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PHOTOPHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF CO2 AND TEMPERATURE LEVELS ON CYSTOSEIRA TAMARISCIFOLIA, COLLECTED ON SOUTHERN SPAIN

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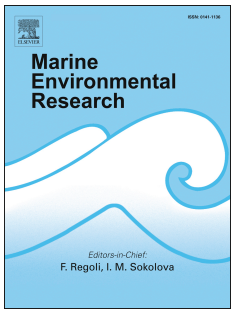

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Seasonal biochemical and photophysiological responses in the intertidal macroalga C*ystoseira tamariscifolia* (Ochrophyta).

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Abstract

Seasonal changes in the biochemistry and photophysiology of the brown macroalga *Cystoseira tamariscifolia* was analyzed in southern Spain. Total carbon and nitrogen contents, phenolic compounds, antioxidant and photosynthetic activities were seasonally determined over two years. Carbon, nitrogen and photoprotective phenolic contents were higher in winter and spring than in summer and autumn. Antioxidant levels were highest in spring and we found a positive correlation between phenolic content and antioxidant activity (EC_{50}). Photosynthetic capacity (ETR_{max}) and photosynthetic efficiency (α_{ETR}) were also highest in spring, and there was a positive correlation between ETR_{max} and the amount of phenols present. Increased irradiance in spring enhanced algal productivity, antioxidant activity and the production of photoprotective compounds but in summer nutrient depletion due to thermal stratification of coastal waters reduced photosynthetic activity and the photoprotective capacity of *C. tamariscifolia*. Electron microscopy showed that phenols occurred in the cytoplasm of cortical cells inside physodes. Spring would be the best period to harvest *C. tamariscifolia* to extract photoprotectors and antioxidants for potential commercial uses, although the environmental impacts would need to be carefully assessed.

Keywords: Algal productivity, antioxidants, *Cystoseira tamariscifolia*, *in vivo* chlorophyll *a* fluorescence, nitrogen, phenols, UV protection, Mediterranean Sea.

INTRODUCTION

Macroalgae in temperate regions, such as southern Spain, are exposed to wide daily and seasonal changes in photosynthetically active radiation (PAR) and ultraviolet (UV) light. They use photoprotective compounds such as carotenoids or polyphenols to help them cope with high light levels (Stengel et al., 2011). Light harvesting carotenoids are highly efficient O_2 scavengers that play an essential role in protection against excess light and photooxidative stress (Cantrell et al., 2003; Stahl and Sies, 2007). Algae can also prevent UV damage using polyphenols which help dissipate light energy (Goss and Jakob, 2010; Hanelt and Figueroa, 2012) and reduce DNA damage (Gómez and Huovinen, 2010); they can decrease metal toxicity by chelating metal ions (Connan et al., 2004; Stengel et al., 2005) and they are effective chemical defences against a wide range of herbivores (Steinberg and Van Altena, 1992).

In stressful conditions, phenolics can be released from algal thalli and react rapidly with proteins and carbohydrates to form UV-absorbing exudates (Koivikko et al., 2005; Celis-Plá et al., 2014a). Phenol content can vary in response to environmental changes in factors such as salinity, nutrients, light, and herbivory (Abdala-Díaz et al., 2006; Celis-Plá et al., 2014b). Brown algal phenols have been investigated for their medical benefits, including anti-inflammatory and hyaluronidase inhibitory activities (Vinay and Kim, 2012). A range of brown algal compounds are used in products as antioxidants Ahn et al., 2007) and for purported benefits as photoprotectors, as antiplasmin inhibitors, to reduce allergies, for skin whitening, anti‑HIV-1, antibacterial, and anticancer activities (Sugiura et al., 2007; Artan et al., 2008; Le et al., 2009; Heo et al., 2010).

Here, we studied *Cystoseira tamariscifolia* (Hudson) Papenfuss, (Phaeophyceae, Fucales) which can be abundant in waters of high ecological status in the Mediterranean, according to the criteria of Water Framework Directive of the European Union (WFD, 2000/60/EC), and it is used as an indicator of waters with high water quality (Ballesteros et al., 2007, Arévalo et al., 2007, Bermejo et al., 2013). In addition, *C. tamariscifolia* was selected since it provides habitat for other species in the Mediterranean Sea (Bermejo et al., 2013). We used the following well established suite of physiological indicators to seasonally evaluate *C. tamariscifolia* on intertidal rocky shores (according to Figueroa and Korbee, 2010): the C: N stoichiometric ratio as an indicator of nutritional status and phenolic content (Celis-Plá et al., 2014a). Maximum quantum yield of PSII (F_v/F_m) was used to determine photoinhibition and the

physiological status of the macroalga (Schreiber et al. 1986). Electron transport rate (ETR) was used to estimate of photosynthetic capacity (Figueroa et al., 2003). In addition, we examined the cell ultrastructure of *C. tamariscifolia* in summer by using both light and transmission electron microcopy to determine the location of phenolic compounds.

Our aim was to assess seasonal variability in photosynthetic production and of commercially valuable compounds in *C. tamariscifolia* to inform potential exploitation of these resources. We also investigated seasonal variations in photosynthetic activity, polyphenol content and antioxidant activity.

MATERIALS AND METHODS

Sampling

Nine *Cystoseira tamariscifolia* thalli were collected at least 2 m apart at 0.1-0.4 m above Chart Datum at 10 am local time monthly from July 2012 to June 2014 in summer, autumn, winter and spring. The samples were collected from rocky shores on La Araña beach, Malaga, Spain (36º 45'N, 4º 18'W). Live material was transported in cooled containers and samples were frozen *in situ* using liquid nitrogen for biochemical analyses. Photographs of the habitat were taken in spring, summer, autumn and winter. A representative image for each season of the intertidal area is shown in Figure 1.

Abiotic parameters

Photosynthetically active radiation (PAR, λ=400-700 nm), Ultraviolet A radiation (λ=320-400 nm) and Ultraviolet B radiation (λ=280-320 nm) were measured using an UV-PAR Multifilter radiometer NILU-6 (Geminali AS, Oslo, Norway) on the roof of the building of Central Services for Research support (SCAI, University of Malaga) located 14 km from the algal collection site. Seawater temperature was logged every minute at a monitoring station (REDCOS buoy number 1514) located at 36º 42'N, 4º 19'W. Seawater nitrate (µmol L^{-1}), ammonium (µmol L^{-1}), phosphate (µmol L^{-1}) and N: P ratio data were obtained from Ramírez et al. (2005) and Mercado et al. (2007, 2012) from 36º 60'N, 4º 10'W.

Histology

105 Macroalgal tissue samples ca 5 mm in length were collected in summer 2013 for
106 Transmission Electron Microscopy, and there were fixed with 2.5% glutaraldehyde,
107 2.0% paraformaldehyde, and 5 mM $CaCl_2$ in 0.075 M sodium cacodylate buffer (pH
108 7.2) plus 0.2 M sucrose and caffeine 1% overnight. The material was then fixed with
109 1% osmium tetroxide for 4 hours, dehydrated in a graded acetone series and embedded
110 in Spurr's resin. Thin sections were stained with aqueous uranyl acetate followed by
111 lead citrate. Four replicates were made for each experimental group; two samples per
112 replicate were then examined under TEM (JEM 1011 JEOL Ltd., Tokyo, Japan, at 80
113 kV).
114
115 ***Biochemical variables***
116 The dry weight of algal carbon and nitrogen contents was determined using an
117 element analyzer (model CNHS-932, LECO Corporation, Michigan, USA). Polyphenol
118 concentrations were measured using 0.25 g fresh weight samples pulverized in a pestle
119 and mortar with sea-sand using 2.5 mL of 80% methanol. This mixture was stored
120 overnight at 4ºC then centrifuged at 4000 rpm for 15 min at 4ºC and the supernatant was
121 collected to measure the phenolic compound content colourimetrically using Folin-
122 Ciocalteu reagent. Phloroglucinol (1,3,5-trihydroxybenzene, Sigma P-3502) was used as
123 standard. Finally, the absorbance was determined at 760 nm using a Shimadzu UVMini-
124 1240 spectrophotometer (Celis-Plá et al. 2014a). Phenolic concentration was expressed
125 as mg g^{-1} dry weight after determining the fresh to dry weight ratio in the tissue (the
126 ratio was 5.6). The results are expressed as average ± Standard Error from 9 replicates.
127 The antioxidant activity DPPH (2,2-diphenyl-1-picrylhydrazyil) assay (i.e. EC_{50})
128 according to Blois (1958) was estimated by reducing the stable free radical DPPH. The
129 supernatant used for phenolic compound measurements was used for DPPH analysis;
130 150 µL of DPPH were added to each extract. This solution of DPPH was prepared in
131 90% methanol (90MeOH: 10H2O) in 20 mL to concentration 1.27 mM. The reaction
132 was complete after 30 min in a dark room at ~20° C and the absorbance was read at 517
133 nm in a spectrophotometer (UVMini-1240 model, Shimadzu, Columbia, USA). A
134 calibration curve made with DPPH was used to calculate the remaining concentration of
135 DPPH in the reaction mixture after incubation. Values of DPPH concentration (mM)
136 were plotted against plant extract concentration (mg DW mL^{-1}) to obtain the oxidation
137 index EC_{50}, which represents the concentration of the extract, expressed as mg DW

138 mL^{-1}, required to scavenge 50% of the DPPH in the reaction mixture. Ascorbic acid was
139 used as positive control (Celis-Plá et al., 2014b).
140
141 ***Photosynthesis and energy dissipation as in vivo chlorophyll a fluorescence***
142 *In vivo* chlorophyll *a* fluorescence by Photosystem II was determined using a
143 portable pulse amplitude modulated fluorometer (Diving-PAM, Walz GmbH,
144 Germany). Apical pieces of macroalgal thalli were put in 10 mL incubation chambers to
145 obtain rapid light curves for each treatment. F_o and F_m were measured after 15 minutes
146 in darkness to obtain the maximum quantum yield (F_v/F_m) being $F_v=F_m-F_o$, F_o the basal
147 fluorescence of 15 min dark adapted thalli and F_m maximal fluorescence after a
148 saturation light pulse of >4000 µmol m^{-2} s^{-1}, with a few seconds of the duration
149 (Schreiber et al., 1995). The electron transport rate (ETR) was determined after 20 s
150 exposure in twelve increasing irradiances of actinic white light (halogen lamp provided
151 by the Diving-PAM) (Celis-Plá et al., 2014a). The ETR was calculated as follows
152 (Schreiber et al., 1995):
153
154 $$ETR\ (\mu mol\ electrons\ m^{-2}\ s^{-1}) = \Delta F/F'_m \times E \times A \times F_{II} \quad (1)$$
155
156 where $\Delta F/F'm$ is the effective quantum yield, being $\Delta F = Fm'-Ft$ (*Ft* is the intrinsic
157 fluorescence of alga incubated in light and *Fm'* is the maximal fluorescence reached
158 after a saturation pulse of algae incubated in light), *E* is the incident PAR irradiance
159 expressed in µmol photons m^{-2} s^{-1}, A is the thallus absorptance as the fraction of
160 incident irradiance that is absorbed by the algae (Figueroa et al., 2003) and F_{II} is the
161 fraction of chlorophyll related to PSII (400-700 nm) being 0.8 in brown macroalgae
162 (Figueroa et al., 2014a). ETR parameters as maximum electron transport rate (ETR_{max})
163 and the initial slope of ETR versus irradiance function (α_{ETR}) as estimator of
164 photosynthetic efficiency were obtained from the tangential function (Eilers and
165 Peeters, 1988). Finally, the saturation irradiance for ETR (Ek_{ETR}) was calculated from
166 the intercept between ETR_{max} and α_{ETR}. Non-photochemical quenching (NPQ) was
167 calculated as (Schreiber et al., 1995):
168
169 $$NPQ= (Fm-Fm')/Fm' \quad (2)$$
170

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 (Eilers and Peeters, 1988).

Statistical analyses

Pearson correlation coefficients were calculated and tested between all measured dependent variables. Interactive effects between physiological variables were analyzed using ANOVA (according to Underwood, 1997). This test was performed for *C. tamariscifolia* including year and season (two-way) as fixed factors for biochemical variables (mean ± SE, n=9) and season (one-way) with four levels, for the photosynthetic variables (mean ± SE, n=9), with a level of probability applied in the statistical analyses at $P<0.05$. Homogeneity of variance was tested using Cochran tests and by visual inspection of the residuals. Student Newman-Keuls tests (SNK) were performed after significant ANOVA interactions. All data conformed to homogeneity of variance. Analyses were carried out using SPSS v.21 (IBM, USA). The general variation patterns between biochemical variables measured in *C. tamariscifolia* were explored using a multivariate approach. A Principal Coordinates Analysis (PCO) was performed for this purpose on the basis of Euclidean distance using PERMANOVA+ for PRIMER6 package (Anderson et al. 2008). Such multivariate ordination was used because it allowed for investigating the variation of the content of biochemical compounds at the same time by looking at the ordination plot. Each one of variables was represented by an arrow in the ordination plot pointing to the samples that showed the highest amount of that particular compound. Each replicate represented the content of all compounds calculated from the three thalli taken at one sampling for each month and grouped for season.

RESULTS

Environmental conditions

Cystoseira tamariscifolia was abundant in all seasons whereas *Ulva rigida* (Chlorophyta) was only abundant in the summer. More *C. tamariscifolia* was present in spring in respect to other species such as e.g., *Ellisolandia elongata* (Figure 1). The seawater temperature ranged from 14-23^{o}C (Table 1) with a peak summer average daily irradiance of *ca*. 10165 kJ m^{-2} for PAR, 1051 kJ m^{-2} for UVA and 57.5 kJ m^{-2} UVB (Figure 2A-C). Seasonal nitrate (NO_3^-) concentrations ranged from 0.6-1.5 mg L^{-1} in

this coastal area according to Ramírez et al. (2005) and Mercado et al. (2007 and 2012). Seawater nitrate concentrations are approximately 2.5 times higher in winter and spring than in summer and autumn (Table 1). Ammonium (NH_4^+) varied through the year from 0.1 to 0.5 mg L^{-1} and was 2.7 times higher in summer than in autumn and winter, 1.4 times higher than in spring (Table 1). The phosphate (PO_4^{3-}) concentration varied little (0.12 to 0.15 mg L^{-1}) in all seasons (Table 1). Chlorophyll *a* concentrations were highest in spring with 1.45 mg L^{-1} and lowest in summer with 0.92 mg L^{-1}, respectively (Table 1).

Morphological observations

The cortical cells of *C. tamariscifolia* had numerous chloroplasts (Figure 3A) and physodes with a thick cell wall (Figures 3A and C) that was embedded with phenolic compounds (Figure 3B). Mitochondria were associated with the chloroplasts (Figure 3D) which had the typical internal organization of brown algae with thylakoids aggregated in bands (Figure 3D). Lipid droplets (plastoglobuli) were situated between the thylakoids (Figure 3D) and there were plasmodesmata cell connections (Figure 3E).

Biochemical responses

Principal Coordinates Analysis (PCO) (Figure 4) revealed a positive correlation of the first axis (43.8% of total variation) with the internal N content. In contrast, the ratio C:N, antioxidant activity and phenolic compounds were negatively correlated with this axis. Seasonality had a marked effect upon these factors (Figure 4). Moreover, the combination of the first two axes explained the 79.5% of the variation in these variables (Figure 4). The small angles between the arrows are indicative of high correlation between the variables. The carbon and nitrogen contents of *C. tamariscifolia* were significantly higher in winter and spring and the C: N ratio was significantly lower in winter (Figure 5, Table 3). Phenol content and antioxidant activity (i.e. less EC_{50}) were significantly higher in spring (Figure 6, Table 3).

Physiological responses

The F_v/F_m ratio was not significantly affected by season, although it tended to be higher in winter. Maximal electron transport rate (ETR_{max}) was highest in spring and photosynthetic efficiency (α_{ETR}) was significantly lower in winter (Tables 2 and 4). The irradiance of saturation of curve (Ek_{ETR}) was not significantly affected by season, but

tended to be higher in winter and spring. The highest non-photochemical quenching (NPQ_{max}) occurred winter, although no statistically significant seasonal differences were found (Tables 2 and 4). The irradiance of saturation of non-photochemical quenching (Ek_{NPQ}) was significantly higher in autumn and the ratio ETR_{max} (production): NPQ_{max} (photoprotection) was highest in spring (Tables 2 and 4). Positive correlations between phenolic compounds and antioxidant activity and between antioxidant activity and nitrogen internal content, through all seasons, were found. There was also a positive correlation between EC_{50}, ETR_{max} and photosynthetic efficiency (Table S1). The absorptance tended to be higher in winter and spring. ETR_{max} and phenolic content was also positively correlated.

DISCUSSION

We found that as the short days of winter lengthened into spring this stimulated an upsurge in photoprotectors, antioxidants, and productivity in *Cystoseira tamariscifolia* as the algae laid down stores of nitrogen and carbon. The phenol and antioxidant capacity of this seaweed fell in summer which we attribute to nutrient depletion as the sea surface waters became gradually more oligotrophic due to thermal stratification. Our analyses of nitrogen and carbon contents revealed nutrient limitation in summer and autumn. This seaweed accumulated nitrogen during winter and spring as a reservoir for periods of the high irradiance when photoprotective mechanisms are most needed (Figueroa et al., 2014b; Celis-Plá et al., 2014a). Increased photosynthetic activity can enhance the accumulation of phenolic compounds in *C. tamariscifolia* as reported in other brown algae (Pavia and Toth 2000) as well as in the green alga *Ulva rigida* (Cabello-Pasini et al. 2011).

We attribute declining phenolic content in *C. tamariscifolia* in the summer to light damage when both PAR and UV radiation peaks (Stengel et al., 2014). Phenol release increases at noon in summer daily cycles (Abdala-Díaz et al., 2006). High PAR irradiances and emersion have been associated with increasing phlorotannin release rates (Ragan and Jensen 1978; Carlson and Carlson 1984). Celis-Plá et al., (2014a) also found a higher release rate of polyphenols form *C. tamariscifolia* in outdoor experiments in summer compared to winter. Phenolic compounds released from the thalli into the seawater can react rapidly with both proteinaceous and carbohydrate substances to form UV-absorbing complexes (Swanson and Druehl 2002; Koivikko et al., 2005). Release of phenolic compounds is thought to be a photoprotection

mechanism due to the transient reduction of UV penetration favored by the accumulation of excreted phenols in the cell wall. Defense against epiphytic algae and bacteria (Koivikko et al., 2005). Karban and Baldwin (1997) is thought to be an indirect effect of excreted phlorotannins in algae which are released into the water when algae are grazed.

The positive correlation between phenolic compounds and C content with maximal ETR indicates a coupling between photosynthesis and carbon accumulation with secondary metabolism (the accumulation of inducible UV photoprotective compounds as polyphenols under stress conditions). In spring, photosynthetic energy can be used for both accumulation of carbon compounds to store energetic and to build up photoprotective compounds. In contrast, antioxidant carotenoids and polyunsaturated fatty acids accumulate in stressful conditions and decreased photosynthetic activity (Stengel et al., 2011; Sharma et al., 2012). Here we found that phenolic compounds were directly related to maximal photosynthetic productivity (ETR_{max}). The accumulation of phenols in *C. tamariscifolia* under increased PAR and UV irradiances has previously been reported (Abdala-Díaz et al., 2006; Figueroa et al., 2014a). *C. tamariscifolia* acclimates to high UVB by up-regulating UV screen substances that also act as antioxidants (Figueroa et al., 2014a). Connan et al., (2004) showed that mid-shore brown algae (such as *Fucus spiralis*, *F. vesiculosus*, *Ascophyllum nodosum*) tend to have higher phenol content and antioxidant activity than those found in the low intertidal or sublittoral zone (such as *F. serratus*, *Bifurcaria bifurcata*, *Himathalia elongata* and *Laminaria digitata*) and suggest that this is to protect them against the higher UV irradiance levels of the mid shore.

Where nutrient levels permit, brown algal phenols are stimulated by high light levels (Pavia and Brock, 2000) but peak phenol content is often not found in summer since nitrate concentrations can become limiting (Pavia and Åberg, 1996). This certainly seems true for *C. tamariscifolia* which has higher phenolic contents when nitrates are most abundant (Celis-Plá et al., 2014b). We found that *C. tamariscifolia* phenol content peaked in spring at about 5-7.0% which is within the range of the highest levels found in brown algae from northwest Europe (Pavia and Åberg, 1996; Connan et al., 2004) and in *Cystoseira* spp. from other areas of Mediterranean sea (Abdala-Díaz et al., 2006; Celis-Plá et al., 2014b and 2015). Phenol-rich vesicles, known as physodes, were mainly located in cortical cells, as is the case in other seaweeds (Schoenwaelder, 2008; Gómez and Huovinen, 2010). This location provides

photoprotection of cytoplasmic organelles and nuclei form both cortical and medullar cells (Schoenwaelder, 2008). In *C. tamariscifolia*, phenols are accumulated preferentially at the apices (Abdala-Díaz et al., 2014), i.e., the part of the thalli with highest light exposure. In our study, in order to avoid the heterogeneity of the phenolic content in the thalli, samples were always collected form the apical part. Phenolic compounds found in *C. tamariscifolia* can be related to photoprotective mechanisms, decreasing the negative effect of increased UVB radiation (Figueroa et al., 2014a). Higher phenol levels occur in brown algae from Southern Chile despite local high light and nutrient levels (Gómez and Huovinen, 2010). These differences can be attributed to higher irradiance in south Spain compared to that of Southern Chile.

Cystoseira tamariscifolia collected in summer, spring and winter had higher NPQ values than those collected in autumn indicating active photoprotective mechanisms related to the xanthophyll cycle (Demmig-Adams and Adams, 2006). Maximal photosynthetic capacity (i.e. ETR_{max}) and photosynthetic efficiency (i.e. α_{ETR}) were highest in spring when high daily PAR (*ca.* 102.72 MJm^{-2}) favored more photosynthetic activity than in winter and autumn (*ca.* 51.5 MJm^{-2}). This indicates that the productivity of *C. tamariscifolia* peaks in spring.

Antioxidant, photoprotective, antiplasmin, antiallergic, antiviral antibacterial and anticancer properties have all been reported in brown algae (Sugiura et al., 2007; Artan et al., 2008; Heo et al., 2010). Several seaweed chemicals, such as phloroglucinols from *Ecklonia cava*, are widely used in Asian medicines, foods and cosmetics (Le et al., 2009). The exploitation of these natural resources requires an evaluation of the biomass and content of the algal compounds through the time (Stengel et al., 2011). The seasonal variations in the biochemical composition and physiology of *C. tamariscifolia* can inform management in this species. Spring would be the best period to harvest *C. tamariscifolia* to extract photoprotectors and antioxidants for commercial products, although the environmental impacts would need to be carefully assessed. Poorly managed harvesting of macroalgae can seriously impact natural populations (Stagnol et al., 2013) so any exploitation would require impact assessments prior to licensing managed activities.

CONCLUSIONS

In *C. tamariscifolia* photosynthetic production peaked in spring when light levels temperature and nutrients were optimal for building up stores of phenols and antioxidants. In summer photoinhibition and low nutrients stressed the *C. tamariscifolia.*

Any harvesting of this seaweed as a source of phenolic with antioxidant capacity would be best carried out in spring when these chemicals peak in abundance. Further work would be needed to establish protocols for the harvesting of *C. tamariscifolia* to avoid adverse environmental impacts.

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543 Table 1. Seasonal changes in surface seawater temperature (mean ± SE, n=2144)
544 according to REDCOS buoy (number 1514) and salinity, nitrate, ammonium, phosphate
545 and N: P ratio (mean values ± SE, n=180) in Málaga bay (Southern Spain) according to
546 Ramirez et al. (2005) and Mercado et al. (2007, 2012).
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	Units	Summer	Autumn	Winter	Spring
Temperature	ºC	18.91 ± 2.09	17.81 ± 1.66	14.88 ± 0.45	15.91 ± 1.14
Salinity		36.87 ± 0.29	36.72 ± 0.34	36.93 ± 0.28	37.14 ± 0.45
Nitrate	µmol L^{-1}	0.58 ± 1.07	0.62 ± 0.77	1.52 ± 1.07	1.59 ± 1.44
Ammonium	µmol L^{-1}	0.53 ± 0.75	0.19 ± 0.27	0.18 ± 0.10	0.35 ± 0.20
Phosphate	µmol L^{-1}	0.12 ± 0.08	0.14 ± 0.01	0.14 ± 0.05	0.15 ± 0.09
N:P molar ratio		4.3 ± 6.6	7.4 ± 10.9	13.4 ± 12.3	16.0 ± 21.3
Chlorophyll *a*	µmol L^{-1}	0.92 ± 0.69	1.21 ± 0.94	1.22 ± 1.14	1.45 ± 0.99

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564 Table 2. Photosynthetic physiology of *Cystoseira tamariscifolia* collected in La Araña
565 beach near Málaga (Southern Spain) in summer, autumn, winter and spring 2013-2014.
566 Maximal quantum yield (F_v/F_m), maximal electron transport rate (ETR_{max}, expressed in
567 µmol electrons m^{-2} s^{-1}), photosynthetic efficiency (α_{ETR}), irradiance of saturation of
568 ETR (Ek_{ETR}) expressed in µmol photons m^{-2} s^{-1}, maximal non-photochemical quenching
569 (NPQ_{max}), irradiance of saturation of NPQ (Ek_{NPQ}) expressed in µmol photons m^{-2} s^{-1}
570 and ETR_{max}/NPQ_{max} ratio (mean ± SE, n=9). Lower-case letters denote significant
571 differences after SNK test.
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	Cystoseira tamariscifolia			
	Summer	Autumn	Winter	Spring
Fv/Fm	0.71 ± 0.01	0.71 ± 0.02	0.69 ± 0.02	0.71 ± 0.01
ETR_{max}	52.18 ± 3.39^{a}	53.01 ± 2.23^{a}	55.14 ± 4.29^{a}	70.65 ± 6.58^{b}
α_{ETR}	0.41 ± 0.02^{b}	0.39 ± 0.01^{b}	0.27 ± 0.01^{a}	0.36 ± 0.03^{b}
Ek_{ETR}	137.95 ± 0.06	136.52 ± 17.81	235.64 ± 49.84	272.38 ± 84.51
NPQ_{max}	1.39 ± 0.11	1.27 ± 0.16	1.61 ± 0.18	1.38 ± 0.14
Ek_{NPQ}	301.28 ± 33.22^{ab}	395.56 ± 48.98^{b}	190.99 ± 27.83^{a}	295.61 ± 66.12^{ab}
ETR_{max}/NPQ_{max}	42.32 ± 4.91	52.14 ± 15.68	38.77 ± 3.93	61.96 ± 7.43
Absorptance	0.76 ± 0.04	0.77 ± 0.02	0.83 ± 0.02	0.79 ± 0.02

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584 Table 3. Seasonal and annual effects on the carbon, nitrogen, C:N ratio, phenolic
585 compounds and antioxidant activity (EC_{50}) of *Cystoseira tamariscifolia* collected in
586 2012-2014 on a rocky shore near Málaga, southern Spain. Significant differences at $P<$
587 0.05 are shown in bold.

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			Cystoseira tamariscifolia		
		Df	**MS**	***F***	***P***
Carbon	*Year*	1	81.83	0.40	0.53
	Season	3	**928.75**	**4.52**	**<0.01**
	*Year*Season*	3	201.34	0.98	0.41
	Res	64	205.41		
Nitrogen	*Year*	1	15.11	1.59	0.21
	Season	3	**163.24**	**17.14**	**<0.01**
	*Year*Season*	3	12.49	1.31	0.28
	Res	64	9.52		
Ratio C:N	*Year*	1	9.83	1.54	0.22
	Season	3	**81.43**	**12.76**	**<0.01**
	*Year*Season*	3	11.12	1.74	0.17
	Res	64	6.38		
Phenolic compounds	*Year*	1	188.25	2.04	0.16
	Season	3	**1763.93**	**19.11**	**<0.01**
	*Year*Season*	3	**1576.40**	**17.07**	**<0.01**
	Res	64	92.33		
EC_{50}	*Year*	1	0.00	0.47	0.49
	Season	3	**0.04**	**4.31**	**<0.01**
	*Year*Season*	3	0.02	1.86	0.15
	Res	64	0.01		

589 *Res: residual*

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Table 4. Seasonal effects on *Cystoseira tamariscifolia* photosynthesis on a rocky shore near Málaga in 2013-2014; maximal quantum yield (F_v/F_m), maximal electron transport rate (ETR_{max}), photosynthetic efficiency (α_{ETR}), irradiance of saturation of ETR (Ek_{ETR}), maximal non-photochemical quenching (NPQ_{max}), irradiance of saturation of NPQ (Ek_{NPQ}) and relationship between ETR_{max}/NPQ_{max} . Significant differences at $P<$ 0.05 are shown in bold.

		Cystoseira tamariscifolia			
		df	**MS**	***F***	***P***
Fv/Fm	*Season*	3	0.00	0.19	0.90
	Res	32	0.00		
ETRmax	*Season*	3	**680.4**	**3.8618**	**0.02**
	Res	32	176.2		
α_{ETR}	*Season*	3	**0.03**	**5.61**	**<0.01**
	Res	32	0.01		
Ek_{ETR}	*Season*	3	42939.9	1.91	0.15
	Res	32	22502.1		
NPQ_{max}	*Season*	3	0.18	0.82	0.49
	Res	32	0.21		
Ek_{NPQ}	*Season*	3	**62901.2**	**3.23**	**0.04**
	Res	32	19464.4		
ETR_{max}/NPQ_{max}	*Season*	3	691.3	0.90	0.45
	Res	32	767.9		
Absorptance	*Season*	3	0.01	1.95	0.14
	Res	32	0.00		

Res: residual

Figure captions

Figure 1. La Araña rocky shore in southern Spain showing high perennial coverage of the brown alga C*ystoseira tamariscifolia* and spring/summer blooms of *Ulva* spp. in 2013.

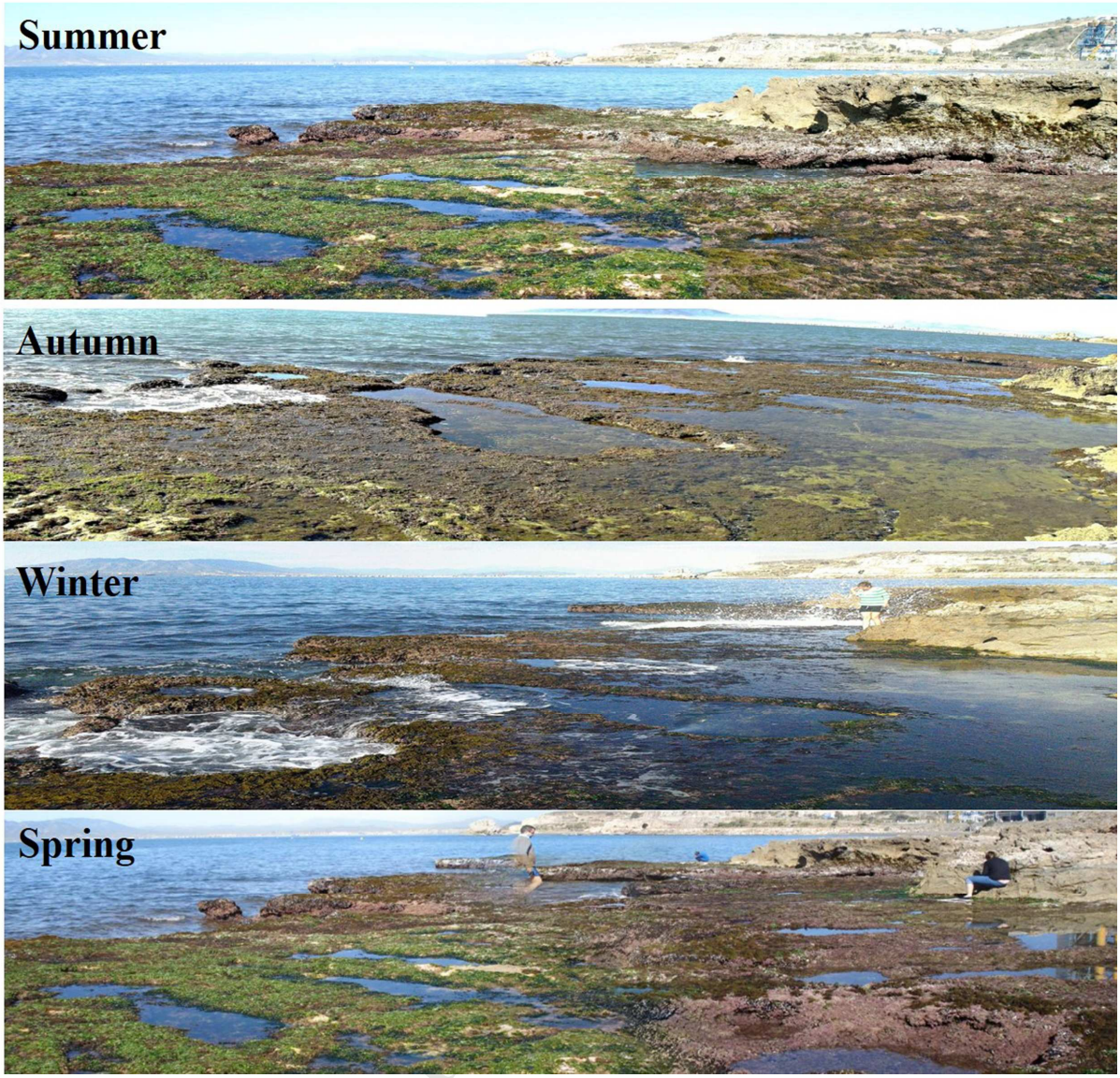
Figure 2. Daily integrated irradiance per month in the period 2012-2014 of A) PAR (400-700 m), B) UVA (320-400 nm) and C) UVB (280-320 nm) in the NILU UV6 station located in the roof Central Services for Research building (University of Malaga).

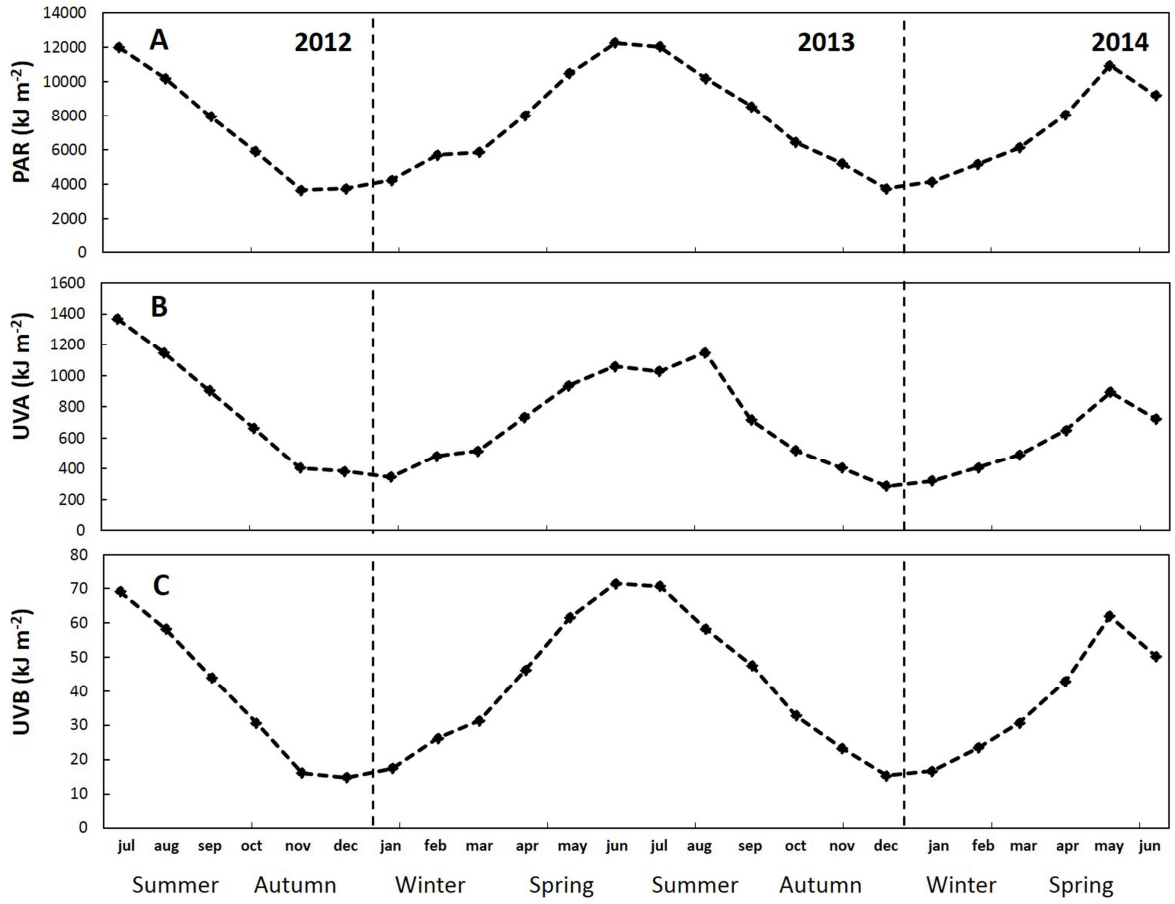
Figure 3. Transmission electron microscopy images of *Cystoseira tamariscifolia* from La Araña in summer 2013. A) Cortical cell with many chloroplasts (C), physodes (Ph) and thick cell wall (CW). B) Arrows indicate presence of phenolic compounds in cell wall. C) Detail of physodes in cortical cell. D) Chloroplast with plastoglobuli (P) and associated mitochondria (M). E) Detail of plasmodesmata (arrows).

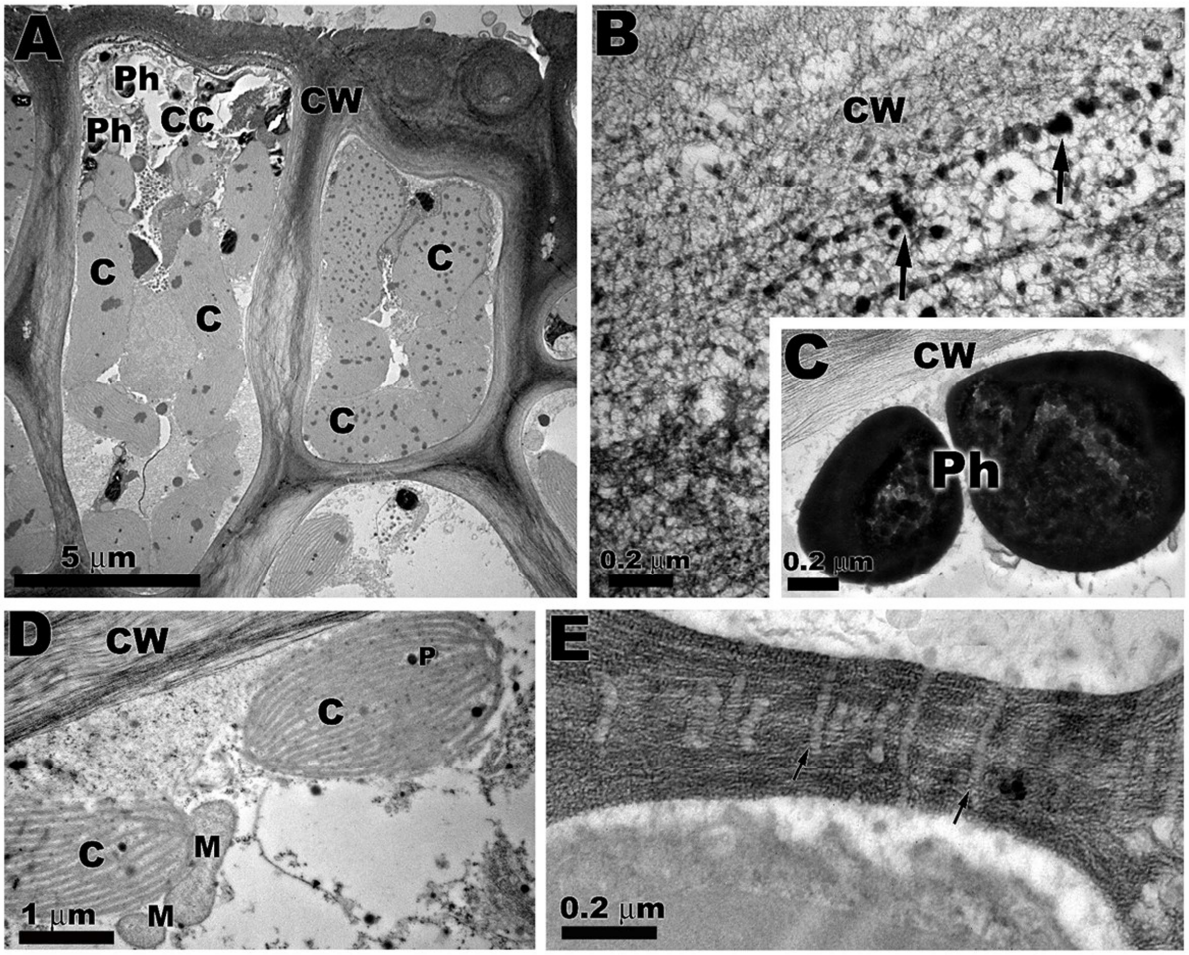
Figure 4. PCO diagram in relation to Season (spring, summer, autumn and winter). Vectors overlay (Spearman rank correlation) indicates the relationship between the PCO axes and the ecophysiologycal variables; C, N: internal conten and C:N ralitionship, respectively, PC: phenolic compounds and AA: such as $1/EC_{50}$ antioxidant axtivity, in the time.

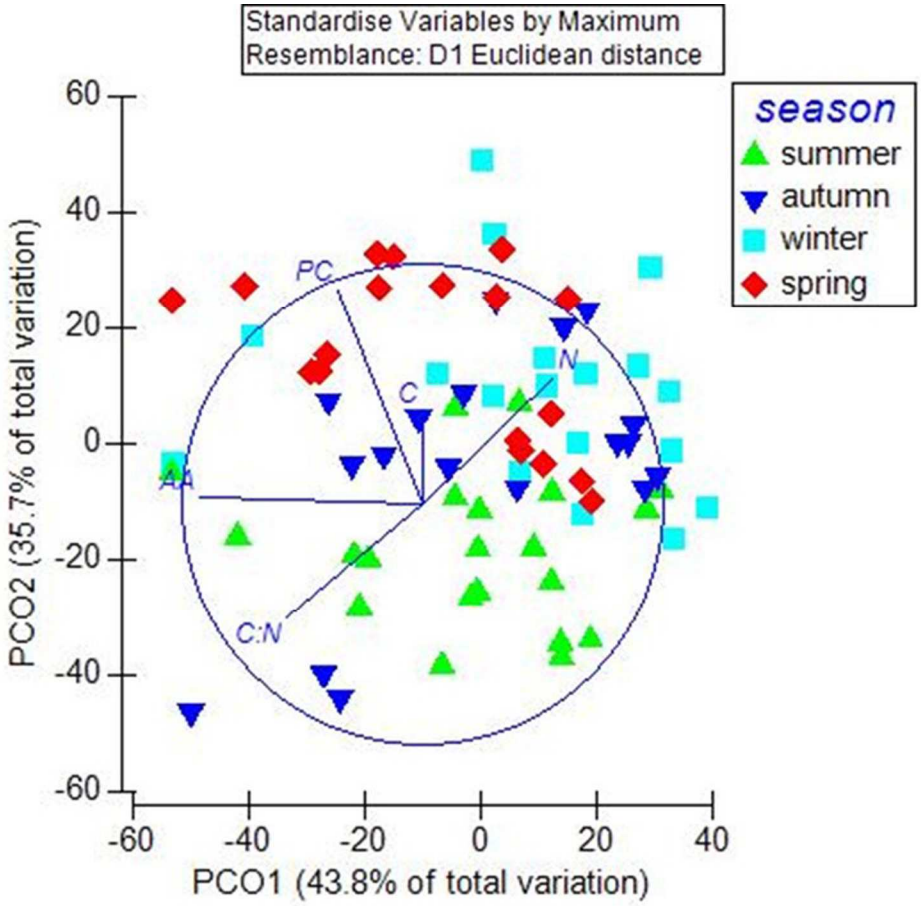
Figure 5. A) Carbon and B) Nitrogen contents expressed as mg g^{-1} DW and C) C:N ratio of *Cystoseira tamariscifolia* in summer, autumn, winter and spring.

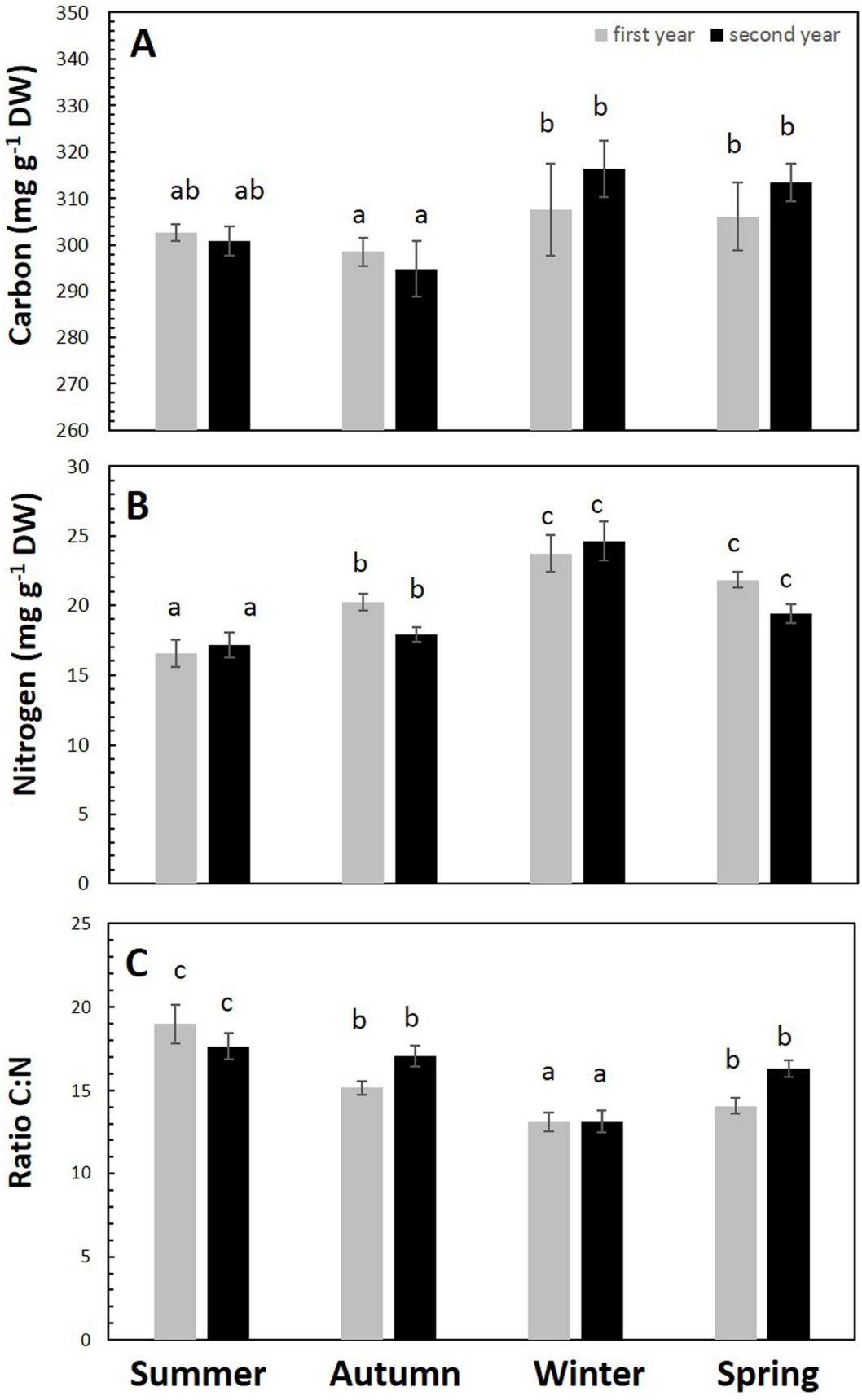
Figure 6. A) Phenolic compounds (expressed as mg g^{-1} DW) and B) Antioxidant activity (EC_{50}; expressed as mg DW mL^{-1}) to *Cystoseira tamariscifolia* through the season (summer, autumn, winter and spring).










A
first year
second year
Carbon (mg g-1 DW)
350
340
330
320
310
300
290
280
270
260
ab
ab
a
a
b
b
b
b
B
Nitrogen (mg g-1 DW)
30
25
20
15
10
5
0
a
a
b
b
c
c
c
c
C
Ratio C:N
25
20
15
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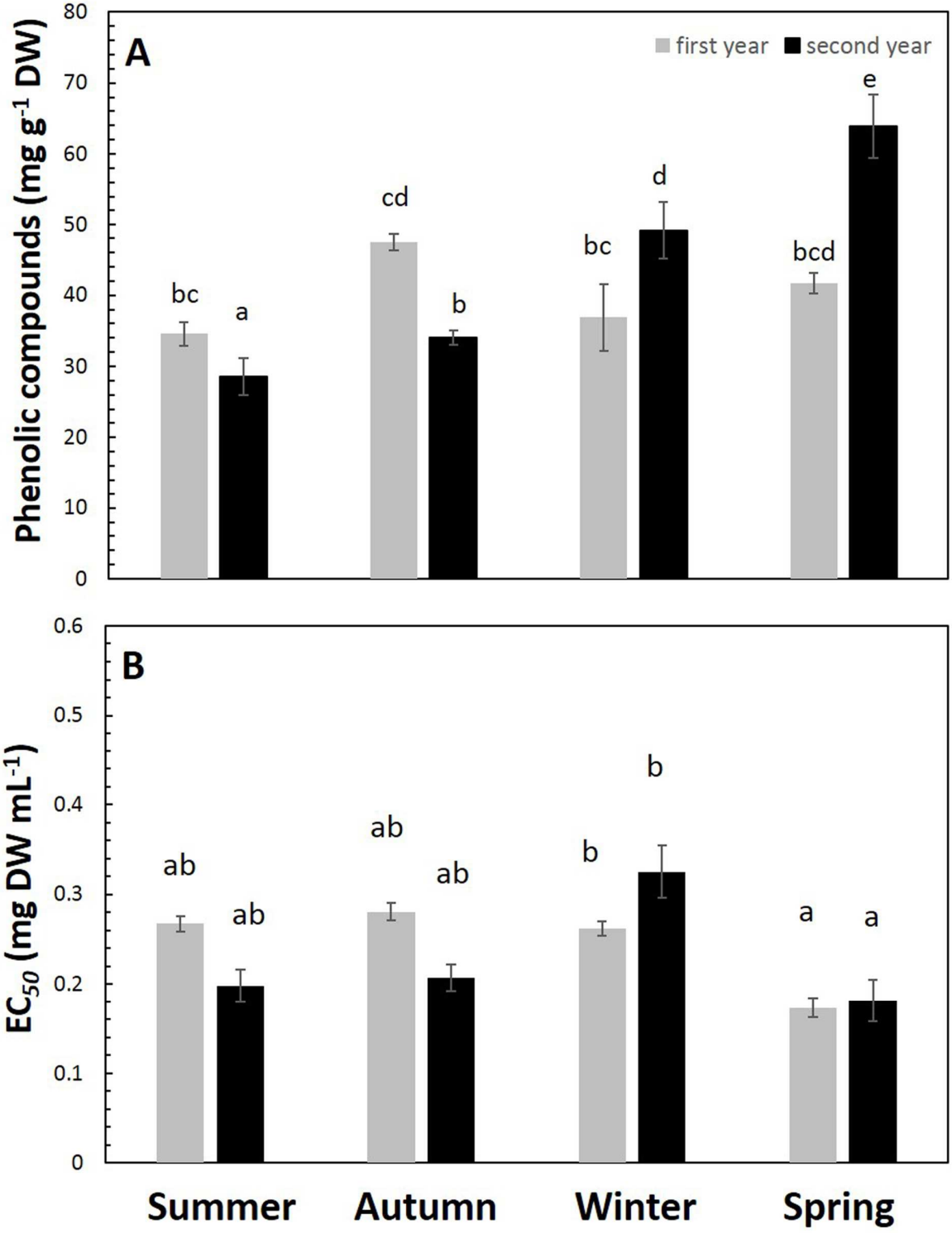
Summer
Autumn
Winter
Spring


A
first year
second year
Phenolic compounds (mg g-1 DW)
80
70
60
50
40
30
20
10
0
bc
a
cd
b
bc
d
bcd
e
B
EC50 (mg DW mL-1)
0.6
0.5
0.4
0.3
0.2
0.1
0
ab
ab
ab
ab
b
b
a
a
Summer
Autumn
Winter
Spring

Seasonal biochemical and photophysiological responses in the intertidal macroalga C*ystoseira tamariscifolia* (Ochrophyta).

Paula S.M. Celis-Plá, Zenilda L. Bouzon, Jason M. Hall-Spencer, Eder C. Schmidt, Nathalie Korbee and Félix L. Figueroa.

Highlights

- Monitoring of the seasonal changes in the biochemistry and photophysiology of the brown macroalga *Cystoseira tamariscifolia*.
- The increased irradiance in spring enhanced the algal productivity, antioxidant activity and the production of photoprotective compounds.
- The monitoring for the best period to harvest *Cystoseira tamariscifolia* to extract potential commercial uses could be in spring.