

2017-03-17

Ecophysiological responses to elevated CO₂ and temperature in *Cystoseira tamariscifolia* (Phaeophyceae)

Celis-Pla, PSM

<http://hdl.handle.net/10026.1/8682>

10.1007/s10584-017-1943-y

Climatic Change

Springer Science and Business Media LLC

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

1 This is the author's accepted manuscript . The final published version of this work (the version of
2 record) is published by Springer in *Climate Change* available on 6 March 2017 at
3 <https://link.springer.com/article/10.1007/s10584-017-1943-y>

4 This work is made available online in accordance with the publisher's policies. Please refer to any
5 applicable terms of use of the publisher.

6
7 **Ecophysiological responses to elevated CO₂ and temperature in**
8 ***Cystoseira tamariscifolia* (Phaeophyceae)**

9
10
11 Paula S. M. Celis-Plá^{1,2*}, Brezo Martínez³, Nathalie Korbee², Jason M. Hall-Spencer^{4,5},
12 and Félix L. Figueroa².

13
14
15 *Corresponding author: paulacelispla@upla.cl

16
17
18
19 ¹Centre of Advanced Studies, University of Playa Ancha, Calle Traslaviña 450, Viña
20 del Mar, Chile

21 ²Department of Ecology, Faculty of Sciences, University of Malaga, 29071 Malaga,
22 Spain

23 ³Biodiversity and Conservation Unit, Rey Juan Carlos University, 28933 Mostoles,
24 Spain

25 ⁴Marine Biology and Ecology Research Centre, Plymouth University, Plymouth PL4
26 8AA, UK

27 ⁵Shimoda Marine Research Centre, Tsukuba University, Japan
28
29
30
31
32
33
34

35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68

Keywords: Climate change, *Cystoseira tamariscifolia*, ocean acidification, temperature, biomass, photosynthesis, phenolic compounds

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 CO₂ (*ca.* 1200-1300 ppm), and at two testing temperatures at 20°C (ambient temperature) and 24°C (ambient temperature + 4°C). Increased CO₂ levels benefited the algae from both origins. Biomass increased in elevated CO₂ 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 CO₂ treatments. The highest polyphenol content and antioxidant activity were observed in ambient temperature/high CO₂ 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 (EC₅₀) 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.

69
70
71
72
73
74

75 INTRODUCTION

76 Ocean acidification due to increased atmospheric CO₂ levels is altering the
77 concentrations of dissolved inorganic carbon (DIC) in surface waters; CO₃²⁻ levels are
78 falling, which can cause dissolution of calcified organisms, whilst CO₂ and HCO₃⁻
79 levels are rising which can stimulate photosynthesis (Connell et al. 2013; Cornwall et al.
80 2012, 2015; Newcomb et al. 2015). Rising atmospheric CO₂ levels have increased
81 seawater temperatures by 0.13°C per decade over the last 50 years (IPCC 2014) and the
82 regression of the seaweeds at their warmest biogeographic limits are being observed
83 (Harley et al. 2006; Brodie et al. 2014; Wernberg et al. 2016). Research into the
84 combined effects of acidification and warming is advancing our understanding of the
85 mechanisms that drive algal responses to global climate change (Figueroa and Korbee
86 2010; Diaz-Pulido et al. 2012; Martínez et al. 2015). Work on brown macroalgae has
87 shown that global climate change can affect a range of key processes, such as nutrient
88 uptake and photosynthesis, with knock-on effects on growth, calcification and
89 reproduction (Betancor et al. 2014; Figueroa et al. 2014; Stengel et al. 2014).

90 Long-term observations in tanks and work at CO₂ seep systems have shown that
91 many calcifying of seaweeds species are more vulnerable to the effects of ocean
92 acidification at warmer seawater temperatures (Martin and Gattuso 2009). Thus, the
93 changes in the structure of the community can be altered by the ocean acidification due
94 to the decrease of growth rates in marine calcifies seaweeds, e.g., coralline algae
95 (Kuffner et al. 2008, Martin and Gattuso 2009), and increase in non-calcifying
96 macroalgae, e.g., kelp forests (Connell and Russell 2010, Celis-Plá et al. 2015). Many
97 brown macroalgae thrive at high CO₂ concentrations (Enochs et al. 2015; Linares et al.
98 2015), often up-regulating their photosynthesis and the nutrient uptake as well as their
99 production of the polyphenols and antioxidants (Figueroa et al. 2014; Celis-Plá et al.
100 2016).

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

103 nutrients levels (Strain et al. 2014; Yesson et al. 2015; Wernberg et al. 2016) yet the
104 canopy-forming brown algae *Cystoseira* spp. and *Sargassum vulgare* proliferate near
105 coastal CO₂ seeps in the Mediterranean (Porzio et al. 2011; Baggini et al. 2014; Celis-
106 Plá et al. 2015) and in mesocosm systems (Ju-Hyoung et al. 2016). *Cystoseira* spp. are
107 used as indicators of high water quality in the Mediterranean as they help maintain the
108 structure and function of coastal ecosystems (Celis-Plá et al. 2016). As Ocean
109 acidification is not happening in isolation, but alongside a plethora of other
110 anthropogenic changes, the study of the combination of the multiple stressors in the
111 Mediterranean Sea, is critical to plan for to explain the disappearance of sensitive
112 ecosystem of the canopy-forming seaweeds. These “habitat-forming” species are
113 suffering a general decline (Fernández 2011, Pérez-Lloréns et al. 2014) and habitat
114 destruction or degradation. Considered as threat to the diversity, structure, functioning
115 and services they provide of marine coastal ecosystems in the Mediterranean Sea
116 (Claudet and Frascchetti 2010, Coll et al. 2010).

117 Our study focuses on the Alboran Sea (western part of Mediterranean Sea), parts of
118 which are so oligotrophic where macroalgal growth is limited (Ferreira et al. 2011;
119 Mercado et al. 2012). We grew *Cystoseira tamariscifolia* in tanks system to examine
120 the combined effects of CO₂ and temperature on thalli collected from ultra-oligotrophic
121 vs less limited nutrient parts of the coast. Here, we analyze ecophysiological responses
122 to ocean acidification at ambient temperature and close to their temperature tolerance
123 limits using standard methods for the study of multiple physical stressors in algae
124 (Stengel et al. 2014; Celis-Plá et al. 2015; Martínez et al. 2015). Our hypothesis is that
125 these macroalgae would benefit from elevated levels of dissolved inorganic carbon
126 (DIC) at ambient conditions of temperature when nutrient levels were sufficient. On the
127 other hand, the temperature increase can produce negative effects since *Cystoseira*
128 *tamariscifolia* of Alboran Sea is located in the southern limit of the distribution of this
129 species (Gómez-Garreta et al. 2001). In spite of the importance of these algal
130 communities, the studies on the vulnerability and acclimation to increased temperature
131 are scarce (Serio et al. 2006, Strain et al. 2014). Thus, we expect that increase of *p*CO₂
132 levels will produce an increase of photosynthetic activity, photoprotectors and
133 antioxidant activity in *C. tamariscifolia* only under ambient temperature.

134

135 MATERIAL AND METHODS

136 *Species and sampling*

137 *Cystoseira tamariscifolia* (Hudson) Papenfuss, (Phaeophyceae, Fucales) (Gómez-
138 Garreta et al. 2001) were collected haphazardly on 25 September 2013 in Cabo de Gata-
139 Nijar Natural Park (36°51'N, 2°6'W) and off La Araña beach, Malaga (36°42'N,
140 4°19'W) both in the Alboran Sea. The waters off the Natural Park had lower
141 concentrations of nitrate and phosphate and lower vertical attenuation coefficient of
142 light (Kd) than La Araña beach (Table S1; according to Ramírez et al. 2005; Mercado et
143 al. 2007, 2012), and the waters can be classified as ultra-oligotrophic and oligotrophic
144 waters, respectively according to the classification proposed by Organization for
145 Economic Cooperation and Development (1982).

146

147 ***Experimental design***

148 *Cystoseira tamariscifolia* (25-30 g fresh weight) were incubated from 27 September
149 to 29 October 2013 (after 48 hours of acclimation), in open tanks system. The
150 experiment was designed to examine interactive effects of current $p\text{CO}_2$ (ca. 400-500
151 ppm, pH target above 8.22 to 8.34) and predicted future concentration (ca.1200-1300
152 ppm, pH target 7.88) in combination with two temperature levels, ambient temperature
153 (target above 19-20°C) and ambient temperature + 4°C (target above 24°C), predicted
154 future temperature for the year 2100 (IPCC 2014). The four treatments were ambient
155 temperature x ambient CO_2 (ATx ACO_2), ambient temperature x high CO_2 (ATx HCO_2),
156 high temperature x ambient CO_2 (HTx ACO_2) and high temperature x high CO_2
157 (HTx HCO_2), in total 24 tanks were used with two replicates per tank, i.e., 6 replicates
158 per treatments; four treatments with three replicate tanks for ultraoligotrophic waters
159 and three replicate tanks for oligotrophic waters.

160

161 ***Experimental conditions***

162 The experimental system consists in 24 open tanks (0.094 m² surface area, 14 L
163 volume), connected in parallel each three tanks to a separate tank of 102 L capacity and
164 these were placed within a tank of 1000 L in water baths. The water flow between each
165 tank and its header tank (102 L capacity) was $0.84 \pm 0.05 \text{ L min}^{-1}$, representing a
166 turnover rate of $26 \pm 1\% \text{ h}^{-1}$. A temperature control System, was monitored T2001HC,
167 Aqua Medic was used in the tanks system (following methods given by Stengel et al.
168 2014). For carbon treatment was operated a computer-operated pH control system (AT)
169 with pH sensors (Aqua Medic T2001HC, Aqua Medic), located inside each of the eight
170 102 L header tanks. The system automatically recorded one measurement every 15 min

171 and was programmed to initiate the supply of pure CO₂ via a solenoid valve as soon as
172 the pH exceeded a threshold of 7.88 in the header tanks (corresponding to 1200 ppm of
173 CO₂, HC treatment). When the pH returned to this value, CO₂ injection stopped. The
174 seawater carbonate system was monitored two times at each week, taking water samples
175 to measure the temperature, salinity, pH_{NBS} and total alkalinity (following methods
176 given by Celis-Plá et al. 2015). Other parameters of the carbonate chemistry were
177 calculated using CO₂SYS. Seawater was enriched with 2 μM nitrate (KNO₃) and 0.1
178 μM phosphate (KH₂PO₄) giving an N:P ratio of 20:1 for oligotrophic waters and with
179 0.5 μM nitrate (KNO₃) and 0.1 μM phosphate (KH₂PO₄) giving an N:P ratio of 5:1 for
180 ultraoligotrophic waters according to Ramírez et al. (2005) and Mercado et al. (2007
181 and 2012) (Table S1). This was assessed by taking triplicate seawater samples from all
182 treatments. Seawater was filtered *in situ* using portable GF/F filters (Whatman
183 International. Ltd., Maidstone, UK), transported to the laboratory inside an isotherm bag
184 (4°C, in darkness), and kept at -20°C. Nitrate (NO₃⁻) and phosphate (P) were determined
185 using an automated wet chemistry analyzer (SanPlus++ System, SKALAR, Breda,
186 Netherlands) applying standard colorimetric procedures (Koroleff 1983).

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

193

194 ***Biomass***

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

198

$$199 \quad \text{Biomass} = (FW_{t=f} - FW_{t=0}) \cdot \text{day}^{-1} \quad (1)$$

200

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

203

204 ***Internal carbon and nitrogen content***

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

210

211 ***Photosynthetic activity***

212 Photosynthetic activity was estimated by *in vivo* chlorophyll a fluorescence
213 associated to Photosystem II (PSII) by using a portable pulse amplitude modulated
214 (PAM) fluorometer (Diving-PAM, Walz GmbH, Germany). Macroalgal thalli were
215 collected from natural populations (initial time). In order to obtain rapid light curves
216 (RLC) for each treatment, apical parts of *C. tamariscifolia* were introduced in 10 mL
217 incubation chambers. F_o and F_m were measured after 15 minutes in darkness to obtain
218 the maximum quantum yield (F_v/F_m) being $F_v = F_m - F_o$, F_o the basal fluorescence of 15
219 min dark adapted thalli and F_m maximal fluorescence after a saturation light pulse of
220 $>4000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Schreiber et al. 1995). The electron transport rate (ETR) as
221 estimator of photosynthetic capacity was determined after 20s exposure in eight
222 increasing irradiances of white light (halogen lamp provided by the Diving-PAM). The
223 ETR was calculated according to Schreiber et al. (1995) as follows:

224

$$225 \quad \text{ETR} (\mu\text{mol electrons m}^{-2} \text{s}^{-1}) = \Delta F/F'_m \times E \times A \times F_{II} \quad (2)$$

226

227 where $\Delta F/F'_m$ is the effective quantum yield, being $\Delta F = F_m' - F_t$ (F_t is the intrinsic
228 fluorescence of alga incubated in light and F_m' is the maximal fluorescence reached
229 after a saturation pulse of algae incubated in light), E is the incident PAR irradiance
230 expressed in $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, A is the thallus absorptance as the fraction of
231 incident irradiance that is absorbed by the algae (Figueroa et al. 2003) and F_{II} is the
232 fraction of chlorophyll related to PSII (400-700 nm) being 0.8 in brown macroalgae
233 (Grzyski et al. 1997). ETR parameters as maximum electron transport rate (ETR_{max})
234 as estimator of photosynthetic efficiency were obtained from the tangential function
235 reported by Eilers and Peeters (1988).

236

237 ***Phenolic compounds and antioxidant activity (EC₅₀)***

238 In brown algae, the UV screen compounds, with antioxidant capacity are the
239 phenolic compounds (PC) were determined using 0.25 g fresh weight samples
240 pulverized with a mortar and pestle with sea-sand and 2.5 mL of 80% methanol. After
241 keeping the samples overnight at 4°C, the mixture was centrifuged at 2253 g for 30 min
242 at 4°C, and then the supernatant was collected. PC were determined colorimetrically
243 using Folin-Ciocalteu reagent and phloroglucinol (1,3,5-trihydroxybenzene, Sigma P-
244 3502) as standard. Finally, the absorbance was determined at 760 nm using a
245 spectrophotometer (UV Mini-1240, Shimadzu) (Celis-Plá et al. 2014). Total phenolic
246 content was expressed as mg g⁻¹ DW after determining the fresh to dry weight ratio in
247 the tissue (4.3 for *C. tamariscifolia* from Cabo de Gata-Níjar Natural Park and 5.6 *C.*
248 *tamariscifolia* from La Araña Beach, respectively). The results are expressed as average
249 ± SE from three replicates of each treatment.

250 Antioxidant activity (EC₅₀) was measured on polyphenol extracts according to
251 Blois (1958); 150 µL of DPPH (2, 2-diphenyl-1-picrylhydrazyl) prepared in 90%
252 methanol were added to each extract. The reaction was complete after 30 min in
253 darkness at ambient temperature (~20°), and the absorbance was read at 517 nm in a
254 spectrophotometer (UVmini-1240, Shimadzu). The calibration curve made from DPPH
255 was used to calculate the remaining concentration of DPPH in the reaction mixture after
256 incubation. Values of DPPH concentration (mM) were plotted against plant extract
257 concentration expressed as the EC₅₀ value (mg DW mL⁻¹) required to scavenge 50% of
258 the DPPH in the reaction mixture. Ascorbic acid was used as a control (according to
259 Celis-Plá et al. 2016).

260

261 ***Statistical analysis***

262 The effects of the combined treatments on growth, photophysiological and
263 biochemical responses of *C. tamariscifolia* were assessed using analysis of variance
264 (ANOVA). For biomass, three fixed factors were considered: temperature with two
265 levels; ambient temperature vs. high temperature (ambient + 4°C temperature), CO₂
266 with two levels in this instance; ca. 400-500 vs. ca. 1200-1300 ppm and origin with two
267 levels; ultraoligotrophic waters (Cabo de Gata-Níjar Natural Park) vs. oligotrophic
268 waters (La Araña beach). For photophysiological and biochemical responses in *C.*
269 *tamariscifolia*, four fixed factors were considered: time, temperature, CO₂ and origin.
270 To ensure the independence of each replica, each thallus was measured once and then
271 was eliminated. This design allowed us to test interactive and additive effects of the

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

277

278 **RESULTS**

279 ***Experimental conditions***

280 The seawater temperatures during the experimental period were $19.80 \pm 0.15^\circ\text{C}$ in
281 ATxACO₂, $20.10 \pm 0.15^\circ\text{C}$ in ATxHCO₂, $23.91 \pm 0.21^\circ\text{C}$ in HTxACO₂ and $23.90 \pm$
282 0.20°C in high temperature and high pCO₂ treatment (mean \pm SE, n = 2232; 279
283 measurements for each tank) and mean pH were 8.34 ± 0.01 in ATxACO₂, 7.88 ± 0.01
284 in ATxHCO₂, 8.22 ± 0.01 in HTxACO₂ and 7.88 ± 0.01 in HTxHCO₂, respectively
285 (mean \pm SE, n = 2232; 279 measurements for each tank) (Table 1). The average daily
286 integrated irradiances for the experimental period were 4238 kJ m^{-2} for PAR, 329 kJ m^{-2}
287 for UVA and 22 kJ m^{-2} for UVB. The nutrients, i.e., nitrate and phosphate
288 concentrations were 2.58 ± 0.52 and $0.16 \pm 0.01 \mu\text{M}$ in ATxACO₂, 1.06 ± 0.03 and
289 $0.13 \pm 0.01 \mu\text{M}$ in ATxHCO₂, 2.02 ± 0.51 and $0.15 \pm 0.02 \mu\text{M}$ in HTxACO₂ and
290 finally, 1.41 ± 0.57 and $0.15 \pm 0.01 \mu\text{M}$ in HTxHCO₂, respectively (mean \pm SE, n = 48
291 measurements) (Table 1).

292

293 ***Biomass***

294 The biomass was significantly different ($p < 0.01$) among all factors; temperature,
295 CO₂ levels and origin (Table S1). *C. tamariscifolia*, only when the thalli came from
296 oligotrophic waters showed enhanced growth under elevated pCO₂ with high
297 temperature treatment (maximal values $0.32 \pm 0.03 \text{ g FW day}^{-1}$, mean \pm SE, n=6)
298 compared to ambient pCO₂ with high temperature (minimal values $-0.50 \pm 0.06 \text{ g FW}$
299 day^{-1} , mean \pm SE, n=6). In addition, under ambient pCO₂, algae from oligotrophic
300 waters lost biomass irrespective of temperature (Fig. 1). Nevertheless, algae from
301 ultraoligotrophic waters showed no changes in biomass accretion irrespective of the
302 treatment conditions they were exposed to. Algae from ultraoligotrophic waters showed
303 maximal values $0.20 \pm 0.07 \text{ g FW day}^{-1}$ (mean \pm SE, n=6) under high
304 temperature/ambient pCO₂ and minimal values $0.07 \pm 0.01 \text{ g FW day}^{-1}$ (mean \pm SE,
305 n=6) under high temperature/high pCO₂ 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 CO₂ and origin. Carbon content in *C. tamariscifolia* only when the
310 thalli came from ultraoligotrophic waters showed increase under elevated $p\text{CO}_2$
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 CO₂ (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 $p\text{CO}_2$ 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 CO₂ 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 CO₂ conditions for both origins (Fig. 3). The antioxidant
334 activity (EC₅₀) was significantly different ($p < 0.01$) for all factors and interaction among
335 temperature, CO₂ and origin (Table S4).

336 EC₅₀ increased (i.e. lower EC₅₀) at the initial time (two first weeks) under high CO₂
337 conditions independent of the temperature, for both origins (Fig. 4).

338

339 **DISCUSSION**

340 Increased levels of dissolved inorganic carbon (DIC) can benefit photosynthesis and
341 growth in *Cystoseira tamariscifolia* in the experimental tanks system and temporal
342 scales of days (28d), confirming expected benefits of ocean acidification already
343 reported for brown macroalgae (Harley et al. 2012; Cornwall et al. 2012; Brodie et al.
344 2014; Koch et al. 2014; Bender et al. 2014; Cornwall et al. 2015). In this study, we
345 show benefits of $p\text{CO}_2$ increase on growth rate in *C. tamariscifolia* being the
346 physiological responses more accelerated in oligotrophic than in ultraoligotrophic
347 harvested algae. Temperature increase has negative effect on growth rate in algae from
348 oligotrophic waters with low $p\text{CO}_2$ conditions and the biomass increased under elevated
349 $p\text{CO}_2$ conditions, in *C. tamariscifolia* of both origins. We found that the full extent of
350 these benefits was only gained at optimal temperatures and if sufficient nutrients were
351 available, building upon work by Celis-Plá et al. (2015) at CO_2 seeps.

352 Reports on non-calcareous macroalgae from other regions have shown that the ocean
353 acidification may increase due to beneficial effects on photosynthesis, production and
354 growth (Harley et al. 2012; Koch et al. 2014; Brodie et al. 2014). Ocean acidification
355 can benefit the physiological state and growth in the field (Johnson et al. 2012; Baggini
356 et al. 2014; Celis-Plá et al. 2015), in laboratory work has shown that increases in
357 dissolved inorganic carbon can benefit species such as *Gracilaria lemaneiformis* in
358 China (Zou and Gao 2009) and *Feldmannia* spp. in Australia (Russell et al. 2011), as
359 well as phaeophytes such as *Nereocystis luetkeana* and *Macrocystis pyrifera* in New
360 Zealand (Swanson and Fox 2007; Roleda et al. 2012). Also in mesocosm systems
361 *Sargassum thunbergii* showed an increased in the photosynthesis activity and growth,
362 when exposed to high $p\text{CO}_2$ conditions (Ju-Hyoung et al. 2016). In this study, fucoid
363 biomass from oligotrophic waters, showed enhanced growth under elevated $p\text{CO}_2$ and
364 the macroalgae from ultraoligotrophic waters showed no changes in biomass
365 irrespective of the treatment conditions they were exposed to. The highest carbon
366 content was observed in *C. tamariscifolia* from ultraoligotrophic waters and incubated
367 in elevated $p\text{CO}_2$ levels.

368 A positive correlation between carbon internal content and ETR_{max} was observed,
369 this suggest that carbon supply increased photosynthetic production expressed as
370 ETR_{max} . The highest ETR_{max} and nitrogen internal content were reached at increased
371 $p\text{CO}_2$ and ambient temperature in algae from both origins being the highest levels
372 reached in algae collected from ultraoligotrophic waters. The carbon content helps
373 explain the dominance of this brown algae at a variety of coastal Mediterranean,

374 probably due to a combination of the direct stimulus of increased dissolved inorganic
375 carbon for photosynthesis (Mercado et al. 1998, Raven and Hurd 2012). Johnson et al.
376 (2012) also showed a significant effect on the photosynthetic responses of *Padina*
377 *pavonica* with CO₂ enrichment. Here, in tanks system seawater with high pCO₂
378 polyphenol content increased, independent of temperature, showing that ocean
379 acidification can benefit algal photoprotection, as shown by Celis-Plá et al. (2015) in
380 *Cystoseira compressa* and *Padina pavonica* at sites with naturally elevated CO₂
381 conditions. Swanson and Fox (2007) showed increased in phenols content in kelp under
382 elevated pCO₂ treatments. The variation of physiological performance of brown
383 macroalgae in long-term period (5 months) due to acidification has been also observed *in*
384 *situ* during a volcanic eruption event in the Canary Islands (Betancor et al. 2014). The
385 brown macroalga *Padina pavonica* suffered decalcification during the eruptive phase
386 and it was directly linked with the acidification of the coastal waters (Betancor et al.
387 2014). This confirms a loss of CaCO₃ for a calcareous seaweed under an ocean
388 acidification scenario, as several studies have reported for seaweeds, and other
389 organisms (Hall-Spencer et al. 2008; Martin et al. 2008; Rodolfo-Metalpa et al. 2009;
390 Fabricius et al. 2011; Hofmann et al. 2012).

391 The polyphenol content was highest in *C. tamariscifolia* from the ultraoligotrophic
392 site which is probably a photoprotective response to the high irradiance level found in
393 highly transparent coastal waters. These intertidal macroalgae presented higher capacity
394 to respond to increased environmental stress for high irradiance, compared to algae
395 from subtidal or from other region with lower daily irradiance levels (Gómez et al.
396 2004, Hanelt and Figueroa 2012). Pérez-Rodríguez (2000) and Figueroa and Gómez
397 (2001) reported vertical attenuation coefficient of light (Kd) in the ultraoligotrophic
398 waters in Eastern of the Mediterranean Sea (Alboran Sea), respect to the West region.
399 The Kd (PAR), Kd (UVA) and Kd (UVB) for the eastern region were 0.070 m⁻¹, 0.105
400 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
401 west. These suggest that the higher concentration of polyphenolic compounds and
402 antioxidant activity in algae from ultraoligotrophic waters (Cabo de Gata-Nijar Natural
403 Park) with high transparency, can increase the photoprotection capacity related to the
404 photoacclimation to high irradiance, thus, preventing the photodamage. The macroalgae
405 can minimize damage from high irradiance not only by down-regulating process in
406 photosystems, also by the production of UV photoprotectors and antioxidant
407 compounds as phenols (Pérez-Rodríguez et al. 1998, Figueroa et al. 2014). In fact, the

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

415 Phenols usually accumulated under elevated $p\text{CO}_2$ treatments, as it has been
416 reported in kelps (Swanson and Fox 2007) and in terrestrial plants (Mattson et al. 2005;
417 Stiling and Cornelissen 2007). However, the effects of $p\text{CO}_2$ on phenol production is
418 not straight forward, as seagrasses decreased the production of phenols when $p\text{CO}_2$
419 increased, indicating these responses are species-specific (Arnold et al. 2012). In
420 addition, in the brown macroalgae *Padina pavonica*, the phenol levels decreased during
421 an submarine eruptive event related to pH decrease, i.e., water acidification up 2.8 unit
422 within 100 m of the water column (Fraile-Nuez et al. 2012), recovering the phenolic
423 levels after the eruptive phase and normal pH values (Betancor et al. 2014). This
424 pattern was related to an increase of excretion rates of polyphenols under reduced pH,
425 condition as it has been reported for the brown alga *Lessonia nigrescens* (Gómez and
426 Huovinen 2010). However, in this study, the content of photoprotectors (phenolic
427 compounds) from both origins was higher under increased $p\text{CO}_2$ conditions in ambient
428 temperature.

429 Celis-Plá et al. (2015) showed also that phenolic compounds in *Cystoseira*
430 *compressa* and *Padina pavonica* were accumulated in elevated $p\text{CO}_2$ treatments with
431 nutrient enrichment conditions, as interactive effects, in a field study with a natural pH
432 gradient (Vulcano Island, Italy). In oligotrophic ambient with natural input of the
433 nutrient (La Araña beach) due to anthropogenic impact, i.e., sewage discharges can
434 increase the photoprotection capacity of seaweeds due to the increase in protein content
435 or polyphenols (Arnold and Targett 2002). We also found antioxidant activity was
436 higher in high $p\text{CO}_2$ treatments in algae from both origins. A positive correlation
437 between antioxidant activity and internal nitrogen content indicates again that nutrient
438 level has a positive effect on photoprotection. We also found antioxidant activity to be
439 higher (i.e. low EC_{50}) under higher $p\text{CO}_2$ treatments in algae from both origins, but in
440 ultraoligotrophic waters the antioxidant activity was higher respect to the oligotrophic
441 waters. It is shown again, that the algae submitted to more stress conditions in the

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

444

445 **CONCLUSION**

446 Elevated $p\text{CO}_2$ levels can clearly enhance brown algal productivity, with
447 implications for furoid forests of the planet, but this will be contingent on other
448 physicochemical parameters. Here, we show that elevated $p\text{CO}_2$ was beneficial to *C.*
449 *tamariscifolia* and that thalli collected from both ultra- and oligotrophic waters were
450 able to acclimate to increased DIC and temperature. The benefits of ocean acidification
451 for furoids worldwide will be contingent on there being enough nutrients and light, and
452 that thermal tolerances are not exceeded. Our study shows that ocean acidification
453 combined with increased temperature had beneficial effects on growth rates,
454 photosynthetic production, antioxidant activity and photoprotection, and shown the
455 importance of light and nutrient history of the macroalgae in the responses to climate
456 change factors. This would ensure the integrity of the communities of rocky shores as
457 these canopy forming species play a profound role in providing habitat and resources to
458 hundreds of accompanying species.

459

460 **ACKNOWLEDGEMENTS**

461 This work was supported by the Junta de Andalucía (Project RNM-5750), by the
462 research group RNM-295 and by the University of Málaga: Programa de
463 Fortalecimiento de Las capacidades de I+D+I en Las universidades 2014-2015,
464 Consejería de Economía, Innovación, Ciencia y Empleo, cofinanciado por el FEDER
465 (Project FC-14CGL-09). Paula S. M. Celis-Plá gratefully acknowledges financial
466 support from ‘Becas-Chile’ (CONICYT) of the Ministry of Education, Republic of
467 Chile and technical support of David Lopez (University of Malaga).

468

469 **Figure Captions**

470 Figure 1 Growth (g d^{-1}) (mean \pm SE, $n=3$) of *Cystoseira tamariscifolia* from two sites at
471 four treatments ATx ACO_2 (ambient T°C x ambient CO_2), ATx HCO_2 (ambient T°C x
472 high CO_2), HT* ACO_2 (high T°C x Ambient CO_2) and HTx HCO_2 (high T°C x high CO_2)
473 after 28 days. Lower-case letters denote significant differences after a SNK test.

474

475 Figure 2 Maximal electron transport rate (ETR_{max} expressed as $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$)
476 (mean \pm SE, n=3) for a) *Cystoseira tamariscifolia* from ultraoligotrophic waters and b)
477 from oligotrophic waters in four treatments ATxACO₂ (ambient T°C x ambient CO₂),
478 ATxHCO₂ (ambient T°C x high CO₂), HTxACO₂ (high T°C x Ambient CO₂) and
479 HTxHCO₂ (high T°C x high CO₂) in relation to time. Upper values in right box indicate
480 initial time values before acclimation time. Lower-case letters denote significant
481 differences after a SNK test.

482

483 Figure 3 Total phenolic compounds (PC expressed as mg g^{-1} DW) (mean \pm SE, n=3) for
484 a) *Cystoseira tamariscifolia* from ultraoligotrophic waters and b) from oligotrophic
485 waters for four treatments ATxACO₂ (ambient T°C x ambient CO₂), ATxHCO₂
486 (ambient T°C x high CO₂), HTxACO₂ (high T°C x Ambient CO₂) and HTxHCO₂ (high
487 T°C x high CO₂) in relation to time. Upper values in right box indicate initial time
488 values before acclimation time.

489

490 Figure 4 Antioxidant activity (EC_{50} expressed as mg DW mL^{-1}) (mean \pm SE, n=3) for a)
491 *Cystoseira tamariscifolia* from ultraoligotrophic waters and b) from oligotrophic waters
492 for four treatments ATxACO₂ (ambient T°C x ambient CO₂), ATxHCO₂ (ambient T°C x
493 high CO₂), HTxACO₂ (high T°C x Ambient CO₂) and HTxHCO₂ (high T°C x high CO₂)
494 in relation to time. Upper values in right box indicate initial time values before
495 acclimation time.

496

497

498

499

500

501

502

503

504

505

506

507

508

509

510

511

512