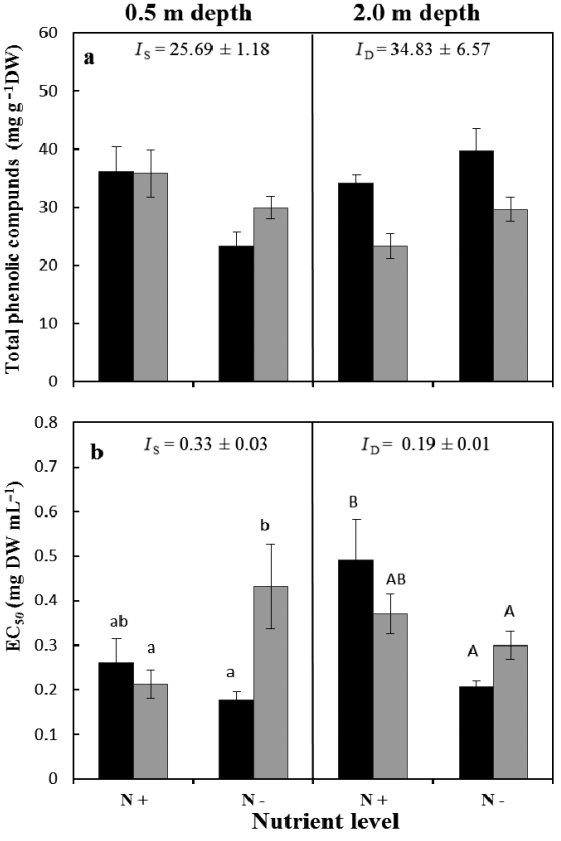

Fig. 4. (a) Total phenolic compounds and (b) antioxidant activity (EC_{50}) (mean ± SE, n = 3) of *Cystoseira tamariscifolia* from 0.5 and 2.0 m depths under irradiance and nutrient treatments. Black bars indicate 100 %$_{PAB}$, and grey bars indicate 70 %$_{PAB}$. N+ and N– indicate nutrient-enriched and non-enriched treatments, respectively. Upper values in each box indicate initial values (I_S: 0.5 m depth; I_D: 2.0 m depth). Lowercase letters denote significant differences after SNK test for algae collected at 0.5 m depth and capital letters for algae collected at 2.0 m

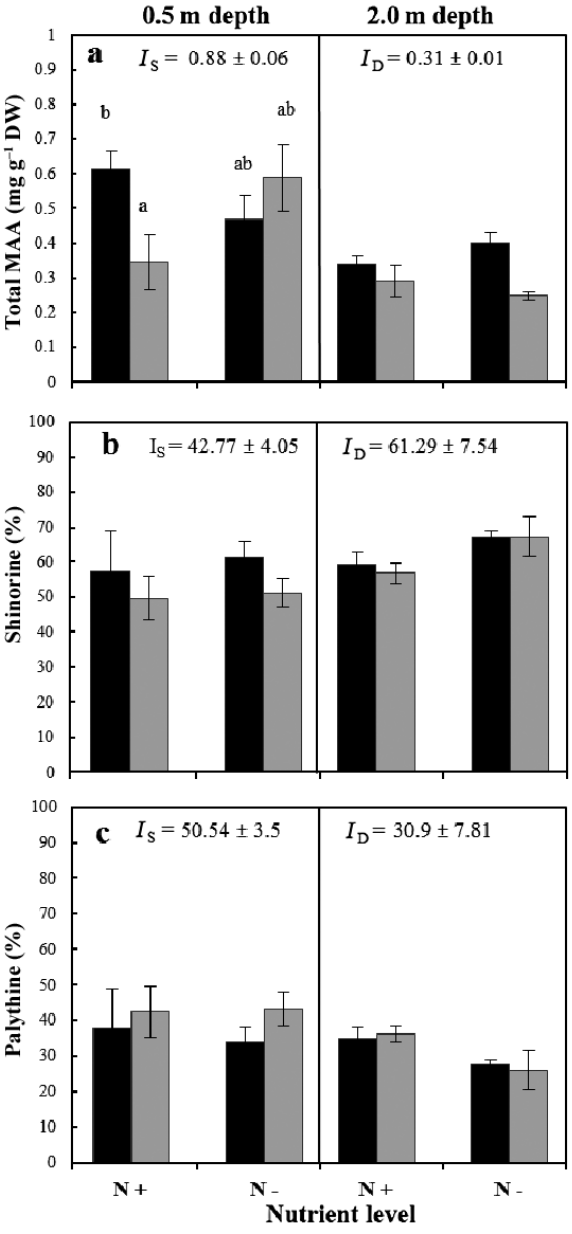

Fig. 5. (a) Total mycosporine-like amino acid (MAA) content and percentages of (b) shinorine and (c) palythine (mean values ± SE, n = 3) in *Ellisolandia elongata* from 0.5 and 2.0 m depth under irradiance and nutrient treatments. Black bars indicate 100 %$_{PAB}$, and grey bars indicate 70 %$_{PAB}$. N+ and N– indicate nutrient-enriched and non-enriched treatments, respectively. Upper values in each box indicate initial values (I_S: 0.5 m depth; I_D: 2.0 m depth). Lowercase letters denote significant differences after SNK test

irradiance, at least in the short-term period analyzed. In any case, prolonged time can eventually reduce the values of ETR_{max} due to less available energy at 2.0 m than that at 0.5 m in spite of photoinhibition. The ETR_{max} of algae collected from 2.0 m depth when transplanted to 100 %$_{PAB}$ increased only in non-

Table 7. ANOVA results after *in situ* experiment testing for the effect of irradiance and nutrients on total mycosporine-like amino acid (MAA) content, and percentages of shinorine and palythine of *Ellisolandia elongata* collected at 2 different depths. We used a significance level of $\alpha = 0.05$, shown in **bold**

	df	0.5 m depth			2.0 m depth		
		MS	*F*	p	MS	*F*	p
Total MAA content							
Nutrients (*N*)	1	0.008	0.427	0.532	0.000	0.096	0.764
Irradiance (*E*)	1	0.016	0.917	0.366	**0.030**	**10.453**	**0.012**
N × *E*	1	**0.114**	**6.471**	**0.035**	0.008	2.857	0.129
Residual	8	0.018			0.003		
% Shinorine							
Nutrients (*N*)	1	24.93	0.151	0.708	**252.61**	**5.92**	**0.041**
Irradiance (*E*)	1	239.16	1.448	0.263	4.81	0.113	0.746
N × *E*	1	6.09	0.037	0.852	4.88	0.114	0.744
Residual	8	165.13			42.69		
% Palythine							
Nutrients (*N*)	1	8.21	0.051	0.827	**230.52**	**6.35**	**0.036**
Irradiance (*E*)	1	143.23	0.885	0.374	0.14	0.004	0.952
N × *E*	1	18.23	0.113	0.746	7.84	0.216	0.654
Residual	8	161.87			36.28		

enriched treatments, but both internal N and phenolic compound contents increased under nutrient enrichment.

PAR and UVR can cause photoinhibition, which can be defined as the light-dependent decline in photosynthetic capacity and maximal photosynthetic efficiency as a consequence of the dominance of photodamage versus photorepair processes (Osmond 1994, Gómez et al. 2004). It is also thought that photoinhibition is a down-regulation mechanism to quench excessive solar energy (Demmig-Adams et al. 2008). However, in *C. tamariscifolia*, no photoinhibition was observed. Intertidal macroalgae from southern Spain have low photoinhibition at noon and high recovery capacity during daily cycles due to high energy dissipation (Figueroa et al. 1997, Häder et al. 1997, 1998).

Photosynthetic efficiency α_{ETR}, ETR_{max} and MAAs in *E. elongata* collected from 0.5 m depth decreased after transfer to 100 %$_{PAB}$ under both nutrient conditions, but internal N contents increased only under nutrient-enriched conditions. The transplant to 70 %$_{PAB}$ provoked an increase of α_{ETR} and ETR_{max} only under nutrient-enriched conditions; however, internal N content and MAAs decreased in both nutrient treatments, indicating that algae grown at 0.5 m depth can be photoinhibited under initial conditions. The level of ETR_{max}, α_{ETR} and MAAs in algae collected from 2.0 m depth increased when they were transplanted to 100 %$_{PAB}$ under both nutrient treatments; however, the internal N content decreased in both nutrient treatments. The transplantation of algae collected from 2.0 m depth to 70 %$_{PAB}$ caused a higher α_{ETR} and ETR_{max} in both nutrient conditions; however, internal N content and MAAs increased in nutrient-enriched conditions.

In general, in both species collected from 0.5 m depth, the addition of nutrients increased their photosynthetic efficiency. The photosynthetic response was also affected by irradiance levels. Although the initial values of NPQ_{max} in *C. tamariscifolia* were similar, NPQ_{max} decayed at both depths under nutrient enrichment and Ek_{NPQ} only increased in the enriched treatment. Furthermore, in *C. tamariscifolia* collected at 2.0 m depth, an interaction between light and nutrients was observed, where transplanted algae (to 100 %$_{PAB}$) under non-enriched treatment showed an increase in NPQ_{max} and Ek_{NPQ} in all treatments. At high nutrient availability, it seems that algae collected from 0.5 m depth had higher levels of photoprotective compounds (phenols) or increased size of antenna (higher content of chl *c* and fucoxanthin were observed). This could be due to high antioxidant activity and less requirement for the dissipation of energy in the form of heat (low NPQ_{max}) or due to less UV radiation that could be reaching the photosynthetic apparatus. However, in *C. tamariscifolia* collected at 2 m depth after the transplant conditions (70 %$_{PAB}$), high levels of accessory pigments were found. These differences were independent of the nutrient treatment. The phenolic compounds and antioxidant activity were affected by irradiance and nutrients as single factors in the first case, and by the interaction of both factors in the second case. For the other carotenoids, similar results were found in *C. tamariscifolia* collected from 0.5 m depth.

Carotenoid contents were less influenced by irradiance or nutrients with the exception of violaxanthin that had higher content after nutrient enrichment. On the other hand, in *C. tamariscifolia* collected from 2.0 m depth, violaxanthin content was higher in the simulated deeper irradiance (70 %$_{PAB}$), as was found in other accessory pigments. However, antheraxanthin and β-carotene were significantly affected by the interaction of irradiance and nutrients. In *E. elongata* collected from 0.5 m depth, an effect of irradiance was found. The responses found in this study for both species are similar to those described by Demmig-Adams & Adams (1996). The response of the xanthophyll cycle and light absorption could re-

flect a regulatory and photoprotective response that down-regulates the delivery of excitation energy into the electron-transport chain to match the rates at which products of electron transport can be consumed in these leaves. Goss & Jakob (2010) indicate that the xanthophyll cycle represents an important photoprotection mechanism in plant cells. This suggests a relationship between higher photosynthetic rates and a higher activity of the xanthophyll cycle. However, the presence of a functional xanthophyll cycle in red algae is uncertain (Andersson et al. 2006, Schubert et al. 2006). In fact, the predominant presence of red algae in intertidal zones and coral reefs suggests a highly efficient capacity to withstand elevated irradiance levels and large diurnal light fluctuations due to tides and aerial exposure (Schubert et al. 2011).

E. elongata possesses high reflectance under high solar radiation, allowing it to live in areas of high radiation and sun exposure due to a skeleton composition of calcium carbonate (Häder et al. 1997). These authors described a high reflectance under high solar radiation exposure in *E. elongata*, which can be advantageous under elevated solar irradiance reducing photoinhibition in this species.

Connan et al. (2004) found higher levels of phenols in summer in several brown macroalgae off Brittany related to higher solar irradiance. Similarly, Abdala-Díaz et al. (2006) found higher phenol content in summer than in winter in *C. tamariscifolia* collected in southern Spain in the morning. However, at noon the levels were similar in both seasons due to the high release of polyphenols in summer. In our study, the phenolic content in *C. tamariscifolia* increased with nutrient enrichment in algae collected at 0.5 m depth in the non-enriched treatment and in transplanted specimens (to 100 $\%_{PAB}$) under non-enrichment treatments in those collected from 2.0 m depth. In brown algae, UV screen compounds (polyphenols) accumulate under high PAR and UVR and these compounds have strong antioxidant activity (Pavia et al. 1997, Connan et al. 2004, Cruces et al. 2012). This may suggest that this is probably more related to the nitrate availability than to solar irradiance conditions. Pavia & Toth (2000) indicate that the N content can enhance the accumulation of phenolic compounds in some brown algae. In fact, concentrations of phenolic compounds show phenotypic plasticity in response to changes in environmental parameters, such as salinity, nutrients, light quality and availability, and intensity of herbivores (Peckol et al. 1996, Pavia et al. 1997, Pavia & Toth 2000, Honkanen et al. 2002, Swanson & Druehl 2002, Amsler & Fairhead 2006). Moreover, *C. tamariscifolia* had higher antioxidant activity at 0.5 m depth in transplanted conditions (70 $\%_{PAB}$) without nutrient enrichment, and also in algae collected from 2.0 m depth in transplant conditions (100 $\%_{PAB}$) with nutrient enrichment.

As has been mentioned, the response of *E. elongata* collected from 0.5 m depth was dependent mostly on irradiance. However, the content of MAAs (UV-screening substance) of algae collected at 0.5 m depth depended on the interaction between irradiance and nutrients, as reported by Korbee-Peinado et al. (2004). Karsten et al. (1998) and Franklin et al. (2001) have shown that accumulation of MAAs depend on both quality and quantity of radiation, with higher accumulation of MAAs with high daily PAR doses and UV exposure. Korbee-Peinado et al. (2004) found that high ammonium concentrations significantly increased the content of MAAs in *Pyropia columbina* (as *Porphyra columbina*). In their study, an interaction between irradiance and nutrients was found. Similar results were found for other *Porphyra* species, *Grateloupia lanceola* and *Gracilaria* spp. (Korbee et al. 2005a, Huovinen et al. 2006, Barufi et al. 2011, Figueroa et al. 2012). In our study, the MAA total content decreased in algae transplanted from 100 $\%_{PAB}$ to 70 $\%_{PAB}$ and after nutrient enrichment, whereas no effect of nutrient was observed in algae collected from 2.0 m depth waters. It seems that the short-term effect of the nutrient addition is not enough to produce an increase of total MAA content under nitrogen-enriched conditions as has been reported in other algae (Barufi et al. 2011, Figueroa et al. 2012). However, the effect of nutrients was reflected by a preferential accumulation of some types of MAAs, but only in *E. elongata* collected from 2.0 m depth. The relative content of palythine increased in nutrient-enriched algae, which has been associated with higher antioxidant activities compared to shinorine (de la Coba et al. 2009).

In conclusion, *C. tamariscifolia* and *E. elongata* showed different physiological responses under different nutrient and irradiance conditions. Few interactive effects between these 2 physical stressors were found, suggesting major additive effects on the responses of both species. In fact, environmental variables acting in additive forms can act as more powerful stress factors (Martínez et al. 2012) leading to changes in the physiology of these macroalgae. Therefore, understanding the physiological consequences of the potential additive effects of these physical stressors on these dominant species is needed to predict future environmental fluctuations related to climate change.

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