Ecophysiological responses to elevated CO_2 and temperature in *Cystoseira tamariscifolia* (Phaeophyceae)

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ABSTRACT

Ocean acidification increases the amount of dissolved inorganic carbon (DIC) available in seawater which can benefit photosynthesis in those algae that are currently carbon limited. Carbon dioxide emissions are causing fundamental changes in surface ocean carbon chemistry that are expected to boost growth rates in some algae but not others, leading to shifts in the structure and function of seaweed communities. Recent studies show ocean acidification driven shifts in seaweed community dominance depend on interactions with other factors such as light and nutrients. The study of interactive effects of ocean acidification and warming can help elucidate the likely effects of climate change on marine primary producers. In this study, we investigated the ecophysiological responses in a brown macroalgae species *Cystoseira tamariscifolia* (Hudson) Papenfuss with important structural role in the coastal Mediterranean communities. Algae were collected in oligotrophic *vs* ultra-oligotrophic waters in the Mediterranean Sea and they were incubated in tanks at ambient (*ca.* 400-500 ppm) and high CO_2 (*ca.* 1200-1300 ppm), and at two testing temperatures at 20ºC (ambient temperature) and 24ºC (ambient temperature + 4ºC). Increased CO_2 levels benefited the algae from both origins. Biomass increased in elevated CO_2 treatments and was similar in algae from both origins. The maximal electron transport rate (ETR_{max}), used to estimate photosynthetic capacity, increased in ambient temperature/high CO_2 treatments. The highest polyphenol content and antioxidant activity were observed in ambient temperature/high CO_2 conditions in algae from both origins being the phenol content higher in algae from ultra-oligotrophic waters (1.5 - 3.0 %) than that from oligotrophic waters (1.0 - 2.2 %). Our study shows that ongoing ocean acidification can be expected to increase algal productivity (ETR_{max}), boost antioxidant activity (EC_{50}) and increase production of photoprotective compounds (polyphenols). *Cystoseira tamariscifolia* collected from oligotrophic and ultra-oligotrophic waters were able to acclimate to increases in DIC with under ambient temperature.

INTRODUCTION

Ocean acidification due to increased atmospheric CO_2 levels is altering the concentrations of dissolved inorganic carbon (DIC) in surface waters; CO_3^{2-} levels are falling, which can cause dissolution of calcified organisms, whilst CO_2 and HCO_3^- levels are rising which can stimulate photosynthesis (Connell et al. 2013; Cornwall et al. 2012, 2015; Newcomb et al. 2015). Rising atmospheric CO_2 levels have increased seawater temperatures by 0.13°C per decade over the last 50 years (IPCC 2014) and the regression of the seaweeds at their warmest biogeographic limits are being observed (Harley et al. 2006; Brodie et al. 2014; Wernberg et al. 2016). Research into the combined effects of acidification and warming is advancing our understanding of the mechanisms that drive algal responses to global climate change (Figueroa and Korbee 2010; Diaz-Pulido et al. 2012; Martínez et al. 2015). Work on brown macroalgae has shown that global climate change can affect a range of key processes, such as nutrient uptake and photosynthesis, with knock-on effects on growth, calcification and reproduction (Betancor et al. 2014; Figueroa et al. 2014; Stengel et al. 2014).

Long-term observations in tanks and work at CO_2 seep systems have shown that many calcifying of seaweeds species are more vulnerable to the effects of ocean acidification at warmer seawater temperatures (Martin and Gattuso 2009). Thus, the changes in the structure of the community can be altered by the ocean acidification due to the decrease of growth rates in marine calcifies seaweeds, e.g., coralline algae (Kuffner et al. 2008, Martin and Gattuso 2009), and increase in non-calcifying macroalgae, e.g., kelp forests (Connell and Russell 2010, Celis-Plá et al. 2015). Many brown macroalgae thrive at high CO_2 concentrations (Enochs et al. 2015; Linares et al. 2015), often up-regulating their photosynthesis and the nutrient uptake as well as their production of the polyphenols and antioxidants (Figueroa et al. 2014; Celis-Plá et al. 2016).

Canopy-forming brown algae have declined in abundance over the past 50 years due to anthropogenic perturbations such as siltation and increased temperature and

nutrients levels (Strain et al. 2014; Yesson et al. 2015; Wernberg et al. 2016) yet the canopy-forming brown algae *Cystoseira* spp. and *Sargassum vulgare* proliferate near coastal CO_2 seeps in the Mediterranean (Porzio et al. 2011; Baggini et al. 2014; Celis-Plá et al. 2015) and in mesocosm systems (Ju-Hyoung et al. 2016). *Cystoseira* spp. are used as indicators of high water quality in the Mediterranean as they help maintain the structure and function of coastal ecosystems (Celis-Plá et al. 2016). As Ocean acidification is not happening in isolation, but alongside a plethora of other anthropogenic changes, the study of the combination of the multiple stressors in the Mediterranean Sea, is critical to plan for to explain the disappearance of sensitive ecosystem of the canopy-forming seaweeds. These "habitat-forming" species are suffering a general decline (Fernández 2011, Pérez-Lloréns et al. 2014) and habitat destruction or degradation. Considered as threat to the diversity, structure, functioning and services they provide of marine coastal ecosystems in the Mediterranean Sea (Claudet and Fraschetti 2010, Coll et al. 2010).

Our study focuses on the Alboran Sea (western part of Mediterranean Sea), parts of which are so oligotrophic where macroalgal growth is limited (Ferreira et al. 2011; Mercado et al. 2012). We grew *Cystoseira tamariscifolia* in tanks system to examine the combined effects of CO_2 and temperature on thalli collected from ultra-oligotrophic *vs* less limited nutrient parts of the coast. Here, we analyze ecophysiological responses to ocean acidification at ambient temperature and close to their temperature tolerance limits using standard methods for the study of multiple physical stressors in algae (Stengel et al. 2014; Celis-Plá et al. 2015; Martínez et al. 2015). Our hypothesis is that these macroalgae would benefit from elevated levels of dissolved inorganic carbon (DIC) at ambient conditions of temperature when nutrient levels were sufficient. On the other hand, the temperature increase can produce negative effects since *Cystoseira tamariscifolia* of Alboran Sea is located in the southern limit of the distribution of this species (Gómez-Garreta et al. 2001). In spite of the importance of these algal communities, the studies on the vulnerability and acclimation to increased temperature are scarce (Serio et al. 2006, Strain et al. 2014). Thus, we expect that increase of pCO_2 levels will produce an increase of photosynthetic activity, photoprotectors and antioxidant activity in *C. tamariscifolia* only under ambient temperature.

MATERIAL AND METHODS

Species and sampling

Cystoseira tamariscifolia (Hudson) Papenfuss, (Phaeophyceae, Fucales) (Gómez-Garreta et al. 2001) were collected haphazardly on 25 September 2013 in Cabo de Gata-Nijar Natural Park (36º51'N, 2º6'W) and off La Araña beach, Malaga (36º42'N, 4º19'W) both in the Alboran Sea. The waters off the Natural Park had lower concentrations of nitrate and phosphate and lower vertical attenuation coefficient of light (Kd) than La Araña beach (Table S1; according to Ramírez et al. 2005; Mercado et al. 2007, 2012), and the waters can be classified as ultra-oligotrophic and oligotrophic waters, respectively according to the classification proposed by Organization for Economic Cooperation and Development (1982).

Experimental design

Cystoseira tamariscifolia (25-30 g fresh weight) were incubated from 27 September to 29 October 2013 (after 48 hours of acclimation), in open tanks system. The experiment was designed to examine interactive effects of current pCO_2 (*ca.* 400-500 ppm, pH target above 8.22 to 8.34) and predicted future concentration (*ca.*1200-1300 ppm, pH target 7.88) in combination with two temperature levels, ambient temperature (target above 19-20ºC) and ambient temperature + 4ºC (target above 24ºC), predicted future temperature for the year 2100 (IPCC 2014). The four treatments were ambient temperature x ambient CO_2 ($ATxACO_2$), ambient temperature x high CO_2 ($ATxHCO_2$), high temperature x ambient CO_2 ($HTxACO_2$) and high temperature x high CO_2 ($HTxHCO_2$), in total 24 tanks were used with two replicates per tank, i.e., 6 replicates per treatments; four treatments with three replicate tanks for ultraoligotrophic waters and three replicate tanks for oligotrophic waters.

Experimental conditions

The experimental system consists in 24 open tanks (0.094 m^2 surface area, 14 L volume), connected in parallel each three tanks to a separate tank of 102 L capacity and these were placed within a tank of 1000 L in water baths. The water flow between each tank and its header tank (102 L capacity) was 0.84 ± 0.05 L min^{-1}, representing a turnover rate of 26 ± 1% h^{-1}. A temperature control System, was monitored T2001HC, Aqua Medic was used in the tanks system (following methods given by Stengel et al. 2014). For carbon treatment was operated a computer-operated pH control system (AT) with pH sensors (Aqua Medic T2001HC, Aqua Medic), located inside each of the eight 102 L header tanks. The system automatically recorded one measurement every 15 min

and was programmed to initiate the supply of pure CO_2 via a solenoid valve as soon as the pH exceeded a threshold of 7.88 in the header tanks (corresponding to 1200 ppm of CO2, HC treatment). When the pH returned to this value, CO_2 injection stopped. The seawater carbonate system was monitored two times at each week, taking water samples to measure the temperature, salinity, pH_{NBS} and total alkalinity (following methods given by Celis-Plá et al. 2015). Other parameters of the carbonate chemistry were calculated using CO_2SYS. Seawater was enriched with 2 µM nitrate (KNO_3) and 0.1 µM phosphate (KH_2PO_4) giving an N:P ratio of 20:1 for oligotrophic waters and with 0.5 µM nitrate (KNO_3) and 0.1 µM phosphate (KH_2PO_4) giving an N:P ratio of 5:1 for ultraoligotrophic waters according to Ramírez et al. (2005) and Mercado et al. (2007 and 2012) (Table S1). This was assessed by taking triplicate seawater samples from all treatments. Seawater was filtered *in situ* using portable GF/F filters (Whatman International. Ltd., Maidstone, UK), transported to the laboratory inside an isotherm bag (4ºC, in darkness), and kept at -20ºC. Nitrate (NO_3^-) and phosphate (P) were determined using an automated wet chemistry analyzer (SanPlus++ System, SKALAR, Breda, Netherlands) applying standard colorimetric procedures (Koroleff 1983).

The outdoor tanks system were shaded using a neutral green mesh reducing photosynthetically active radiation (PAR; 400-700 nm) by 35%, and UVA (320-400 nm) and UVB (280-320 nm) by 39%, as reported by Stengel et al. (2014). Incident irradiance was monitored continuously in air using an UV-PAR Multifilter radiometer NILU-6 (Geminali AS, Oslo, Norway). The irradiances of UVA and UVB were calculated using methods provided by Høiskar et al. (2003).

Biomass

Fronds of the *C. tamariscifolia* were blotted dry and weighed immediately before being transferred to the experimental tanks and after the experimental period (28 days). Growth was calculated according to Martínez et al. (2015)

$$Biomass = (FW_{t=f} - FW_{t=0}) \cdot day^{-1} \quad (1)$$

where $FW_{t=f}$ is fresh weight measured to final and $FW_{t=0\ is}$ fresh weight measured before the start of the experiment (Fig. 1).

Internal carbon and nitrogen content

Stoichiometric ratios (C:N) were determined to estimate the physiological status of the seaweeds (according to Figueroa and Korbee 2010). Seaweed samples (1-2 g FW) were dried for 48 hours in an oven at 60°C. Total internal C and N contents on a dry weight (DW) basis were determined using a CNHS-932 elemental analyzer (Leco Corporation, Michigan, USA).

Photosynthetic activity

Photosynthetic activity was estimated by *in vivo* chlorophyll a fluorescence associated to Photosystem II (PSII) by using a portable pulse amplitude modulated (PAM) fluorometer (Diving-PAM, Walz GmbH, Germany). Macroalgal thalli were collected from natural populations (initial time). In order to obtain rapid light curves (RLC) for each treatment, apical parts of *C. tamariscifolia* were introduce in 10 mL incubation chambers. F_o and F_m were measured after 15 minutes in darkness to obtain the maximum quantum yield (F_v/F_m) being $F_v=F_m-F_o$, F_o the basal fluorescence of 15 min dark adapted thalli and F_m maximal fluorescence after a saturation light pulse of >4000 µmol m^{-2} s^{-1} (Schreiber et al. 1995). The electron transport rate (ETR) as estimator of photosynthetic capacity was determined after 20s exposure in eight increasing irradiances of white light (halogen lamp provided by the Diving-PAM). The ETR was calculated according to Schreiber et al. (1995) as follows:

$$ETR\ (\mu mol\ electrons\ m^{-2}\ s^{-1}) = \Delta F/F'_m \times E \times A \times F_{II} \quad (2)$$

where $\Delta F/F'm$ is the effective quantum yield, being $\Delta F = Fm'-Ft$ (*Ft* is the intrinsic fluorescence of alga incubated in light and *Fm'* 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 (Figueroa et al. 2003) and F_{II} is the fraction of chlorophyll related to PSII (400-700 nm) being 0.8 in brown macroalgae (Grzymski et al. 1997) . ETR parameters as maximum electron transport rate (ETR_{max}) as estimator of photosynthetic efficiency were obtained from the tangential function reported by Eilers and Peeters (1988).

Phenolic compounds and antioxidant activity (EC_{50})

In brown algae, the UV screen compounds, with antioxidant capacity are the phenolic compounds (PC) were determined using 0.25 g fresh weight samples pulverized with a mortar and pestle with sea-sand and 2.5 mL of 80% methanol. After keeping the samples overnight at 4ºC, the mixture was centrifuged at 2253 g for 30 min at 4ºC, and then the supernatant was collected. PC were determined colorimetrically using Folin-Ciocalteu reagent and phloroglucinol (1,3,5-trihydroxybenzene, Sigma P-3502) as standard. Finally, the absorbance was determined at 760 nm using a spectrophotometer (UV Mini-1240, Shimadzu) (Celis-Plá et al. 2014). Total phenolic content was expressed as mg g^{-1} DW after determining the fresh to dry weight ratio in the tissue (4.3 for *C. tamariscifolia* from Cabo de Gata-Nijar Natural Park and 5.6 *C. tamariscifolia* from La Araña Beach, respectively). The results are expressed as average ± SE from three replicates of each treatment.

Antioxidant activity (EC_{50}) was measured on polyphenol extracts according to Blois (1958); 150 µL of DPPH (2, 2-diphenyl-1-picrylhydrazyil) prepared in 90% methanol were added to each extract. 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 expressed as the EC_{50} value (mg DW mL^{-1}) required to scavenge 50% of the DPPH in the reaction mixture. Ascorbic acid was used as a control (according to Celis-Plá et al. 2016).

Statistical analysis

The effects of the combined treatments on growth, photophysiological and biochemical responses of *C. tamariscifolia* were assessed using analysis of variance (ANOVA). For biomass, three fixed factors were considered: temperature with two levels; ambient temperature *vs*. high temperature (ambient + 4ºC temperature), CO_2 with two levels in this instance; *ca.* 400-500 *vs. ca.* 1200-1300 ppm and origin with two levels; ultraoligotrophic waters (Cabo de Gata-Nijar Natural Park) *vs*. oligotrophic waters (La Araña beach). For photophysiological and biochemical responses in *C. tamariscifolia*, four fixed factors were considered: time, temperature, CO_2 and origin. To ensure the independence of each replica, each thallus was measured once and then was eliminated. This design allowed us to test interactive and additive effects of the

variables on the physiological responses. Student Newman Keuls tests (SNK) were performed after significant ANOVA interactions (Underwood 1997). The homogeneity of variance of all data was confirmed by using Cochran tests and by visual inspection of the residuals (Underwood 1997). Analyses were performed by using SPSS v.21 (IBM, USA).

RESULTS

Experimental conditions

The seawater temperatures during the experimental period were 19.80 ± 0.15ºC in $ATxACO_2$, 20.10 ± 0.15ºC in $ATxHCO_2$, 23.91 ± 0.21ºC in $HTxACO_2$ and 23.90 ± 0.20ºC in high temperature and high pCO_2 treatment (mean ± SE, n = 2232; 279 measurements for each tank) and mean pH were 8.34 ± 0.01 in $ATxACO_2$, 7.88 ± 0.01 in $ATxHCO_2$, 8.22 ± 0.01 in $HTxACO_2$ and 7.88 ± 0.01 in $HTxHCO_2$, respectively (mean ± SE, n = 2232; 279 measurements for each tank) (Table 1). The average daily integrated irradiances for the experimental period were 4238 kJ m^{-2} for PAR, 329kJ m^{-2} for UVA and 22kJ m^{-2} for UVB. The nutrients, i.e., nitrate and phosphate concentrations were 2.58 ± 0.52 and 0.16 ± 0.01 µM in $ATxACO_2$, 1.06 ± 0.03 and 0.13 ± 0.01µM in $ATxHCO_2$, 2.02 ± 0.51 and 0.15 ± 0.02µM in $HTxACO_2$ and finally, 1.41 ± 0.57 and 0.15 ± 0.01 µM in $HTxHCO_2$, respectively (mean ± SE, n = 48 measurements) (Table 1).

Biomass

The biomass was significantly different ($p<0.01$) among all factors; temperature, CO_2 levels and origin (Table S1). *C. tamariscifolia*, only when the thalli came from oligotrophic waters showed enhanced growth under elevated pCO_2 with high temperature treatment (maximal values 0.32 ± 0.03 g FW day^{-1}, mean ± SE, n=6) compared to ambient pCO_2 with high temperature (minimal values -0.50 ± 0.06 g FW day^{-1}, mean ± SE, n=6). In addition, under ambient pCO_2, algae from oligotrophic waters lost biomass irrespective of temperature (Fig. 1). Nevertheless, algae from ultraoligotrophic waters showed no changes in biomass accretion irrespective of the treatment conditions they were exposed to. Algae from ultraoligotrophic waters showed maximal values 0.20 ± 0.07 g FW day^{-1} (mean ± SE, n=6) under high temperature/ambient pCO_2 and minimal values 0.07 ± 0.01 g FW day^{-1} (mean ± SE, n=6) under high temperature/high pCO_2 treatments (Fig. 1).

306

307 ***Internal Carbon (C) and Nitrogen (N) content***

308 Carbon internal content was significantly different (p<0.05) (Table S3) for
309 interaction between CO_2 and origin. Carbon content in *C. tamariscifolia* only when the
310 thalli came from ultraoligotrophic waters showed increase under elevated pCO_2
311 independent of the temperature levels, in first two weeks (Table 2). Nitrogen content
312 was significantly different (p<0.01) among time, temperature and CO_2 (Table S3).
313 Algae from ultraoligotrophic and oligotrophic waters showed changes to increase in
314 nitrogen internal content, at the end the experimental period, irrespective of the
315 treatment conditions they were exposed to.

316

317 ***Photosynthetic responses***

318 The maximal electron transport (ETR_{max}) as indicator of photosynthetic capacity
319 had interactive effects (p<0.01) among all factors (Table S3). The ETR_{max} data
320 increased under high pCO_2 conditions with ambient and high temperature in *C.*
321 *tamariscifolia* from oligotrophic waters respect to ultraoligotrophic waters, at the
322 second week during the experimental period (Fig. 2). Algae from both origins showed a
323 decreased in the ETR_{max} values at the end the experimental period, irrespective of the
324 treatment conditions they were exposed to (Fig. 2).

325

326 ***Total Polyphenolic compounds and antioxidant activity***

327 Polyphenolic compound (PC) was significantly (p<0.01) for all factors, and showed
328 interactive effects (p<0.05) between CO_2 and origin (Table S4). Polyphenols were *ca.*
329 1.5 - 3.0 % in algae collected from ultraoligotrophic waters (Fig. 3a) and in *C.*
330 *tamariscifolia* from oligotrophic waters, polyphenols were *ca.* 1.0 - 2.2 % (Fig. 3b), this
331 suggest more phenolic production, such as, photoprotection in the macroalgae collected
332 from ultraoligotrophic waters (Fig. 3a). In *C. tamariscifolia* the polyphenolic
333 compounds increased in high CO_2 conditions for both origins (Fig. 3). The antioxidant
334 activity (EC_{50}) was significantly different (p<0.01) for all factors and interaction among
335 temperature, CO_2 and origin (Table S4).
336 EC_{50} increased (i.e. lower EC_{50}) at the initial time (two first weeks) under high CO_2
337 conditions independent of the temperature, for both origins (Fig. 4).

338

339 **DISCUSSION**

Increased levels of dissolved inorganic carbon (DIC) can benefit photosynthesis and growth in *Cystoseira tamariscifolia* in the experimental tanks system and temporal scales of days (28d), confirming expected benefits of ocean acidification already reported for brown macroalgae (Harley et al. 2012; Cornwall et al. 2012; Brodie et al. 2014; Koch et al. 2014; Bender et al. 2014; Cornwall et al. 2015). In this study, we show benefits of pCO_2 increase on growth rate in *C. tamariscifolia* being the physiological responses more accelerated in oligotrophic than in ultraoligotrophic harvested algae. Temperature increase has negative effect on growth rate in algae from oligotrophic waters with low pCO_2 conditions and the biomass increased under elevated pCO_2 conditions, in *C. tamariscifolia* of both origins. We found that the full extent of these benefits was only gained at optimal temperatures and if sufficient nutrients were available, building upon work by Celis-Plá et al. (2015) at CO_2 seeps.

Reports on non-calcareous macroalgae from other regions have shown that the ocean acidification may increase due to beneficial effects on photosynthesis, production and growth (Harley et al. 2012; Koch et al. 2014; Brodie et al. 2014). Ocean acidification can benefit the physiological state and growth in the field (Johnson et al. 2012; Baggini et al. 2014; Celis-Plá et al. 2015), in laboratory work has shown that increases in dissolved inorganic carbon can benefit species such as *Gracilaria lemaneiformis* in China (Zou and Gao 2009) and *Feldmannia* spp. in Australia (Russell et al. 2011), as well as phaeophytes such as *Nereocystis luetkeana* and *Macrocystis pyrifera* in New Zealand (Swanson and Fox 2007; Roleda et al. 2012). Also in mesocosm systems *Sargassum thunbergii* showed an increased in the photosynthesis activity and growth, when exposed to high pCO_2 conditions (Ju-Hyoung et al. 2016). In this study, fucoid biomass from oligotrophic waters, showed enhanced growth under elevated pCO_2 and the macroalgae from ultraoligotrophic waters showed no changes in biomass irrespective of the treatment conditions they were exposed to. The highest carbon content was observed in *C. tamariscifolia* from ultraoligotrophic waters and incubated in elevated pCO_2 levels.

A positive correlation between carbon internal content and ETR_{max} was observed, this suggest that carbon supply increased photosynthetic production expressed as ETR_{max}. The highest ETR_{max} and nitrogen internal content were reached at increased pCO_2 and ambient temperature in algae from both origins being the highest levels reached in algae collected from ultraoligotrophic waters. The carbon content helps explain the dominance of this brown algae at a variety of coastal Mediterranean,

probably due to a combination of the direct stimulus of increased dissolved inorganic carbon for photosynthesis (Mercado et al. 1998, Raven and Hurd 2012). Johnson et al. (2012) also showed a significant effect on the photosynthetic responses of *Padina pavonica* with CO_2 enrichment. Here, in tanks system seawater with high pCO_2 polyphenol content increased, independent of temperature, showing that ocean acidification can benefit algal photoprotection, as shown by Celis-Plá et al. (2015) in *Cystoseira compressa* and *Padina pavonica* at sites with naturally elevated CO_2 conditions. Swanson and Fox (2007) showed increased in phenols content in kelp under elevated pCO_2 treatments. The variation of physiological performance of brown macroalgae in long-term period (5 moths) due to acidification has been also observed *in situ* during a volcanic eruption event in the Canary Islands (Betancor et al. 2014). The brown macroalga *Padina pavonica* suffered decalcification during the eruptive phase and it was directly linked with the acidification of the coastal waters (Betancor et al. 2014). This confirms a loss of $CaCO_3$ for a calcareous seaweed under an ocean acidification scenario, as several studies have reported for seaweeds, and other organisms (Hall-Spencer et al. 2008; Martin et al. 2008; Rodolfo-Metalpa et al. 2009; Fabricius et al. 2011; Hofmann et al. 2012).

The polyphenol content was highest in *C. tamariscifolia* from the ultraoligotrophic site which is probably a photoprotective response to the high irradiance level found in highly transparent coastal waters. These intertidal macroalgae presented higher capacity to respond to increased environmental stress for high irradiance, compared to algae from subtidal or from other region with lower daily irradiance levels (Gómez et al. 2004, Hanelt and Figueroa 2012). Pérez-Rodríguez (2000) and Figueroa and Gómez (2001) reported vertical attenuation coefficient of light (Kd) in the ultraoligotrophic waters in Eastern of the Mediterranean Sea (Alboran Sea), respect to the West region. The Kd (PAR), Kd (UVA) and Kd (UVB) for the eastern region were 0.070 m^{-1}, 0.105 m^{-1} and 0.220 m^{-1}, compared to 0.102 m^{-1}, 0.275 m^{-1} and 0.378 m^{-1}, respectively in the west. These suggest that the higher concentration of polyphenolic compounds and antioxidant activity in algae from ultraoligotrophic waters (Cabo de Gata-Nijar Natural Park) with high transparency, can increase the photoprotection capacity related to the photoacclimation to high irradiance, thus, preventing the photodamage. The macroalgae can minimize damage from high irradiance not only by down-regulating process in photosystems, also by the production of UV photoprotectors and antioxidant compounds as phenols (Pérez-Rodríguez et al. 1998, Figueroa et al. 2014). In fact, the

phenol content was higher in algae from ultraoligotrophic waters, i.e., with highest values of 3.0 % than that in oligotrophic water with highest values of 2.2 %. Polyphenolic accumulation in brown seaweeds is stimulated under high solar PAR and UVR (Connan et al. 2006) protecting the cells against photo-oxidative stress (Schoenwaelder et al. 2002). Abdala et al. (2006) showed in *Cystoseira tamariscifolia* that the hourly and monthly variations of phenolic compounds were related to daily integrated irradiance in the ultraoligotrophic waters of Cabo de Gata Natural Park.

Phenols usually accumulated under elevated pCO_2 treatments, as it has been reported in kelps (Swanson and Fox 2007) and in terrestrial plants (Mattson et al. 2005; Stiling and Cornelissen 2007). However, the effects of pCO_2 on phenol production is not straight forward, as seagrasses decreased the production of phenols when pCO_2 increased, indicating these responses are species-specific (Arnold et al. 2012). In addition, in the brown macroalgae *Padina pavonica*, the phenol levels decreased during an submarine eruptive event related to pH decrease, i.e., water acidification up 2.8 unit within 100 m of the water column (Fraile-Nuez et al. 2012), recovering the phenolic levels after the eruptive phase and normal pH values (Betancor et al. 2014). This pattern was related to an increase of excretion rates of polyphenols under reduced pH, condition as it has been reported for the brown alga *Lessonia nigrescens* (Gómez and Huovinen 2010). However, in this study, the content of photoprotectors (phenolic compounds) from both origins was higher under increased pCO_2 conditions in ambient temperature.

Celis-Plá et al. (2015) showed also that phenolic compounds in *Cystoseira compressa* and *Padina pavonica* were accumulated in elevated pCO_2 treatments with nutrient enrichment conditions, as interactive effects, in a field study with a natural pH gradient (Vulcano Island, Italy). In oligotrophic ambient with natural input of the nutrient (La Araña beach) due to anthropogenic impact, i.e., sewage discharges can increases the photoprotection capacity of seaweeds due to the increase in protein content or polyphenols (Arnold and Targett 2002). We also found antioxidant activity was higher in high pCO_2 treatments in algae from both origins. A positive correlation between antioxidant activity and internal nitrogen content indicates again that nutrient level has a positive effect on photoprotection. We also found antioxidant activity to be higher (i.e. low EC_{50}) under higher pCO_2 treatments in algae from both origins, but in ultraoligotrophic waters the antioxidant activity was higher respect to the oligotrophic waters. It is shown again, that the algae submitted to more stress conditions in the

natural environment, i.e., ultraoligotrophic *vs* oligotrophic presented the highest acclimation capacity.

CONCLUSION

Elevated pCO_2 levels can clearly enhance brown algal productivity, with implications for fucoid forests of the planet, but this will be contingent on other physicochemical parameters. Here, we show that elevated pCO_2 was beneficial to *C. tamariscifolia* and that thalli collected from both ultra- and oligotrophic waters were able to acclimate to increased DIC and temperature. The benefits of ocean acidification for fucoids worldwide will be contingent on there being enough nutrients and light, and that thermal tolerances are not exceeded. Our study shows that ocean acidification combined with increased temperature had beneficial effects on growth rates, photosynthetic production, antioxidant activity and photoprotection, and shown the importance of light and nutrient history of the macroalgae in the responses to climate change factors. This would ensure the integrity of the communities of rocky shores as these canopy forming species play a profound role in providing habitat and resources to hundreds of accompanying species.

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Figure Captions

Figure 1 Growth (g d^{-1}) (mean ± SE, n=3) of *Cystoseira tamariscifolia* from two sites at four treatments ATxACO_2 (ambient TºC x ambient CO_2), ATxHCO_2 (ambient TºC x high CO_2), HT*ACO_2 (high TºC x Ambient CO_2) and HTxHCO_2 (high TºC x high CO_2) after 28 days. Lower-case letters denote significant differences after a SNK test.

Figure 2 Maximal electron transport rate (ETR_{max} expressed as µmol electrons m^{-2} s^{-1}) (mean ± SE, n=3) for a) *Cystoseira tamariscifolia* from ultraoligotrophic waters and b) from oligotrophic waters in four treatments $ATxACO_2$ (ambient TºC x ambient CO_2), $ATxHCO_2$ (ambient TºC x high CO_2), $HTxACO_2$ (high TºC x Ambient CO_2) and $HTxHCO_2$ (high TºC x high CO_2) in relation to time. Upper values in right box indicate initial time values before acclimation time. Lower-case letters denote significant differences after a SNK test.

Figure 3 Total phenolic compounds (PC expressed as mg g^{-1} DW) (mean ± SE, n=3) for a) *Cystoseira tamariscifolia* from ultraoligotrophic waters and b) from oligotrophic waters for four treatments $ATxACO_2$ (ambient TºC x ambient CO_2), $ATxHCO_2$ (ambient TºC x high CO_2), $HTxACO_2$ (high TºC x Ambient CO_2) and $HTxHCO_2$ (high TºC x high CO_2) in relation to time. Upper values in right box indicate initial time values before acclimation time.

Figure 4 Antioxidant activity (EC_{50} expressed as mg DW mL^{-1}) (mean ± SE, n=3) for a) *Cystoseira tamariscifolia* from ultraoligotrophic waters and b) from oligotrophic waters for four treatments $ATxACO_2$ (ambient TºC x ambient CO_2), $ATxHCO_2$ (ambient TºC x high CO_2), $HTxACO_2$ (high TºC x Ambient CO_2) and $HTxHCO_2$ (high TºC x high CO_2) in relation to time. Upper values in right box indicate initial time values before acclimation time.

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