



Short-term effects of increasing CO₂, nitrate and temperature on three Mediterranean macroalgae: biochemical composition

Fl Figueroa, J Bonomi Barufi, Ej Malta, R Conde-Álvarez, U Nitschke, F Arenas, M Mata, S Connan, Mh Abreu, R Marquardt, et al.

► To cite this version:

Fl Figueroa, J Bonomi Barufi, Ej Malta, R Conde-Álvarez, U Nitschke, et al.. Short-term effects of increasing CO₂, nitrate and temperature on three Mediterranean macroalgae: biochemical composition. *Aquatic Biology*, 2014, 22, pp.177-193. <10.3354/ab00610>. <hal-04652550>

HAL Id: hal-04652550

<https://hal.science/hal-04652550v1>

Submitted on 19 Jul 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY 4.0 - Attribution - International License



Short-term effects of increasing CO₂, nitrate and temperature on three Mediterranean macroalgae: biochemical composition

F. L. Figueroa¹, J. Bonomi Barufi², E. J. Malta^{3,*}, R. Conde-Álvarez¹, U. Nitschke⁴, F. Arenas⁵, M. Mata¹, S. Connan^{6,7}, M. H. Abreu⁸, R. Marquardt⁹, F. Vaz-Pinto⁵, T. Konotchick¹⁰, P. S. M. Celis-Plá¹, M. Hermoso¹, G. Ordoñez¹, E. Ruiz¹, P. Flores¹, J. de los Ríos¹, D. Kirke⁴, F. Chow¹¹, C. A. G. Nassar¹², D. Robledo¹³, Á. Pérez-Ruzafa¹⁴, E. Bañares-España¹⁵, M. Altamirano¹⁵, C. Jiménez¹, N. Korbee¹, K. Bischof¹⁶, D. B. Stengel⁴

¹Department of Ecology, Faculty of Sciences, University of Malaga, 29071 Malaga, Spain

Complete list of author addresses is in the Appendix

ABSTRACT: Short-term effects of increasing $p\text{CO}_2$; 380 ppm (LC) vs. 700 ppm (HC); at different nitrogen levels; 5 μM nitrate (LN) vs. 50 μM (HN); on the contents of protein, mycosporine-like amino acids (MAAs), phenolic compounds and total fatty acids, antioxidant activity, calcification and C:N ratios were analyzed in 3 eulittoral Mediterranean macroalgae with different bio-optical characteristics and carbon assimilation efficiencies: *Cystoseira tamariscifolia* (Heterokontophyta), *Ulva rigida* (Chlorophyta) and *Ellisolandia elongata* (Rhodophyta). After acclimation to different $p\text{CO}_2$ and nitrogen conditions for 6 d, the algae were subjected to a 4°C temperature increase for 3 d. Increasing temperature and $p\text{CO}_2$ produced alterations in the biochemical composition of the 3 macroalgae. Short-term variations of protein levels were observed in *U. rigida*, with clearly decreased values in the HCLN treatment. In *C. tamariscifolia*, protein decreased after the temperature increase but only under LC. The interaction of temperature and N affected phenolic compounds only in *U. rigida* and the content of MAAs in *E. elongata*. The functional patterns of the 3 macroalgae in response to the $p\text{CO}_2$, nitrogen and temperature regimes may be explained in terms of their bio-optical characteristics and antioxidant activity. The vulnerability and acclimation of the 3 species to the expected variations of climate change factors are discussed.

KEY WORDS: Acidification · Climate change · *Cystoseira tamariscifolia* · *Ellisolandia elongata* · Nitrate · Short-term experiment · Temperature · *Ulva rigida*

INTRODUCTION

Sea surface temperature has been increasing by 0.13°C per decade over the last 50 yr, mainly due to the increase of CO₂ levels in the atmosphere (IPCC 2013). One-third of the atmospheric CO₂ produced by human activity has been absorbed by the oceans in the past 200 yr, which has led to a reduction in the pH of surface seawater by 0.1 units (Orr et al. 2005).

Oceans will be further acidified by 0.3 to 0.4 units by 2100 due to the expected rise in the atmospheric CO₂ level to 800–1000 ppmv by the end of this century, according to the 'business-as-usual' CO₂ emission scenario (Brewer 1997, Caldeira & Wickett 2003). This ocean acidification process may significantly change the water carbonate chemistry (Sabine et al. 2004, Pelejero et al. 2010) and may harm marine calcifying organisms (Gao et al. 1993, Riebesell et al.

*Corresponding author: ejmalta@hotmail.com

2000). Ocean acidification has been consistently related to reduced growth rates in calcified macroalgae (Kroeker et al. 2010). Reductions in calcification rate at elevated $p\text{CO}_2$ have been demonstrated for crustose and articulated coralline red algae, as well for the calcified green algae of the genus *Halimeda* (Gao et al. 1993, Büdenbender et al. 2011, Price et al. 2011). However, reduced calcification at increased $p\text{CO}_2$ did not emerge as a general pattern in a meta-analysis of multiple seaweed studies (Kroeker et al. 2010). This may partly be because the effects of ocean acidification on calcification vary among seaweeds due the diversity of calcification strategies amongst macroalgae, e.g. many species are able to create microclimates of chemistry favorable for calcification regardless of ambient conditions (Price et al. 2011, Roleda et al. 2012a). Reduced pH may have important consequences for non-calcifying taxa as well (Roleda et al. 2012b), although the cumulative effects of climatically realistic, CO_2 -driven pH change on non-calcifying seaweeds remain poorly understood (Kroeker et al. 2010). Studies on the ecological and physiological impacts of elevated CO_2 concentrations on macroalgae were initiated in the early 1990s: growth of *Pyropia* (*Porphyra*) *yezoensis* juveniles was significantly enhanced in cultivation enriched with CO_2 up to 1000 ppmv (Gao et al. 1991, 1993). The photosynthetic carbon fixation rate of some intertidal macroalgal species increased during low-tide periods when they were exposed to air containing high CO_2 concentration (Gao et al. 1991, Zou & Gao 2002). Macroalgae are a highly diverse group with complex functional morphologies and varied ecological roles. Thus, morphological plasticity in addition to physiological mechanisms may determine their capacity to acclimate to global climate changes (Falkenberg et al. 2013).

In recent years, there has been a significant effort to predict the future impact of climate change on seaweed communities (Graham et al. 2007, Halpern et al. 2008, Wernberg et al. 2010). Two main approaches have been followed: (1) experimental approaches designed as factorial experiments, incubating macroalgae for days or months at different growth temperatures according to the future predicted scenarios, and evaluating the interactive responses with other variables, such as acidification, UV radiation (UVR), and nutrient availability, amongst others (Baulch et al. 2003, Hoppe et al. 2008, Porzio et al. 2011); and (2) field studies of seaweeds growing at their temperature limit for growth and reproduction, while monitoring the temporal and spatial variation of temperature and other variables (Viejo et al. 2011,

Martínez et al. 2012). Most investigations have been conducted on individual species separately, rather than communities (Olabarria et al. 2013), although it has been reported that community-level impacts might be less noticeable (Kroeker et al. 2010). Moreover, studies on the effect of global climate changes on aquatic organisms have mostly been conducted with 1 or 2 variables, and interactions between multiple factors have scarcely been studied (Franklin & Foster 1997, Gordillo et al. 2001, Bischof et al. 2006a, Häder et al. 2007). For example, the effect of increased UVR, temperature and CO_2 on photosynthetic metabolism, nutrient incorporation and growth has been studied in algae from Mediterranean and Atlantic waters off the southern Iberian Peninsula (reviewed in Häder & Figueroa 1997, Figueroa & Gómez 2001), but also in algae from the North Sea (Hanelt et al. 1997b) and the Arctic region (Hanelt et al. 1997a, Hanelt 1998, Roleda 2009). Investigations have been conducted in the field (*in situ*) and in experimentally controlled conditions under solar radiation (outdoor) and under artificial conditions (indoor). Mechanisms for acclimation to global climate changes have been evaluated as the effect on photoinhibition, photoprotection, nutrient uptake systems and patterns of growth, reproduction and morphogenesis of different developmental stages of macroalgae (Häder & Figueroa 1997, Gómez & Figueroa 1998, Figueroa et al. 2003b, Villafañe et al. 2003, De la Coba et al. 2009).

To date, few short-term (<1 yr) algal manipulation experiments have been performed. Previous studies have revealed mixed responses depending on the algal species and the culture conditions applied (Porzio et al. 2011, Zou et al. 2011, Cornwall et al. 2012). In macroalgae, doubling the CO_2 level caused an increase in photosynthetic activity of 52 to 130 %, depending on the species (Gao et al. 1993, Kübler et al. 1999, Riebesell et al. 2007). Overall, the sensitivity of algae to acidification is expected to be complex, due to interactions between the effects of pH and CO_2 on the enhancement of photosynthesis. Although increasing ocean CO_2 concentration may enhance rates of photosynthesis and growth, particularly in species without carbon concentrating mechanisms (CCMs), such increases may be limited by the availability of other limiting nutrients (Raven et al. 2005). Mercado et al. (1998) reported a relationship between CCMs and light energy availability in intertidal macroalgae, but not with inorganic carbon availability: intertidal algae with emersion periods presented higher photosynthetic rates (Mercado et al. 1998) and carbon uptake (Flores-Moya et al. 1998)

due to the higher availability of CO₂ compared to submerged algae. Seasonal changes in temperature, nutrient availability and light are also likely to interact with the effect of CO₂ on metabolic processes in algae (Tyrell et al. 2008, Martin & Gattuso 2009, Mercado & Gordillo 2011, Hofmann et al. 2013). As calcification, photosynthesis, nutrient uptake, growth, and other metabolic processes are affected by temperature, light and nutrient availability, changes in these parameters are likely to have a strong influence on the enzymatic response of macroalgae to increasing CO₂. Therefore, outdoor mesocosm studies are useful for monitoring CO₂ effects over time during natural temperature, nutrient and light fluctuations.

The aim of this study was to evaluate the short-term (6 d) vulnerability and acclimation to increasing pCO₂ (low CO₂, LC: 380 ppm vs. high CO₂, HC: 700 ppm), under different nitrogen levels through nitrate pulses: high nitrate level (HN, 50 µM) vs. low nitrate level (LN, 5 µM) on 3 common eulittoral macroalgal species from the coastal area of Málaga in the Alborán Sea (Mediterranean Sea). In this region, nitrate concentrations are decreasing as a consequence of the weakening of wind-induced upwelling; thus, oligotrophication is becoming more recurrent (Mercado et al. 2012). The Mediterranean Sea is a priority area for studies on climate change due to the well-recognized increase in average seawater temperatures and the 200 to 500 % increase in the number of days with heat extremes (Mieszkowska et al. 2006, Diffenbaugh et al. 2007, Louanchi et al. 2009).

The vulnerability of seaweeds to climate change factors in the short-term was evaluated by using physiological indicators including stoichiometry (C:N), quality of biomass (soluble proteins, photoprotectors and lipids) and antioxidant substances according to Figuerola & Korbee (2010) and Figuerola et al. (2014b). The experiments were conducted in the framework of the International Workshop of the Group on Aquatic Primary Productivity (GAP 9) hosted in September 2012, in Málaga. In addition to this study, the results of the experiments on these macroalgae have been presented in other reports (Stengel et al. 2014, Figuerola et al. 2014a, Parages et al. 2014; all this Theme Section).

MATERIALS AND METHODS

Algae and experimental design

Macroalgae from different morpho-functional groups defined according to Littler et al. (1983) were col-

lected in La Araña (36° 45' N, 4° 18' W) on the Málaga coastline (Alborán Sea, southern Iberian Peninsula) characterized by a good ecological status of coastal waters as defined by the European Water Framework Directive (2000/60/EC) (Mercado et al. 2012, Bermejo et al. 2013).

Ulva rigida C. Agardh (Chlorophyta) is a distromatic laminar alga consisting of 2 cell layers, characterized by rapid growth and a nitrophilic pattern; the leathery *Cystoseira tamariscifolia* (Hudson) Papenfuss (Phaeophyceae) displays a complex parenchymatic morphology with a thick, non-calcareous thallus with cortical and medullar cells, and presents a low growth rate. Both *U. rigida* and *C. tamariscifolia* contain phenolic compounds. Finally, the calcareous *Ellisolandia elongata* (J. Ellis & Solander, K. R. Hind & G. W. Saunders), formerly *Corallina elongata* J. Ellis et Solander (Rhodophyta) according to Hind & Saunders (2013), presents a complex pseudoparenchymatic morphology with a thick calcareous thallus, cortical and medullar cells, slow growth rate, and contains mycosporine-like amino acids (MAAs), as do other red algae. These macroalgal species were selected since they are dominant species on the Mediterranean coast of the southern Iberian Peninsula and possess different morphologies, bio-optical properties and carbon acquisition efficiencies; in addition, their ecophysiological responses have been extensively studied in these or similar species (Mercado et al. 1998, Figuerola & Viñegla 2001, Gordillo et al. 2001, Abdala-Díaz et al. 2006, Cabello-Pasini et al. 2011, amongst others).

The collected algae were transported to the laboratory in an icebox, cleaned of epibiota and acclimated for 4 d to low C and low N conditions prior to experimental treatment conditions. The experimental design is described in detail by Stengel et al. (2014). Briefly, the 3 species were cultivated in a mesocosm system, with either high or low C and N conditions: high CO₂ (HC): 700 ppm vs. low CO₂ (LC): 380 ppm; high nitrate level (HN): 50 µM vs. low nitrate level (LN): 5 µM. The level of 380 ppm used in the experiment as ambient level (LC) of CO₂ is the range surface pCO₂ of the Alborán Sea (367 to 394 ppm of pCO₂) according to Takahashi et al. (2011) and McElhany & Busch (2013). This region can be characterized as oligotrophic (Ramírez et al. 2005, Mercado et al. 2012). The level of 5 µM nitrate is the highest range of the natural levels found during certain periods of the year (Ramírez et al. 2005). Thus, the LCLN treatment can be considered a control treatment because it was closest to natural conditions. In addition to the different C and N conditions, the algae

were subjected to a 4°C temperature increase for 3 d after a 6 d acclimation to the different carbon and nitrogen conditions. As a result, temperature fluctuated between 19°C (08:00 h GMT min.) and 24.5°C (17:00 h GMT max.) during the acclimation phase, and between 24°C (08:00 h GMT min.) and 29°C (17:00 h GMT max.) during the +4°C treatment. Detailed hourly temperature fluctuations can be found in Stengel et al. (2014). Orthophosphate at 2 µM was included in both LN and HN treatments.

Biochemical composition

Variables including accumulation of secondary metabolites, antioxidant capacity and stoichiometry were used as physiological indicators and were estimated at different time periods (morning, noon, and evening) (Figueroa & Korbee 2010, García-Sánchez et al. 2012, Figueroa et al. 2014b) as follows.

Soluble proteins (SPs). Total SPs were extracted in a mortar from 50 mg dry weight (DW) of macroalgae in a 0.1 M phosphate buffer (pH 6.5). After centrifugation at 13 000 rpm (15493 g), 50 µl of supernatant was mixed with 750 µl of 0.1 M phosphate buffer (pH 6.5) and 200 µl of assay mix (Bio-Rad Protein Assay). The absorbance at 595 nm was determined using a spectrophotometer (UVMini-1240 model, Shimadzu) after 15 min of incubation at room temperature (Bradford 1976). Concentrations were calculated by means of standards prepared with bovine serum albumin (Sigma).

Total Fatty Acids (Total FAs). Total FA content was determined by gas chromatography-flame ionization detector (GC/FID) after direct transmethylation of the freeze-dried biomass as described previously by Schmid et al. (2014). For quantification, pentadecanoic acid 15:0 (99%, Alfa Aesar) was added as an internal standard.

MAAs. MAAs of *E. elongata* were determined using HPLC (Waters 600) as described in Korbee-Peinado et al. (2004). Total MAAs were expressed in mg g⁻¹ DW, and the different MAAs identified as shinorine, palythine, asterina-330 and palythanol as % of total MAAs.

Phenolic compounds and antioxidant activity (DPPH method). Phenolic compounds of *U. rigida* and *C. tamariscifolia* were extracted using 0.25 g fresh weight (FW) of macroalga and 2.5 ml of 80% methanol (80% MeOH:20% H₂O). After being kept overnight, the mixture was centrifuged at 4000 rpm (2253 g) for 15 min at 4°C and the supernatant was collected. Total phenolic compounds were determined

colorimetrically using the Folin-Ciocalteu reagent (Folin & Ciocalteu 1927). Phloroglucinol (1,3,5-trihydroxybenzene, Sigma) was used as a standard. Finally, the absorbance was read at 760 nm using a spectrophotometer (UVMini-1240 model, Shimadzu). Phenolic concentrations were expressed as mg g⁻¹ DW.

The antioxidant activity was measured on the phenolic compound extracts using the reduction of the stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) according to Blois (1958): 150 µl of DPPH (1.27 mM) prepared in 90% methanol (90% MeOH:10% H₂O) was added to each extract. The reaction was completed after 30 min in the dark at room temperature (~20°C), and the absorbance was read at 517 nm using a spectrophotometer (UVMini-1240 model). The calibration curve made with DPPH was used to calculate the remaining concentration of DPPH in the reaction mixture after incubation. Values of DPPH concentration (mM) were plotted against algal extract concentration (mg DW ml⁻¹) in order to obtain the oxidation index EC₅₀, which represents the concentration of algal extract required to scavenge 50% of the DPPH in the reaction mixture. Ascorbic acid was used as positive control (Connan et al. 2006).

Calcification. Samples of *E. elongata* were kept at 40°C until desiccated. Initial dry weight (DW_I) was obtained and then 10% HCl was added in order to initiate CaCO₃ dissolution, maintaining the same weight/volume ratio. After complete CaCO₃ removal, samples were washed with distilled water, dried again at 40°C and final dry weight (DW_F) was measured. CaCO₃ content (%) was calculated according to the formula:

$$\text{CaCO}_3 = [(DW_I - DW_F) / DW_F] \times 100 \quad (1)$$

Carbon (C) and nitrogen (N) content. Seaweed samples were dried for 24 h at 60°C and kept desiccated until analyses. Total internal C and N contents on a DW basis were determined using a CNHS-932 elemental analyzer (Leco Corporation). The molar ratio between C and N was obtained by dividing total internal C by total internal N content.

FW/DW ratio. To acquire the FW:DW ratio, 15 to 20 samples with known FW of each algal species collected at each sampling time and under each treatment were dried at 60°C for 24 h, reaching constant DW.

Statistical analyses. After visually checking for normality, data were tested for homogeneity of variances using Cochran's test. Logarithm transformations were used when the assumptions were not achieved. Multi-factorial ANOVAs were performed including temperature (ambient and high), nitrogen (low and high), CO₂ (low and high) and time of sam-

pling (morning, noon, evening), all considered fixed crossed factors, and tank as a random factor nested in the interaction of $N \times C$ and crossed with the other 2 factors. Tank (alone or in interactions) was not significant ($p > 0.25$) and thus this factor was not included in the final models (Underwood 1997). When significant differences were detected, post hoc tests were performed using a Student-Newman-Keuls (SNK) test. The significance level was set to $\alpha = 0.05$. Data sets of the different variables corresponded to 2 measurement days ($n = 6$). In addition, Pearson correlation coefficients were calculated and tested between all measured dependent variables.

RESULTS

SPs

In the 'initial' samples after 6 d of acclimation, SP concentrations were similarly high in *Ulva rigida* and *Cystoseira tamariscifolia*, and about 3 times higher than that of *Ellisolandia elongata* (Table 1). During the experimental period, the highest protein levels were reached in *C. tamariscifolia* ($15.02 \pm 1.48 \text{ mg g}^{-1} \text{ DW}$), followed by *U. rigida* ($9.27 \pm 0.57 \text{ mg g}^{-1} \text{ DW}$) and *E. elongata* ($5.91 \pm 0.30 \text{ mg g}^{-1} \text{ DW}$) (Fig. 1). In *C. tamariscifolia*, the interactive effect of the 4 factors was significant (Table 2). C and N treatments also produced a significant effect as did C and time (Table 2). In general, SP values were lower at noon and in the evening under the HCHN treatment (Fig. 1a). In *U. rigida*, 2 significant interactions were observed: time and temperature, and C and N (Table 3). For both the ambient and increased temperature, we found the highest SP contents in the morning and at noon respectively (Fig. 1b). The lowest value was observed in HCLN, and it was lower at LCLN than at HCHN (Fig. 1c). Finally, in *E. elongata*, N and temperature and the interaction of time $\times$ temperature had significant effects on protein levels (Table 4). As was expected, the lowest content of SP was observed at LN and for the interactive effect, the highest value was detected at ambient temperature and during the evening (Fig. 1d).

Total FAs

The average levels of total FAs were 0.94 ± 0.01 and $1.07 \pm 0.03 \text{ \% g}^{-1} \text{ DW}$ in *C. tamariscifolia*, 1.03 ± 0.02 and $0.90 \pm 0.03 \text{ \% g}^{-1} \text{ DW}$ in *U. rigida* and 0.24 ± 0.01 and $0.11 \pm 0.005 \text{ \% g}^{-1} \text{ DW}$ in *E. elongata* under ambi-

ent and ambient + 4°C temperatures respectively. Temperature produced significant effects on total FA contents in the 3 species (Tables 2–4). The increased temperature caused a decrease of total FAs in *C. tamariscifolia* and *E. elongata*, whereas in *U. rigida* it resulted in an increase in FAs (data not shown).

MAAs

E. elongata contained $0.23 \text{ mg total MAAs g}^{-1} \text{ DW}$ at the initial time period (Table 1). The interactive effects of $N \times$ temperature, time $\times$ temperature and the triple interaction of $C \times$ time $\times$ temperature were significant (Table 5); at ambient temperature, no significant differences through the day between LC and HC treatments were found except in the evening (Fig. 2). No differences were found at ambient temperature +4°C amongst treatments nor over time. Shinorine was the dominant MAA in general, with proportions higher than 40%. Palythine was also present (>25%) and palythanol (around 10 to 20%). Asterina-330 presented the lowest proportion (2 to 5%, data not shown). Asterina-330 and palythine presented a similar pattern with the most double (5 for both) and triple (4 and 3 respectively) interactions among variables, whereas in shinorine, only the double interactions $C \times$ time, $N \times$ time and time $\times$ temperature, and the triple interactions $C \times N \times$ time and $C \times$ time $\times$ temperature were found (Table 5). Finally, the palythanol level was significantly affected by the double interaction $N \times$ temperature, and the triple interaction $C \times N \times$ temperature (Table 5).

Phenolic compounds and antioxidant activity (DPPH scavenger capacity)

Phenolic contents in *C. tamariscifolia* at the initial time were about 4 times higher than in *U. rigida* (Table 1). For *C. tamariscifolia*, an interactive effect of $C \times$ temperature was found (Table 2), with phenolic compounds (plentics) being highest at ambient temperature and LC (Fig. 3a). EC_{50} values presented a complex pattern (Fig. 3b), with a significant interactive effect between C, N time and temperature (Table 2). At ambient temperature, the maximal values (lowest antioxidant activity) were reached at HCLN in the morning and HC at noon, whereas the maximal antioxidant activity was reached under LCLN in the morning (Fig. 3b). Under ambient +4°C, the minimal value of EC_{50} was reached at noon under the LCHN treatment (Fig. 3b).

Table 1. Means $\pm$ SD ($n = 3$) of different variables measured in *Cystoseira tamariscifolia*, *Ulva rigida* and *Ellisolandia elongata* after 6 d of acclimation to ambient CO_2 (LC), LN condition (5 μM nitrate) and ambient temperature (23 to 26°C) (i.e., conditions at initial time). SPs = total soluble proteins; C = total internal carbon; N = total internal nitrogen; phenolics = phenolic compounds; EC_{50} = concentration of the methanolic extract required to scavenge 50% of the DPPH. Total MAAs = total content of mycosporine-like amino acids, shinorine, palythine, asterina-330 and palythiol are expressed as of total MAAs

	SPs (mg g^{-1} DW)	C (mg g^{-1} DW)	N (mg g^{-1} DW)	Phenolics (mg g^{-1} DW)	EC_{50} (mg DW ml^{-1})	Total MAAs (mg g^{-1} DW)	Shinorine (%)	Palythine (%)	Asterina (%)	Palythiol (%)
<i>C. tamariscifolia</i>	8.85 ± 3.11	309.33 ± 2.21	14.16 ± 2.28	23.20 ± 1.15	0.64 ± 0.19	–	–	–	–	–
<i>U. rigida</i>	10.13 ± 0.62	236.93 ± 4.42	17.82 ± 0.62	6.48 ± 1.01	–	–	–	–	–	–
<i>E. elongata</i>	3.85 ± 0.4	153.90 ± 0.85	7.16 ± 0.49	–	–	0.23 ± 0.04	57.3 ± 3.21	24.76 ± 0.75	2.70 ± 0.10	15.25 ± 2.53

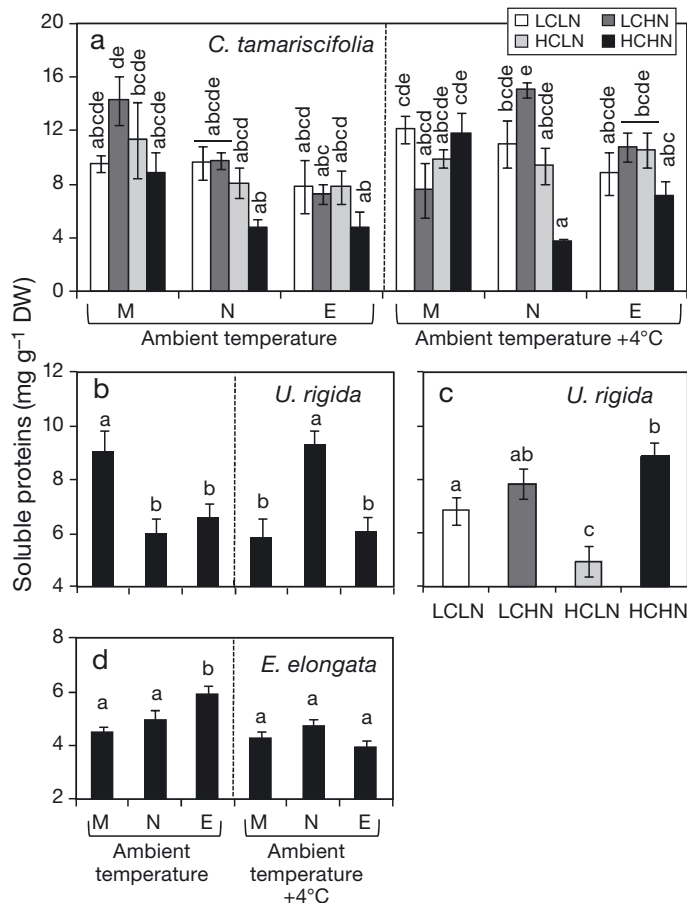


Fig. 1. Soluble protein content of (a) *Cystoseira tamariscifolia*, (b,c) *Ulva rigida*, and (d) *Ellisolandia elongata* exposed to combined conditions of C (high carbon, HC: air enriched with CO_2 at 700 ppm; low carbon, LC: air without CO_2 addition, i.e. 390 ppm), N (high nutrient enrichment, HN: 50 μM nitrate; low nutrient enrichment, LN: 5 μM), and temperature (first 6 d at ambient temperature followed by 3 d at an increase of 4°C). Measurements were conducted with samples collected in the morning (M), noon (N) and evening (E) of the experimental period. Data are pooled means $\pm$ SE, in accordance with significant effects obtained by ANOVA: *C. tamariscifolia*, interactive significant effects of C and N concentrations, time, and temperature (a, $n = 6$); *U. rigida*, interactive significant effects of time and temperature (b, $n = 24$) and interactive effects of C and N concentrations (c, $n = 36$); *E. elongata*, interactive effects of time and temperature (d, $n = 24$). Different letters above the histograms indicate significant differences ($p < 0.05$, SNK post hoc test)

In *U. rigida*, phenolic contents were significantly affected by the interactions C $\times$ time, C $\times$ temperature, N $\times$ temperature, time $\times$ temperature and the triple interaction C $\times$ time $\times$ temperature (Table 3), with the lowest values found in the LC treatments in the morning and the LN treatments at noon (Fig. 4). In this species, no antioxidant activity of methanolic extracts was found as EC_{50} values were below the detection limit of the DPPH method.

Table 2. ANOVA results for experiment with *Cystoseira tamariscifolia* after being exposed to combined conditions of C (high carbon, HC; air enriched with CO₂ at 700 ppm; low carbon, LC; air without CO₂ addition, i.e. 390 ppm), N (high nutrient enrichment, HN; 50 µM; low nutrient enrichment, LN; 5 µM), time (morning, noon and evening) and temperature (ambient temperature and ambient temperature +4°C) on the soluble proteins (SPs), internal C and N, phenolic compounds (phenolics) and antioxidant activity (EC₅₀). Results for the fatty acids (FAs) were for the effects of C, N, and temperature only. Significant differences at $p < 0.05$ are shown in **bold**

Source	df	SPs			C			N			Phenolics			EC ₅₀			FAs		
		MS	F	p	MS	F	p	MS	F	p	MS	F	p	MS	F	p	MS	F	p
C (1)	1	159.28	14.58	<0.001	148.93	1.32	0.25	2.99	0.91	0.34	202.55	26.48	<0.001	0.30	12.33	<0.001	0.00	0.11	0.74
N (2)	1	25.52	2.34	0.13	50.94	0.45	0.50	10.79	3.29	0.07	90.34	11.81	<0.001	0.01	0.42	0.52	0.03	0.87	0.35
Time (3)	2	81.12	7.42	<0.001	2182.90	19.38	<0.001	6.34	1.94	0.15	16.72	2.19	0.12	0.03	1.17	0.31	0.00	0.06	0.81
Temperature (4)	1	49.11	4.49	0.04	19.19	0.17	0.68	15.30	4.67	0.03	161.64	21.13	<0.001	0.00	0.06	0.81	0.29	8.13	0.01
(1) × (2)	1	117.03	10.71	<0.001	591.15	5.25	0.02	5.73	1.75	0.19	5.12	0.67	0.42	0.02	0.85	0.36	0.01	0.26	0.61
(1) × (3)	2	69.34	6.35	<0.001	108.28	0.96	0.39	0.21	0.06	0.94	23.23	3.04	0.05	0.36	14.74	<0.001	0.04	1.09	0.30
(1) × (4)	1	0.00	0.00	0.99	36.84	0.33	0.57	1.70	0.52	0.47	68.92	9.01	<0.001	0.19	7.93	0.01	0.04	1.09	0.30
(2) × (3)	2	5.24	0.48	0.62	25.21	0.22	0.80	9.94	3.03	0.05	2.88	0.38	0.69	0.10	4.25	0.02	0.01	0.22	0.64
(2) × (4)	1	0.37	0.03	0.85	14.80	0.13	0.72	22.74	6.94	0.01	1.01	0.13	0.72	0.15	6.34	0.01	0.01	0.22	0.64
(3) × (4)	2	30.25	2.77	0.07	154.45	1.37	0.26	3.74	1.14	0.32	15.93	2.08	0.13	0.29	11.97	<0.001	0.13	3.76	0.06
(1) × (2) × (3)	2	29.31	2.68	0.07	1.78	0.10	0.90	4.45	1.36	0.26	2.56	0.33	0.72	0.31	12.75	<0.001	0.02	0.69	0.41
(1) × (2) × (4)	1	5.04	0.46	0.50	3.23	0.03	0.87	0.14	0.04	0.84	6.16	0.81	0.37	0.02	0.69	0.41	0.02	0.69	0.41
(1) × (3) × (4)	2	27.78	2.54	0.08	19.58	0.17	0.84	0.78	0.24	0.79	10.27	1.34	0.27	0.43	18.01	<0.001	0.15	6.41	<0.001
(2) × (3) × (4)	2	11.38	1.04	0.36	394.65	3.50	0.03	18.38	5.61	<0.001	2.88	0.38	0.69	0.15	6.41	<0.001	0.22	9.12	<0.001
(1) × (2) × (3) × (4)	2	84.11	7.70	<0.001	55.15	0.49	0.61	1.06	0.32	0.72	19.53	2.55	0.08	0.22	9.12	<0.001	0.02	0.04	0.04
Residual	1093	10.93			112.64			3.28			7.65								

Calcification

CaCO₃ content of *E. elongata* was greater than 70% of the total algal DW in all treatments both under ambient and increased temperature. No effect of C and N treatments were found (data not shown).

Total internal C and N

In the 'initial' samples (after 6 d acclimatisation) C content was higher in *C. tamariscifolia* than that in *U. rigida*, followed by *E. elongata* (Table 1). In *C. tamariscifolia*, C content was significantly affected by the interactions of C × N and N × time × temperature (Table 2). The internal C content was significantly higher in the LCHN treatment than in HCHN or LCLN (Fig. 5b). Among the triple interactions, significantly higher values for both temperature treatments during the evening were found for the N treatment (Fig. 5a, Table 2). In *U. rigida*, total internal C did not vary as a function of time. It was only impacted significantly by C and temperature as single factors (Table 3). Under ambient temperature, the C content was 278 mg g⁻¹ DW, and 286 mg g⁻¹ DW under increased temperature. C content increased under the HCHN treatment (Fig. 5c). Finally, in *E. elongata*, C content was significantly affected by time, temperature and the interaction of C × temperature (Table 4); the values were maintained close to 150 mg C g⁻¹ DW (Fig. 5d,e). Under HC, the C content was higher at ambient than at increased temperature (Fig. 5d), and higher in the evening than in the morning or at noon (Fig. 5e).

In *C. tamariscifolia*, internal N content was affected by the interactive effects of N × temperature and N × time × temperature (Table 2). No significant variations in N content throughout the time and treatments were observed, except in the evening under ambient temperature (Fig. 5f). In *U. rigida*, the interaction of C × N had a significant effect on the internal level of N (Table 3). The lowest value was detected at the HCLN treatment followed by LCLN; N levels were similar under LCHN and HCHN (Fig. 5g). Finally, in *E. elongata*, although an interactive effect of C × time was detected by the ANOVA for internal N content, the SNK test did not detect

Table 3. ANOVA results for *Ulva rigida*, details as in Table 2

Source	df	SPs			C			N			Phenolics			FAs		
		MS	F	p	MS	F	p	MS	F	p	MS	F	p	MS	F	p
C (1)	1	7.44	0.84	0.36	7194.92	20.77	<0.001	13.82	4.41	0.04	7.90	10.02	<0.001	0.04	1.25	0.27
N (2)	1	212.70	24.14	<0.001	906.28	2.62	0.11	387.66	123.67	<0.001	0.86	1.09	0.30	0.00	0.10	0.75
Time (3)	2	24.14	2.74	0.07	51.84	0.15	0.86	2.14	0.68	0.51	4.05	5.14	0.01			
Temperature (4)	1	0.83	0.09	0.76	1897.94	5.48	0.02	6.98	2.23	0.14	9.86	12.51	<0.001	0.25	7.02	0.01
(1) × (2)	1	74.23	8.42	<0.001	2235.46	6.45	0.01	45.24	14.43	<0.001	0.37	0.46	0.50	0.00	0.06	0.80
(1) × (3)	2	10.38	1.18	0.31	180.87	0.52	0.59	3.74	1.19	0.31	6.62	8.40	<0.001			
(1) × (4)	1	9.31	1.06	0.31	13.20	0.04	0.85	0.09	0.03	0.87	13.66	17.32	<0.001	0.03	0.82	0.37
(2) × (3)	2	9.57	1.09	0.34	854.50	2.47	0.09	2.06	0.66	0.52	1.09	1.38	0.26			
(2) × (4)	1	3.44	0.39	0.53	47.86	0.14	0.71	3.22	1.03	0.31	3.61	4.58	0.04	0.01	0.16	0.69
(3) × (4)	2	127.67	14.49	<0.001	890.59	2.57	0.08	3.88	1.24	0.30	29.46	37.36	<0.001			
(1) × (2) × (3)	2	11.17	1.27	0.29	178.01	0.51	0.60	7.29	2.33	0.10	0.94	1.19	0.31			
(1) × (2) × (4)	1	20.68	2.35	0.13	6.95	0.02	0.89	1.79	0.57	0.45	0.05	0.06	0.80	0.04	1.04	0.31
(1) × (3) × (4)	2	13.04	1.48	0.23	11.85	0.03	0.97	0.33	0.11	0.90	8.49	10.77	<0.001			
(2) × (3) × (4)	2	5.76	0.65	0.52	411.68	1.19	0.31	1.61	0.52	0.60	0.10	0.13	0.88			
(1) × (2) × (3) × (4)	2	14.56	1.65	0.20	186.46	0.54	0.59	0.72	0.23	0.80	0.19	0.25	0.78			
Residual		8.81			346.49			3.13			0.79			0.04		

the differences among data (Table 4); all N data were around 7 mg N g⁻¹ DW (Fig. 5h).

Relationships among the variables (Pearson correlation)

SP content was positively related with phenolic content in both *C. tamariscifolia* and *U. rigida*, and with antioxidant activity in *C. tamariscifolia* (Table 6) and N content in *U. rigida* (Table 7). Total C content was related to total N content in *C. tamariscifolia*

(Table 6) and *U. rigida* (Table 7). In *C. tamariscifolia*, phenolic content and antioxidant activity were positively related (Table 6), whereas MAAs in *E. elongata* were negatively correlated to N content (Table 8); however when each MAA is analyzed separately, a negative correlation with N was found for palythine. A positive correlation for shinorine and asterina-330 with N was also found (Table 8). Total FA content was positively related to phenolic content only in *C. tamariscifolia* (Table 6). In *E. elongata*, total FA content was positively related to total SP and C contents (Table 8).

Table 4. ANOVA results for *Ellisolandia elongata*, details as in Table 2

Source	df	SPs			C			N			FAs		
		MS	F	p	MS	F	p	MS	F	p	MS	F	p
C (1)	1	5.93	3.45	0.07	5.07	0.17	0.68	0.08	0.09	0.77	0.01	2.13	0.15
N (2)	1	6.76	3.93	0.05	32.18	1.08	0.30	1.89	2.11	0.15	0.00	0.35	0.56
Time (3)	2	4.34	2.53	0.08	1785.58	59.70	<0.001	0.67	0.75	0.47			
Temperature (4)	1	24.03	13.99	<0.001	139.40	4.66	0.03	2.12	2.37	0.13	0.29	47.81	<0.001
(1) × (2)	1	0.53	0.31	0.58	50.64	1.69	0.20	0.73	0.81	0.37	0.01	1.67	0.20
(1) × (3)	2	3.69	2.15	0.12	48.62	1.63	0.20	5.00	5.57	0.01			
(1) × (4)	1	1.23	0.72	0.40	124.16	4.15	0.04	1.31	1.46	0.23	0.00	0.02	0.90
(2) × (3)	2	1.25	0.73	0.49	75.38	2.52	0.09	0.59	0.66	0.52			
(2) × (4)	1	1.77	1.03	0.31	66.98	2.24	0.14	0.05	0.05	0.82	0.01	1.17	0.28
(3) × (4)	2	12.31	7.17	<0.001	6.68	0.22	0.80	0.56	0.62	0.54			
(1) × (2) × (3)	2	1.05	0.61	0.55	18.42	0.62	0.54	1.05	1.17	0.32			
(1) × (2) × (4)	1	1.74	1.01	0.32	3.26	0.11	0.74	0.39	0.44	0.51	0.00	0.34	0.56
(1) × (3) × (4)	2	1.67	0.97	0.38	5.98	0.20	0.82	0.74	0.83	0.44			
(2) × (3) × (4)	2	0.07	0.04	0.96	11.75	0.39	0.68	0.87	0.97	0.38			
(1) × (2) × (3) × (4)	2	1.33	0.77	0.46	43.78	1.46	0.24	1.86	2.07	0.13			
Residual		1.72			29.91			0.90			0.01		

Table 5. ANOVA results for *Ellisolandia elongata* (for details see Table 2), testing for effects on total mycosporine-like amino acids (MAAs), % shinorine, % asterina, % palythine and % palythanol

Source	df	Total MAAs			% Shinorine			% Asterina			% Palythine			% Palythanol		
		MS	F	p	MS	F	p	MS	F	p	MS	F	p	MS	F	p
C (1)	1	0.01	2.96	0.09	1002.80	6.76	0.01	18.32	26.53	<0.001	1808.40	20.67	<0.001	201.33	3.07	0.08
N (2)	1	0.00	0.01	0.92	133.36	0.90	0.35	0.29	0.41	0.52	75.74	0.87	0.36	1.85	0.03	0.87
Time (3)	2	0.00	0.66	0.52	828.71	5.59	0.01	2.38	3.45	0.04	415.05	4.74	0.01	754.90	11.50	<0.001
Temperature (4)	1	0.00	0.22	0.64	0.01	0.00	0.99	0.55	0.80	0.37	197.62	2.26	0.14	194.99	2.97	0.09
(1) × (2)	1	0.00	0.01	0.94	2.12	0.01	0.91	0.72	1.04	0.31	54.34	0.62	0.43	49.38	0.75	0.39
(1) × (3)	2	0.01	1.52	0.23	653.58	4.41	0.02	2.88	4.17	0.02	324.50	3.71	0.03	51.98	0.79	0.46
(1) × (4)	1	0.00	1.13	0.29	132.35	0.89	0.35	9.81	14.20	<0.001	419.20	4.79	0.03	124.38	1.89	0.17
(2) × (3)	2	0.00	0.74	0.48	1511.60	10.19	<0.001	10.63	15.39	<0.001	1631.99	18.65	<0.001	54.08	0.82	0.44
(2) × (4)	1	0.01	4.09	0.05	128.52	0.87	0.36	6.35	9.20	<0.001	1450.05	16.57	<0.001	801.67	12.21	<0.001
(3) × (4)	2	0.02	5.52	0.01	683.38	4.61	0.01	10.36	15.00	<0.001	1454.15	16.62	<0.001	202.28	3.08	0.05
(1) × (2) × (3)	2	0.00	1.06	0.35	670.92	4.52	0.01	6.42	9.30	<0.001	1141.30	13.05	<0.001	96.26	1.47	0.24
(1) × (2) × (4)	1	0.00	0.05	0.83	512.66	3.46	0.07	6.46	9.36	<0.001	1383.90	15.82	<0.001	260.86	3.97	0.05
(1) × (3) × (4)	2	0.02	6.36	<0.001	586.23	3.95	0.02	3.45	5.00	0.01	984.94	11.26	<0.001	182.48	2.78	0.07
(2) × (3) × (4)	2	0.00	1.06	0.35	210.27	1.42	0.25	2.55	3.69	0.03	200.89	2.30	0.11	28.78	0.44	0.65
(1) × (2) × (3) × (4)	2	0.00	0.39	0.68	358.12	2.41	0.10	1.59	2.30	0.11	265.77	3.04	0.05	36.85	0.56	0.57
Residual		0.00			148.35			0.69			87.49			65.64		

DISCUSSION

In this study, the results of short-term effects of $p\text{CO}_2$, nitrate and temperature increases on the physiology of 3 abundant eulittoral Mediterranean macroalgae with different morphological, bio-optical and physiological characteristics (Mercado et al. 1998, Figuerola et al. 2003a, 2014b, Abdala-Díaz et al. 2006) are presented. Combined effects of CO_2 (LC and HC) and nitrogen (LN and HN nitrate concentration through morning pulses) under 2 temperature conditions were tested (6 d at ambient temperature followed by 3 d after a 4°C increase). Most studies on the effects of global climate change on aquatic organisms have been conducted with 1 or 2 experimental variables, and studies on the interaction of multiple factors are very scarce (Gao & Zheng 2010, Cornwall et al. 2012, Koch et al. 2013, Yildiz et al. 2013).

N levels and the interaction C × N in *Ulva rigida* had significant effects on protein concentration, as previously reported (Gordillo et al. 2001, 2003). However, in *Cystoseira tamariscifolia*, N levels did not affect the protein level in contrast to C, temperature and the interaction of C × N, whereas in *Ellisolandia elongata*, only N and temperature as single factors influenced the protein level. Burkhardt & Riebesell (1997) observed a decrease in the C:N ratio at high CO_2 in different phytoplankton species. However, this ratio increased in the macroalgae *U. rigida* and *Pyropia (Porphyra) leucostica* grown under high CO_2 (Mercado et al. 1999, Gordillo et al. 2001). Algae grown at a high inorganic C concentration (5 % CO_2)

displayed a higher soluble carbohydrate concentration and maximum photosynthesis rate but had a lower photosynthetic affinity for inorganic C, and lower phycobiliprotein and Rubisco contents than those algae cultured at low inorganic C levels (air CO_2) (Andría et al. 1999). Inorganic C enrichment also affected N uptake and assimilation in *Gracilaria* sp., causing a decrease in N uptake rate even under N-replete conditions (Andría et al. 1999).

In *C. tamariscifolia*, the temperature increase produced a decrease in total FAs and phenolic com-

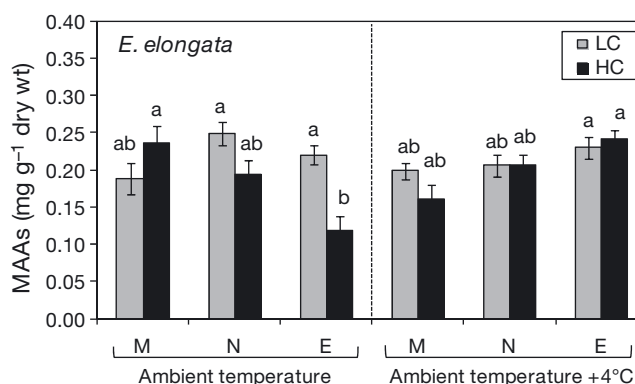


Fig. 2. Total mycosporine-like amino acid (MAA) content of *Ellisolandia elongata* exposed to combined conditions of C (LC and HC), N (HN and LN), and temperature (see Fig. 1 for description of experimental conditions). Measurements were conducted with samples collected in the morning (M), noon (N) and evening (E). Data are pooled means $\pm$ SE, in accordance with significant effects obtained by ANOVA, considering interactive significant effects of carbon, time and temperature ($n = 12$). Different letters above the histograms indicate significant differences ($p < 0.05$, SNK post hoc test)

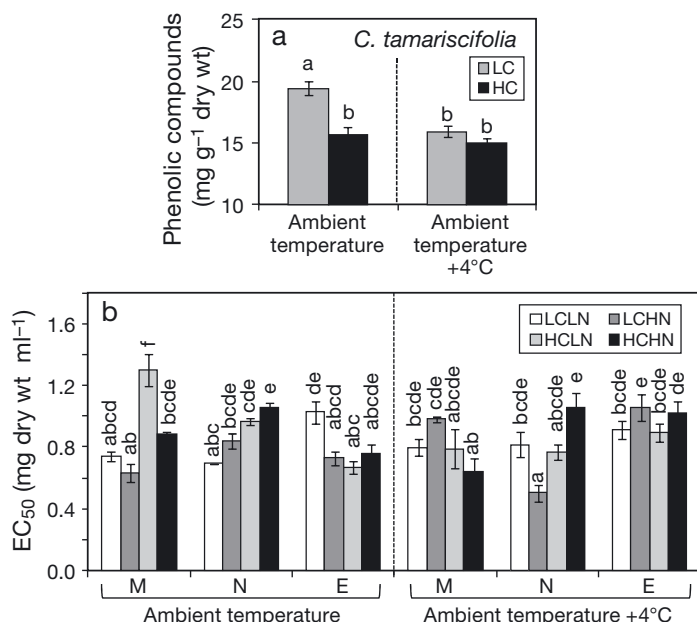


Fig. 3. (a) Total phenolic compounds and (b) oxidation index (EC_{50}) of *Cystoseira tamariscifolia* exposed to combined conditions of C (HC and LC), N (HN and LN), and temperature (see Fig. 1 for description of experimental conditions). Measurements were conducted with samples collected in the morning (M), noon (N) and evening (E) of experimental period. Data are pooled means $\pm$ SE, in accordance with significant effects obtained by ANOVA: phenolic compounds, interactive significant effects of carbon concentrations, and temperature (a, $n = 36$); EC_{50} , interactive significant effects of C and N concentrations, time, and temperature (b, $n = 6$). Different letters above the histograms indicate significant differences ($p < 0.05$, SNK post hoc test)

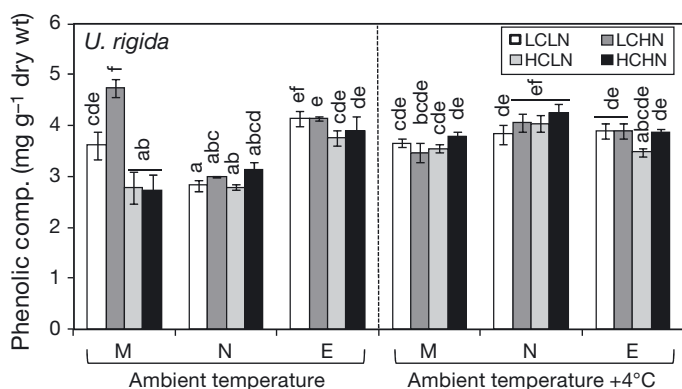


Fig. 4. Total phenolic compounds of *Ulva rigida* exposed to combined conditions of C (HC and LC), N (HN and LN), and temperature (see Fig. 1 for description of experimental conditions). Measurements were conducted with samples collected in the morning (M), noon (N) and evening (E) of experimental period. Data are pooled means $\pm$ SE, in accordance with significant effects obtained by ANOVA, with interactive significant effects of C concentrations, time and temperature ($n = 3$). Different letters above the histograms indicate significant differences ($p < 0.05$, SNK post hoc test)

pound levels and an increase in protein content (at noon and under HN). In *E. elongata*, a decrease in total FAs and SPs (in the evening) was observed. In contrast, in *U. rigida*, total FAs, SPs and phenolic compounds (at noon) increased under ambient + 4°C temperature. Thus, in *U. rigida* the internal biochemical compounds seem to be accumulated under stress, as has been previously shown under different stress conditions in algae (Abdala-Díaz et al. 2006, Stengel et al. 2011, Sharma et al. 2012). The effect of increased temperature in this study took into account the day/light fluctuations including the decrease in temperature during the night compared to the day period. Temperature is a major factor controlling the rate of photosynthesis in algae (Davison 1991). Temperature affects the availability of inorganic C (Surif & Raven 1989) and the rate of C fixation by Rubisco (Suklenik et al. 1987). Several primary sites of temperature sensitivity have been proposed to initiate the process of photoinhibition, including damage to PSII, in particular degradation of D1 protein (Warner et al. 1999), disintegration of the thylakoid membrane (Tchernov et al. 2004), generation of reactive oxygen species concomitant to photooxidative damage to the photosynthetic apparatus (Lesser 2006), and impairment of the Calvin-Benson cycle (Jones et al. 1998). Overall, the mechanisms of thermal tolerance are not well understood in macrophytes, but studies indicate comparable responses to heat stress amongst temperate marine macrophytes and terrestrial plants.

The different responses amongst the species to C, N and temperature treatments could be explained by the different bio-optical pattern: i.e. *C. tamariscifolia* and *E. elongata* have a very high absorbance (0.8 or 0.9) due to their thickness and complex morphology and structure, whereas the absorbance of *U. rigida* thalli, with just 2 cell layers, ranges from 0.40 to 0.65 (Salles et al. 1996). In addition, the reflectance is high in *E. elongata* (15 to 20%). In this study, the effect of increased CO₂ and temperature under different N supply was very different in thin and laminar species (*U. rigida*) compared to the shrubby macroalga *C. tamariscifolia*, or the jointed calcareous alga *E. elongata*. Additionally, different biochemical constituents were affected to different degrees by the 4 treatments. Biochemical composition of *C. tamariscifolia* responded (directly or in interaction) mainly to temperature for nearly all variables; effects of CO₂ increase were also detected, whereas composition was hardly affected by nutrient supply. In contrast, the biochemical composition of *U. rigida* was affected by increases in temperature, CO₂ and nutrient supply

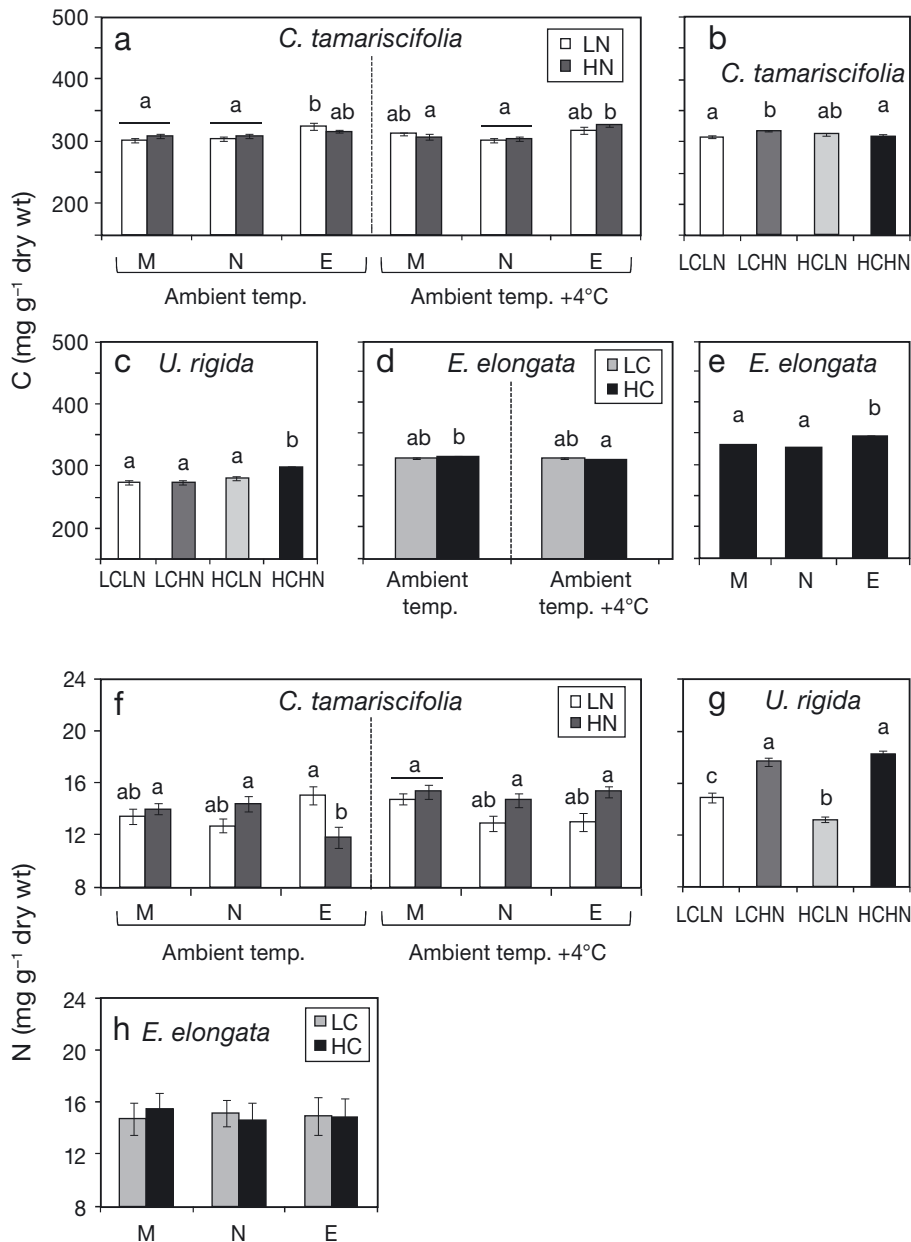


Fig. 5. Total internal (a–e) carbon content and (f–h) nitrogen content of (a,b,f) *Cystoseira tamariscifolia*, (c,g) *Ulva rigida*, and (d,e,h) *Ellisolandia elongata* exposed to combined conditions of C (HC and LC), N (HN and LN), and temperature (see Fig. 1 for description of experimental conditions). Measurements were conducted with samples collected in the morning (M), noon (N) and evening (E) of experimental period. Data are pooled means $\pm$ SE, in accordance with significant effects obtained by ANOVA. Carbon content: *C. tamariscifolia*, interactive significant effects of C and N concentrations, time, and temperature (a, $n = 12$), and interactive effects of C and N concentrations (b, $n = 30$); *U. rigida*, interactive significant effects of C and N concentrations (c, $n = 30$); *E. elongata*, interactive effects of C concentrations and temperature (d, $n = 30$), and isolated effect of time (e, $n = 60$). N content: *C. tamariscifolia*, interactive significant effects of N concentrations, time, and temperature (f, $n = 12$); *U. rigida*, interactive significant effects of C and N concentrations (g, $n = 30$); *E. elongata*, interactive effects of C concentrations and time (h, $n = 24$). Different letters above the histograms indicate significant differences ($p < 0.05$, SNK post hoc test)

and their interactions, although the responses were different for each variable. In addition, the light climate in the tanks depended on the positioning of the species. *U. rigida*, with its freely moving thalli in the tank's water flow, presented more variable canopy episodes than *C. tamariscifolia* (fixed position) or than *E. elongata*, which was located at the bottom (see Stengel et al. 2014). Thus, in addition to the bio-optical characteristic of the thalli, the canopy effect in the tanks must be taken into account to explain light absorption. Field studies on mats and canopies confirm this conclusion; for example, Malta et al. (2003) reported strong vertical heterogeneity in physiological characteristics of *Ulva* sp. organized in mats. Bischof et al. (2006b) also showed the photoacclimation within mats of the filamentous green macroalga *Chaetomorpha linum*, presenting much less photoinhibition than the first layers of thalli with high light exposure. Falkenberg et al. (2013) reported that turf algae responded to enrichment of CO₂ and nutrients, whereas canopy-forming species responded only to nutrient enrichment; they concluded that the identification of how these conditions modify resource availability may help predict to what extent such major ecosystem components and the communities they support may adapt to future conditions.

According to morphology, thickness and reflectance, *U. rigida* may be expected to be more vulnerable to high solar irradiance than *C. tamariscifolia* and *E. elongata*. Algal phenolic compounds are photoprotective due to their UV-screen and antioxidant capacities (Connan et al. 2006). In this study, *C. tamariscifolia* not only presented much higher levels of phenolic compounds and antioxidant capacity than *U. rigida*, but also the release of phenolic com-

Table 6. Pearson correlation values between dependent variables in *Cystoseira tamariscifolia*. Values in **bold** show significant relationships ($p < 0.05$). (*) $n = 144$; (**) $n = 120$; (***) $n = 72$. SPs = total soluble proteins; phenolics = phenolic compounds; FAs = fatty acids; EC_{50} = concentration of the methanolic extract required to scavenge 50% of the DPPH

	C**	N**	Phenolics*	EC_{50} *	FAs***
SPs*	-0.018 $p = 0.845$	0.093 $p = 0.312$	0.206 $p = 0.013$	-0.203 $p = 0.015$	-0.062 $p = 0.603$
C**		0.565 $p < 0.001$	0.185 $p = 0.043$	0.032 $p = 0.725$	0.188 $p = 0.114$
N**			-0.023 $p = 0.807$	-0.031 $p = 0.736$	0.122 $p = 0.308$
Phenolics*				-0.211 $p = 0.011$	0.244 $p = 0.039$
EC_{50} *					0.117 $p = 0.330$

Table 7. Pearson correlation values between dependent variables in *Ulva rigida*. Values in **bold** show significant relationships ($p < 0.05$). (*) $n = 144$; (**) $n = 120$; (***) $n = 72$

	C***	N***	Phenolics**	FAs***
SPs*	-0.1699 $p = 0.064$	0.2517 $p = 0.006$	0.2274 $p = 0.018$	-0.0069 $p = 0.958$
C**		0.4015 $p < 0.001$	-0.0695 $p = 0.530$	0.3412 $p = 0.008$
N**			-0.0648 $p = 0.558$	0.0692 $p = 0.599$
Phenolics				-0.0852 $p = 0.621$

Table 8. Pearson correlation values between dependent variables in *Ellisolandia elongata*. Values in **bold** show significant relation ($p < 0.05$). (*) $n = 144$; (**) $n = 120$; (***) $n = 72$. SPs = total soluble proteins; FAs = fatty acids; MAAs = mycosporine-like amino acids

	C**	N**	Total MAAs***	% Palythine***	% Shinorine***	% Asterina***	% Palythanol***	% FAs****
SPs*	0.1659 $p = 0.070$	0.0656 $p = 0.476$	0.0917 $p = 0.374$	-0.2452 $p = 0.016$	0.1025 $p = 0.320$	0.1382 $p = 0.179$	0.2264 $p = 0.027$	0.5334 $p < 0.001$
C**		0.0477 $p = 0.605$	-0.0795 $p = 0.441$	-0.252 $p = 0.013$	0.2383 $p = 0.019$	0.2393 $p = 0.019$	0.0035 $p = 0.973$	0.2483 $p = 0.037$
N**			-0.2446 $p = 0.016$	-0.3915 $p < 0.001$	0.3883 $p < 0.001$	0.3011 $p = 0.003$	-0.0305 $p = 0.768$	-0.1618 $p = 0.178$
Total MAAs***				0.2826 $p = 0.005$	-0.5196 $p < 0.001$	-0.4311 $p < 0.001$	0.4425 $p < 0.001$	0.1318 $p = 0.273$
% Palythine***					-0.8271 $p < 0.001$	-0.8112 $p < 0.001$	-0.1928 $p = 0.060$	0.0638 $p = 0.597$
% Shinorine***						0.8918 $p < 0.001$	-0.3896 $p < 0.001$	0.0141 $p = 0.907$
% Asterina***							-0.2526 $p = 0.013$	0.0054 $p = 0.964$
% Palythanol***								-0.1515 $p = 0.207$

pounds into the seawater in the case of *C. tamariscifolia* considerably reduced the UVR in the experimental tanks (Stengel et al. 2014). In this study, the concentration of phenolic compounds (expressed as phloroglucinol equivalent) in the seawater (experimental vessel) under ambient temperature was higher than under increased temperature. The release of phenolic compounds from *C. tamariscifolia* thalli at noon has been suggested to be a photoprotective mechanism under high irradiance conditions (Abdala-Díaz et al. 2006, Celis-Plá et al. 2014). In the green macroalga *Dasycladus vermicularis*, phenolic compounds (trihydroxycoumarins) with antioxidant capacity are released under stressful conditions, reducing photoinhibition and increasing the recovery capacity of the photosynthetic yield and productivity (Pérez-Rodríguez et al. 1998, 2001, 2003). Thus, the combination of thallus thickness, phenolic compounds (and their positive response to temperature increase), antioxidant activity and the reduction in UV penetration by the release of phenolic compounds might give *C. tamariscifolia* an advantage over *U. rigida* in high light environments that could even result in a higher production of the first species, especially with increasing temperatures. An indication of the relationship between phenolic compounds and the good physiological status of *C. tamariscifolia* and *U. rigida* is the linear and positive relationship of their phenolic content with the SP content. The increase in total FA content was also related to phenolic content only in *C. tamariscifolia*. These results indicate a link between primary metabolism, i.e. protein or lipid accumulation,

and secondary metabolism, i.e. accumulation of phenolic compounds.

E. elongata also contains UV-absorbing compounds involved in photoprotection, i.e. MAAs. C and N internal contents were positively related to some MAAs (i.e. shinorine and asterina-330) but negatively to palythine, indicating that the C and N enrichment favored the accumulation of only some MAAs. This can be advantageous under stressful conditions, since asterina-330 presented a high antioxidant activity against hydrosoluble radicals (ABTS assay) and lipid peroxidation (β -carotene oxidation assay) (De la Coba et al. 2009). N enrichment increases the accumulation of MAAs in different species of red macroalgae of the genus *Porphyra* (*Pyropia*) (Korbee-Peinado et al. 2004, Korbee et al. 2005), *Gracilaria* (Zheng & Gao 2009, Figuerola et al. 2010) and *Grateloupia* (Huovinen et al. 2006). In spite of the fact that MAAs are N-containing compounds, no significant effect of N as a single factor was found for the accumulation of MAAs, except for the double interaction of N $\times$ time for all MAAs except palythanol, and the double interaction N $\times$ temperature for all MAAs except shinorine. MAAs are synthesized from mycoporine-glycine and they are inter-converted between them under the influence of light quality and N availability (Carreto & Carignan 2011). The accumulation of photoprotectors as MAAs and phenolic compounds and the high antioxidant capacity of methanolic extracts are useful indicators of the capacity of the algae to acclimate to increased UV-B radiation or other stressful conditions (Pérez-Rodríguez et al. 2003, Connan et al. 2004, Abdala-Díaz et al. 2006, Carreto & Carignan 2011, García-Sánchez et al. 2012, Figuerola et al. 2014b).

In conclusion, the 3 algal species investigated here displayed high biochemical plasticity in response to changes in nutrient concentration and temperature. Responses were species-specific and could, in part, be related to differences in their bio-optical properties and ecological strategies. Links between primary (productivity, protein production) and secondary metabolisms (antioxidant production) were established for *Ulva* and *Cystoseira*.

Acknowledgements. The financial contributions for the GAP 9 workshop 'Influence of the pulsed-supply of nitrogen on primary productivity in phytoplankton and marine macrophytes: an experimental approach' by Walz GmbH facilitated the use of several PAM fluorometers. Redox, University of Málaga, Ministry of Economy and Competitiveness of Spanish Government (Acción Complementaria CTM2011-15659-E), the Project Interacid (RNM5750) of Junta de Andalucía and Spanish Institute of Oceanography are grate-

fully acknowledged. U.N. gratefully acknowledges financial support from the Marine Institute and the Marine Research Sub-programme of the (Irish) National Development Plan 2007-2013. K.B. acknowledges funding by the German Ministry for Research and Education (BMBF) in the frame of the BIOACID Project. E.M. acknowledges funding by the project 'The Salty Gold' SIA Foundation, The Netherlands. M.A. acknowledges funding from Junta de Andalucía (P09-RNM-5187). The economical contribution for the biochemical analyses by the authors F.C., C.A.G.N., D.R. (CONACYT CB-86933.Z), D.B.S. and E.J.M. is thankfully acknowledged. C.J. was supported by the Ministry of Science and Innovation of Spain (Grant CTM2011 24007). F.A. was funded by FCT-Portugal, project Physiography (PTDC/MAR/105147/2008), co-funded by FEDER through the program Compete-Qren. T.K. was supported by US NSF grant DBI-1103652. P.S.M.C.P. gratefully acknowledges financial support 'Becas-Chile' (CONICYT) from the Ministry of Education of the Republic of Chile.

LITERATURE CITED

- Abdala-Díaz RT, Cabello-Pasini A, Pérez-Rodríguez E, Conde-Álvarez RM, Figuerola FL (2006) Daily and seasonal variations of optimum quantum yield and phenolic compounds in *Cystoseira tamariscifolia* (Phaeophyta). *Mar Biol* 148:459–465
 - Andría J, Vergara JJ, Pérez-Lloréns JL (1999) Biochemical responses and photosynthetic performance of *Gracilaria* sp. (Rhodophyta) from Cádiz, Spain, cultured under different inorganic and nitrogen levels. *Eur J Phycol* 34: 497–504
 - Baulch HM, Nord TW, Ackerman MY, Dale JD, Hazewinkel RRO, Schindler D, Vinebrooke RD (2003) Climate warning experiments: design of a mesocosm heating system. *Limnol Oceanogr Methods* 1:10–15
 - Bermejo R, de la Fuente G, Vergara JJ, Hernández I (2013) Application of the CARLIT index along a biogeographical gradient in the Alboran Sea (European Coast). *Mar Pollut Bull* 72:107–118
 - Bischof K, Gómez I, Molis M, Hanelt D and others (2006a) Ultraviolet radiation shapes seaweed communities. *Rev Environ Sci Biotechnol* 5:141–166
 - Bischof K, Rautenberg R, Brey L, Pérez-Lloréns JL (2006b) Physiological acclimation along gradients of solar irradiance within mats of the filamentous green macroalga *Chaetomorpha linum* from southern Spain. *Mar Ecol Prog Ser* 306:165–175
 - Blois MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature* 181:1199–1200
 - Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254
 - Brewer PG (1997) Ocean chemistry of the fossil fuel CO₂ signal: the haline signal of 'business as usual'. *Geophys Res Lett* 24:1367–1369
 - Büdenbender J, Riebesell U, Form A (2011) Calcification of the Arctic coralline red algae *Lithothamnion glaciale* in response to elevated CO₂. *Mar Ecol Prog Ser* 441:79–87
 - Burkhardt S, Riebesell U (1997) CO₂ availability affects elemental composition (C:N:P) of the marine diatom *Skeletonema costatum*. *Mar Ecol Prog Ser* 155:67–76
- Cabello-Pasini A, Macías-Carranza V, Abdala-Díaz R, Kor-

- bee N, Figueroa FL (2011) Effect of nitrate concentration and UVR on photosynthesis, respiration, nitrate reductase activity and phenolic compounds in *Ulva rigida* (Chlorophyta). J Appl Phycol 23:363–369
- Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425:365
- Carreto JI, Carignan M (2011) Mycosporine-like amino acids: relevant secondary metabolites. Chemical and ecological aspects. Mar Drugs 9:387–446
- Celis-Plá PSM, Korbee N, Gómez-Garreta A, Figueroa FL (2014) Seasonal photoacclimation patterns in the intertidal macroalga *Cystoseira tamariscifolia* (Ochrophyta). Sci Mar 7:377–388
- Connan S, Goulard F, Stiger V, Deslandes E, Ar Gall E (2004) Interspecific and temporal variation in phlorotannin levels in an assemblage of brown algae. Bot Mar 47: 410–416
- Connan S, Delisle F, Deslandes E, Ar Gall E (2006) Intrathallus phlorotannin content and antioxidant activity in Phaeophyceae of temperate waters. Bot Mar 49:39–46
- Cornwall CE, Hepburn CD, Pritchard D, Currie KI, McGraw CM, Hunter KA, Hurd CL (2012) Carbon-use strategies in macroalgae: differential responses to lowered pH and implications for ocean acidification. J Phycol 48:137–144
- Davison IR (1991) Environmental effects on algal photosynthesis: temperature. J Phycol 27:2–8
- De la Coba F, Aguilera J, Figueroa F, de Gálvez MV, Herrera E (2009) Antioxidant activity of mycosporine-like amino acids isolated from three red macroalgae and one marine lichen. J Appl Phycol 21:161–169
- Diffenbaugh S, Pal JS, Giorgi F, Gao X (2007) Heat stress intensification in the Mediterranean climate change hotspot. Geophys Res Lett 34:L11706, doi:10.1029/2007 GL030000
- Falkenberg LJ, Rusell BD, Connell SD (2013) Contrasting resource limitations of marine primary producers: implications for competitive interactions under enriched CO₂ and nutrient regimes. Oecologia 172:575–583
- Figueroa FL, Gómez I (2001) Photoacclimation to solar UV radiation in red macroalgae. J Appl Phycol 13:235–248
- Figueroa FL, Korbee N (2010) Interactive effects of UV radiation and nutrients on ecophysiology: vulnerability and adaptation to climate change. In: Israel A, Einav R, Seckbach J (eds) Seaweeds and their role in globally changing environments. Springer, Dordrecht, p 157–182
- Figueroa FL, Viñegla B (2001) Effects of solar UV radiation on photosynthesis and enzyme activities (carbonic anhydrase and nitrate reductase) in marine macroalgae from southern Spain. Rev Chil Hist Nat 74:237–249
- Figueroa FL, Conde-Álvarez R, Gómez I (2003a) Relations between electron transport rates determined by pulse amplitude modulated chlorophyll fluorescence and oxygen evolution in macroalgae under different light conditions. Photosynth Res 75:259–275
- Figueroa FL, Nygard C, Ekelund N, Gómez I (2003b) Photobiological characteristics and photosynthetic UV responses in two *Ulva* species (Chlorophyta) from southern Spain. J Photochem Photobiol B Biol 72:35–44
- Figueroa FL, Israel A, Neori A, Martínez B and others (2010) Effect of nutrient supply on photosynthesis and pigmentation to short-term stress (UV radiation) in *Gracilaria conferta* (Rhodophyta). Mar Pollut Bull 60:1768–1778
- Figueroa FL, Conde-Álvarez R, Bonomi Barufi J, Celis-Plá PSM and others (2014a) Continuous monitoring of *in vivo* chlorophyll *a* fluorescence in *Ulva rigida* (Chlorophyta) submitted to different CO₂, nutrient and temperature regimes. Aquat Biol 22:195–212
- Figueroa FL, Domínguez-González B, Korbee N (2014b) Vulnerability and acclimation to increased UVB in the three intertidal macroalgae of different morpho-functional groups. Mar Environ Res 97:30–38
- Flores-Moya A, Gómez I, Viñegla B, Altamirano M and others (1998) Effects of solar radiation on the endemic Mediterranean red alga *Rissoella verrucosa*: photosynthetic performance, pigment content and the activities of enzymes related to nutrient uptake. New Phytol 139:673–683
- Folin O, Ciocalteu V (1927) On tyrosine and tryptophane determinations in proteins. J Biol Chem 73:627–650
- Franklin LA, Foster RM (1997) The changing irradiance environment: consequences for marine macrophytes physiology, productivity and ecology. Eur J Phycol 32:207–232
- Gao K, Zheng Y (2010) Combined effects of ocean acidification and solar UV radiation on photosynthesis, growth, pigmentation and calcification in the coralline alga *Corallina sessilis* (Rhodophyta). Glob Change Biol 16: 2388–2398
- Gao K, Aruga Y, Asada K, Ishihara T, Akano T, Kiyohara M (1991) Enhanced growth of the red alga *Porphyra yezoensis* Ueda in high CO₂ concentrations. J Appl Phycol 3:355–362
- Gao K, Aruga Y, Asada K, Ishihara T, Akano T, Kiyohara M (1993) Calcification in the articulated coralline alga *Corallina pilulifera* with special reference to the effect of elevated CO₂ concentration. Mar Biol 117:129–132
- García-Sánchez M, Korbee N, Pérez-Ruzafa IM, Marcos C, Domínguez B, Figueroa FL, Pérez-Ruzafa A (2012) Physiological response and photoacclimation capacity of *Caulerpa prolifera* (Forsskål) J.V. Lamouroux and *Cymodocea nodosa* (Ucria) Ascherson meadows in the Mar Menor lagoon (SE Spain). Mar Environ Res 79:37–47
- Gómez I, Figueroa FL (1998) Effects of solar UV stress on chlorophyll fluorescence kinetics of intertidal macroalgae from southern Spain: a case study in *Gelidium* species. J Appl Phycol 10:285–294
- Gordillo FJL, Niell FX, Figueroa FL (2001) Non-photosynthetic enhancement of growth by high CO₂ level in the nitrophilic seaweed *Ulva rigida* C. Agardh (Chlorophyta). Planta 213:64–70
- Gordillo FJL, Figueroa F, Niell FX (2003) Photon and carbon use efficiency in *Ulva rigida* at different CO₂ and N levels. Planta 218:315–322
- Graham MH, Kinlan BP, Druehl LD, Garske LE, Banks S (2007) Deep-water refugia as potential hotspots of tropical marine diversity and productivity. Proc Natl Acad Sci USA 104:16576–16580
- Häder DP, Figueroa FL (1997) Photoecophysiology of marine macroalgae. Photochem Photobiol 66:1–14
- Häder DP, Kumar HD, Smith RC, Worest RC (2007) Effect of solar UV radiation on aquatic ecosystems and interactions with climate change. Photochem Photobiol Sci 6: 267–285
- Halpern BS, Walbridge S, Selkoe KA, Kappel CV and others (2008) A global map of human impact on marine ecosystems. Science 319:948–952
- Hanelt D (1998) Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. Mar Biol 131:361–369
- Hanelt D, Wiencke C, Nultsch W (1997a) Influence of UV radiation on photosynthesis of Arctic macroalgae in the

- field. *J Photochem Photobiol B Biol* 38:40–47
- Hanelt D, Wiencke C, Karsten U, Nultsch W (1997b) Photo-inhibition and recovery after high light stress in different developmental and life-history stages of *Laminaria saccharina* (Phaeophyta). *J Phycol* 33:387–395
 - Hind KR, Saunders GW (2013) A molecular phylogenetic study of the tribe Corallineae (Corallinales, Rhodophyta) with the assessment of the genus-level taxonomic features and description of novel genera. *J Phycol* 49: 103–114
 - Hofmann LC, Straub S, Bischof K (2013) Elevated CO₂ levels affect the activity of nitrate reductase and carbonic anhydrase in the calcifying rhodophyte *Corallina officinalis*. *J Exp Bot* 64:899–908
 - Hoppe HG, Breithaupt P, Walther K, Koppe R, Bleck S, Sommer U, Jürgens K (2008) Climate warming in winter affects the coupling between phytoplankton and bacteria during the spring bloom: a mesocosm study. *Aquat Microb Ecol* 51:105–115
 - Huovinen P, Matos J, Sousa-Pinto I, Figuerola FL (2006) The role of nitrogen in photoprotection against high irradiance in the Mediterranean red alga *Grateloupia lanceola*. *Aquat Bot* 84:308–316
 - IPCC (2013) Ocean acidification. In: Stocker TF, Qin D, Plattner GK, Tignor M and others (eds) *Climate Change 2013: The physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, p 37–41
 - Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impairment of the CO₂ fixation mechanism in zooxanthellae. *Plant Cell Environ* 21:1219–1230
 - Koch M, Bowes G, Ross C, Zhang XH (2013) Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Glob Change Biol* 19:103–132
 - Korbee N, Huovinen P, Figuerola FL, Aguilera J, Karsten U (2005) Availability of ammonium influences photosynthesis and the accumulation of mycosporine-like amino acids in two *Porphyra* species (Bangiales, Rhodophyta). *Mar Biol* 146:645–654
 - Korbee-Peinado N, Abdala-Díaz R, Figuerola FL, Helbling EW (2004) Ammonium and UVR stimulate the accumulation of mycosporine-like amino acids (MAAs) in *Porphyra columbina* (Rhodophyta) from Patagonia, Argentina. *J Phycol* 40:248–259
 - Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol Lett* 13:1419–1434
 - Kübler JE, Johnston AM, Raven JA (1999) The effects of reduced and elevated CO₂ and O₂ on the seaweed *Lomentaria articulata*. *Plant Cell Environ* 22:1303–1310
 - Lesser MP (2006) Oxidative stress in marine environments: biochemistry and physiological ecology. *Annu Rev Physiol* 68:253–278
 - Littler MM, Littler DS, Taylor PR (1983) Evolutionary strategies in a tropical barrier reef system: functional form groups of marine macroalgae. *J Phycol* 19:229–237
 - Louanchi F, Boudjakdji M, Nacef N (2009) Decadal changes in surface carbon dioxide and related variables in the Mediterranean Sea as inferred from a coupled data diagnostic model approach. *ICES J Mar Sci* 66:1538–1546
 - Malta EJ, Rijstenbil JW, Brouwer PEM, Kromkamp J (2003) Vertical heterogeneity in physiological characteristics of *Ulva* spp. mats. *Mar Biol* 143:1029–1038
 - Martin S, Gattuso JP (2009) Response of Mediterranean coralline algae to ocean acidification and elevated temperature. *Glob Change Biol* 15:2089–2100
 - Martínez B, Arenas F, Rubal M, Burgués S and others (2012) Physical factors driving intertidal macroalgae distribution: physiological stress of a dominant furoid at its southern limit. *Oecologia* 170:341–353
 - McElhany P, Busch DS (2013) Appropriate pCO₂ treatments in ocean acidification. *Mar Biol* 160:1807–1812
 - Mercado JM, Gordillo FJ (2011) Inorganic carbon acquisition in algal communities: Are the laboratory data relevant to the natural ecosystems? *Photosynth Res* 109: 257–267
 - Mercado JM, Gordillo FJ, Figuerola FL, Niell FX (1998) External carbonic anhydrase and affinity to inorganic carbon in intertidal macroalgae. *J Exp Mar Biol Ecol* 221: 209–220
 - Mercado JM, Gordillo FJL, Niell FX, Figuerola FL (1999) Regulation of photosynthetic quantum yields by inorganic carbon in *Porphyra leucosticta*. *J Appl Phycol* 11: 455–461
 - Mercado JM, Cortés D, Ramírez T, Gómez F (2012) Decadal weakening of the wind-induced upwelling reduces the impact of nutrient pollution in the Bay of Málaga (western Mediterranean Sea). *Hydrobiologia* 680:91–107
 - Mieszkowska N, Kendall MA, Hawkins SJ, Leaper R, Williamson P, Hardman-Mountford NJ, Southward AJ (2006) Changes in the range of some common rocky shore species in Britain—a response to climate change? *Hydrobiologia* 555:241–251
 - Olabarria C, Arenas F, Viejo RM, Gestoso I and others (2013) Response of macroalgal assemblages from rockpools to climate change: effects of persistent increase in temperature and CO₂. *Oikos* 122:1065–1079
 - Orr JC, Fabry VJ, Aumont O, Bopp L and others (2005) Anthropogenic ocean acidification over the twenty-first century and its impacts on calcifying organisms. *Nature* 437:681–686
 - Parages ML, Figuerola FL, Conde-Álvarez RM, Jiménez C (2014) Phosphorylation of MAPK-like proteins in three intertidal macroalgae under stress conditions. *Aquat Biol* 22:213–226
 - Pelejero C, Calvo E, Hoegh-Guldberg O (2010) Paleoperspectives on ocean acidification. *Trends Ecol Evol* 25:332–344
 - Pérez-Rodríguez E, Gómez I, Figuerola FL (1998) Effects of UV radiation on photosynthesis and excretion of UV-absorbing pigments of *Dasycladus vermicularis* (Chlorophyta, Dasycladales) from southern Spain. *Phycologia* 37:379–387
 - Pérez-Rodríguez E, Aguilera J, Gómez I, Figuerola FL (2001) Excretion of coumarins by the Mediterranean green alga *Dasycladus vermicularis* in response to environmental stress. *Mar Biol* 139:633–639
 - Pérez-Rodríguez E, Aguilera J, Figuerola FL (2003) Tissular localization of coumarins in the green alga *Dasycladus vermicularis* (Scopoli) Krasser: a photoprotective role? *J Exp Bot* 54:1093–1100
 - Porzio L, Buia MC, Hall-Spencer JM (2011) Effects of ocean acidification on macroalgal communities. *J Exp Mar Biol Ecol* 400:278–287
 - Price NN, Hamilton SL, Tootell JS, Smith JE (2011) Species-specific consequences of ocean acidification for the calcareous tropical green algae *Halimeda*. *Mar Ecol Prog Ser* 440:67–78

- Ramírez T, Cortés D, Mercado JM, Vargas-Yáñez M, Sebastián M, Liger E (2005) Seasonal dynamics of inorganic nutrients and phytoplankton biomass in the NW Alboran Sea. *Estuar Coast Shelf Sci* 65:654–670
- Raven JA, Ball LA, Beardall J, Giordano M, Maberly SC (2005) Algae lacking carbon concentrating mechanisms. *Can J Bot* 83:879–890
- Riebesell U, Zondervan I, Rost B, Tortell PD, Zeebe RE, Morel FMM (2000) Reduced calcification of marine plankton in response to increased atmospheric CO₂. *Nature* 407:364–367
- Riebesell U, Schulz KG, Bellerby RGJ, Botros B and others (2007) Enhanced biological carbon consumption in a high CO₂ ocean. *Nature* 450:545–548
- Roleda MY (2009) Photosynthetic response of Arctic kelp zoospores exposed to radiation and thermal stress. *Photochem Photobiol Sci* 8:1302–1312
- Roleda MY, Boyd PW, Hurd CL (2012a) Before ocean acidification: calcifier chemistry lessons. *J Phycol* 48:840–843
- Roleda MY, Morris JN, McGraw CM, Hurd CL (2012b) Ocean acidification and seaweed reproduction: increased CO₂ ameliorates the negative effect of lowered pH on meiospore germination in the giant kelp *Macrocystis pyrifera* (Laminariales, Phaeophyceae). *Glob Change Biol* 18:854–864
- Sabine CL, Feely RA, Gruber N, Key RM and others (2004) The oceanic sink for anthropogenic CO₂. *Science* 305: 367–371
- Salles S, Aguilera J, Figueroa FL (1996) Light field in algal canopies: changes in spectral light ratios and growth of *Porphyra leucosticta* Thur. *Sci Mar* 60:29–38
- Schmid M, Guihéneuf F, Stengel DB (2014) Fatty acid contents and profiles of 16 macroalgae collected from the Irish coast at two seasons. *J Appl Phycol* 26:451–463
- Sharma KK, Schuhmann H, Schenk P (2012) High lipid induction in microalgae for biodiesel production. *Energies* 5:1532–1553
- Stengel DB, Connan S, Popper ZA (2011) Algal chemodiversity and bioactivity: sources of natural variability and implication for commercial application. *Biotechnol Adv* 29:483–501
- Stengel DB, Conde-Álvarez R, Connan S, Nitschke U and others (2014) Short-term effects of CO₂, nutrients and temperature on three marine macroalgae under solar radiation. *Aquat Biol* 22:159–176
- Sukenik A, Bennet J, Falkowski P (1987) Light-saturated photosynthesis: limitation by electron transport or carbon fixation? *Biochim Biophys Acta* 891:205–215
- Surif MB, Raven JA (1989) Exogenous inorganic carbon sources for photosynthesis in seawater by members of Fucales and Laminariales (Phaeophyta): ecological and taxonomic implications. *Oecologia* 78:97–105
- Takahashi T, Sutherland SC, Kozyr A (2011) Global ocean surface water partial pressure of CO₂ database: measurements performed during 1957–2010 (version 2010). Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Dep of Energy, Oak Ridge, TN
- Tchernov D, Gorbunov M, de Vargas C, Narayan Yadav S, Milligan A, Hagglblom M, Falkowski P (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proc Natl Acad Sci USA* 101: 13531–13535
- Tyrrell T, Schneider B, Charalampopoulou A, Riebesell U (2008) Coccolithophores and calcite saturation state in the Baltic and Black Seas. *Biogeosciences* 5:485–494
- Underwood AJ (1997) Experiments in ecology: their logical design and interpretation using analysis of variance. Cambridge University Press, New York, NY
- Viejo RM, Martínez B, Arrontes J, Astudillo C, Hernández L (2011) Reproductive patterns in central and marginal populations of large brown seaweeds: drastic changes at the southern range limit. *Ecography* 34:75–84
- Villafañe VE, Sundbäck K, Figueroa FL, Helbling EW (2003) Photosynthesis in the aquatic environment as affected by UVR. In: Helbling EW, Zagarese H (eds) UV effects in aquatic organisms and ecosystems. The Royal Society of Chemistry, Cambridge, p 357–397
- Warner ME, Fitt WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: a determinant coral bleaching. *Proc Natl Acad Sci USA* 96:8007–8012
- Wernberg T, Thomsen MS, Tuya F, Kendrick GA, Staehr PA, Toohey BD (2010) Decreasing resilience of kelp beds along a latitudinal temperature gradient: potential implications for a warmer future. *Ecol Lett* 13:685–694
- Yildiz G, Hofmann LC, Bischof K, Dere S (2013) Ultraviolet radiation modulates the physiological responses of the calcified rhodophyte *Corallina officinalis* to elevated CO₂. *Bot Mar* 56:161–168
- Zheng Y, Gao K (2009) Impacts of solar UV radiation on the photosynthesis, growth and UV-absorbing compounds in *Gracilaria lemaneiformis* (Rhodophyta) grown at different nitrate concentrations. *J Phycol* 45:314–323
- Zou DH, Gao KS (2002) Effects of elevated CO₂ concentration on the photosynthesis and related physiological processes in marine macroalgae. *Acta Ecol Sin* 22: 1750–1757
- Zou D, Gao K, Luo H (2011) Short- and long-term of elevated CO₂ on photosynthesis and respiration in the marine macroalgae *Hizikia fusiforme* (Sargassaceae, Phaeophyta) grown at low and high nitrogen supply. *J Phycol* 47:87–97

Appendix. Complete list of authors' addresses

F. L. Figuerola¹, J. Bonomi Barufi², E. J. Malta^{3,*}, R. Conde-Álvarez¹, U. Nitschke⁴, F. Arenas⁵, M. Mata¹, S. Connan^{6,7}, M. H. Abreu⁸, R. Marquardt⁹, F. Vaz-Pinto⁵, T. Konotchick¹⁰, P. S. M. Celis-Plá¹, M. Hermoso¹, G. Ordoñez¹, E. Ruiz¹, P. Flores¹, J. de los Ríos¹, D. Kirke⁴, F. Chow¹¹, C. A. G. Nassar¹², D. Robledo¹³, Á. Pérez-Ruzafa¹⁴, E. Bañares-España¹⁵, M. Altamirano¹⁵, C. Jiménez¹, N. Korbee¹, K. Bischof¹⁶, D. B. Stengel⁴

¹Department of Ecology, University of Málaga, 29071 Malaga, Spain

²Departamento de Botânica, Universidade Federal de Santa Catarina, 88040-970 Trindade, Florianópolis, SC, Brazil

³Centro IFAPA Agua del Pino, Crtra. El Rompido – Punta Umbría, 21459 Cartaya (Huelva), Spain

⁴Botany and Plant Science, School of Natural Sciences, Ryan Institute for Environmental, Marine and Energy Research, National University of Ireland Galway, Galway, Ireland

⁵CIIMAR/CIMAR – Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal

⁶Photobiotechnology, INTECHMER, Conservatoire National des Arts et Métiers, BP 324, 50103 Cherbourg Cedex, France

⁷CNRS, GEPEA, UMR6144, Boulevard de l'Université, CRTT BP 406, 44602 Saint Nazaire Cedex, France

⁸ALGApplus Ltd – R&D Department, Rua António Castilho, 3850-405 Angeja, Portugal

⁹Department of Aquaculture, Fraunhofer Institution for Marine Biotechnology, 23562 Lübeck, Germany

¹⁰Environmental & Microbial Genomics Department, J. Craig Venter Institute, 4120 Capricorn Lane, La Jolla, CA 92037, USA

¹¹Department of Botany, University of São Paulo, Rua do Matão 277, CEP 05508-090, São Paulo, SP, Brazil

¹²Departamento de Botânica, Instituto de Biologia, Universidade Federal do Rio de Janeiro, Brazil

¹³CINVESTAV-IPN, Unidad Mérida Km 6 Antigua Carretera a Progreso Apartado Postal 73 Cordemex 97310, Mérida, Yucatán, Mexico

¹⁴Departamento de Ecología e Hidrología, Facultad de Biología, Campus de Excelencia Mare Nostrum, Universidad de Murcia, 30100 Murcia, Spain

¹⁵Departamento de Biología Vegetal (Botánica), Facultad de Ciencias, Universidad de Málaga, Campus de Teatinos s/n, 29071 Málaga, Spain

¹⁶Marine Botany, Bremen Marine Ecology Centre for Research and Education, University of Bremen, Leobener Str. NW2, 28359 Bremen, Germany