PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Physical factors driving intertidal macroalgae distribution: physiological stress of a dominant fucoid at its southern limit

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Abstract Climate change is driving species range shifts worldwide. However, physiological responses related to distributional changes are not fully understood. Oceanographers have reported an increase in ocean temperature in the northwest Iberian Peninsula that is potentially related to the decline in some cold-temperate intertidal macroalgae in the Cantabrian Sea, namely *Fucus serratus*. Low tide stress could also play a role in this decline. We performed one mensurative (in situ) and two manipulative (in culture) experiments designed to evaluate the interactive effects of some physical factors. The first experiment analysed field response to low tide stress in marginal (mid-Cantabrian Sea and northern Portugal) versus central (Galicia) populations of *F. serratus*. Then a second experiment was performed that utilized either harsh or mild summer conditions of atmospheric temperature, irradiance, humidity, and wind velocity to compare the responses of individuals from one marginal and one central population to low tide stress. Finally, the combined effect of sea temperature and the other factors was evaluated to detect interactive effects. Changes in frond growth, maximal photosynthetic quantum yield (F_v/F_m), temperature, and desiccation were found. Three additive factors (solar irradiation, ocean and air temperatures) were found to drive *F. serratus* distribution, except under mildly humid conditions that ameliorated atmospheric thermal stress (two additive factors). Mid-Cantabrian Sea temperatures have recently increased, reaching the inhibitory levels suggested in this study of *F. serratus*. We also expect an additive secondary contribution of low tide stress to this species decline. On the northern Portugal coast, ocean warming plus low tide stress has not reached this species' inhibition threshold. No significant differential responses attributed to the population of origin were found. Mechanistic approaches that are designed to analyse the interactive effects of physical stressors may improve the levels of confidence in predicted range shifts of species.

Keywords Biogeography · Climate change · Ecophysiology · *Fucus serratus* · Seaweed

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Introduction

Climate is considered a major determinant of species distribution (Grinnell 1917). Recently, there has been growing concern about the effect of global warming on species distribution, and numerous studies have suggested climate-driven species distribution shifts both in terrestrial (e.g. Thomas et al. 2004; Parmensan 2006) and marine (e.g. Lima et al. 2007; Hawkins et al. 2009) systems. However, few studies have focussed on the mechanistic processes involved behind species range shifts, particularly at low-latitude retracting margins (Hampe and Petit 2005; Jump et al. 2006; Lima et al. 2007). Synergistic effects of

temperature and other climatic and nonclimatic physical factors on species physiology may cause unexpected distributional responses (Darling and Côté 2008; Kearney and Porter 2009; Austin and Van Niel 2011). The interaction between stressors remains largely unexplored, thereby limiting the capacity of ecologists to predict the effects of climate change (reviewed in Darling and Côté 2008). Moreover, differences in physical factors between the global, local, and regional scales may constrain the accuracy of predictions based solely on the latitudinal variation of climate (Christensen et al. 2006; Helmuth et al. 2006; Jump et al. 2006; Austin and van Niel 2011).

Apart from ocean temperature, other climatic and non-climatic physical factors are critical in shaping the vertical and latitudinal distributions of marine intertidal organisms (e.g. Harley 2003). When emerged, intertidal species undergo water loss and significant overheating, eventually leading to major physiological damage (Chapman 1995; Davison and Pearson 1996; Livingstone 2001). Regional differences in the timing of low tide may create "hot spots" of atmospheric thermal stress associated with the mortality of intertidal organisms, which cannot be predicted on the basis of latitudinal temperature patterns (Helmuth et al. 2006). Photoinhibition due to high irradiances of photosynthetic active radiation (PAR) and ultraviolet radiation (UVR) during low tide is also common in intertidal macroalgae (reviewed in Häder and Figueroa 1997; Figueroa and Viñegla 2001). The negative effects of UVR have been found to increase at elevated temperatures in intertidal fucoid algae (Altamirano et al. 2003). This may be altered by regional or local cloudiness and wind regimes, which modify air temperature, humidity, and irradiance conditions (Dromgoole 1980; Helmuth et al. 2006). Nevertheless, traditional distribution models for intertidal species have mostly ignored these physical factors and only account for large-scale changes in ocean temperature (reviewed in Lüning 1990). Physiological responses to other physical stressors have been poorly related to their geographic distribution (but see Harley 2003). Ecological forecasting requires precise knowledge of the mechanistic responses of organisms to an assortment of environmental factors that vary at different spatial scales and may operate in an interactive way (Darling and Côté 2008; Kearney and Porter 2009; Austin and Van Niel 2011).

In the northwest (NW) Iberian Peninsula (southern Europe), oceanographers have recorded mean annual sea-surface temperature increases of between 0.1 and 0.5 °C per decade from the mid-1970s (Lemos and Pires 2004; Llope et al. 2006; Gómez-Gesteira et al. 2008; Michel et al. 2009). Lima et al. (2007) linked this ocean warming to shifts in the distribution margins of some intertidal organisms on the Portuguese shores, but stated that the overall retraction of northern-type macroalgae was not clear, i.e.

similar numbers were shifting northward and southward. Several species studied by Lima et al. (2007) are also absent in the inner part of the Gulf of Biscay and thus show a second Iberian distributional limit in the mid-Cantabrian Sea (Fischer-Piette 1957; Lüning 1990). Westward and eastward historical displacements of this limit have traditionally been related to warming and cooling cycles, suggesting more dynamic climate-driven changes than on the Portuguese coast (Fischer-Piette 1957; Arrontes 2002; Lima et al. 2007). Evidence from the last decade suggests that the distributional retraction and drastic decline in abundance of several cold-temperate fucoids in this area is associated with the ongoing warming (Fernández and Anadón 2008; Viejo et al. 2011). However, this has not been observed for dominant fucoids in northern Portugal (authors' pers. obs.). Traditional biogeographic models that related these seaweeds' southern limits to the August oceanic isotherm (reviewed in Lüning 1990) cannot explain these two distinctive trends.

In the NW Iberian Peninsula, maximum air temperatures are driven by latitude, while oceanic temperatures are driven by coastal upwellings (Gómez-Gesteira et al. 2008; Michel et al. 2009). As a result, the mid-Cantabrian limit is the area within these seaweeds' distribution range that shows the highest sea temperatures. However, the maximum air temperature is lower than in north (N) Portugal. The ongoing decline of the cold-temperate fucoid *Fucus serratus* in N Spain has primarily been related to ocean warming, but low tide stress has also been suggested to make a significant contribution (Viejo et al. 2011). Cantabrian Sea edge populations are composed of low-density dwarf individuals, showing reduced reproductive potential, except for dispersed individuals in shaded microhabitats with a more common morphology (Viejo et al. 2011). The response of *F. serratus* can be used as a model for other cold-temperate macroalgae that also appear to be declining in N Spain. These canopy-forming species play key ecological roles as foundation species and ecosystem engineers (sensu Jones et al. 1994) at their corresponding intertidal fringe. Thus, they are of major importance for the whole coastal ecosystem.

We performed the mensurative and two manipulative experiments to analyse the roles of temperature and other major physical factors, as well as their interaction, in determining the southern geographic limit of *F. serratus*. In situ responses to low tide stress were analysed in one mensurative field experiment that aimed to compare algae responses in marginal areas (mid-Cantabrian and N Portugal) with those in central areas (Lugo, Galicia) in the Iberian Peninsula. The working hypothesis was that individuals from marginal populations would show a reduced physiological performance compared to central populations (see Pearson et al. 2009), particularly the dwarf morphs that survived in

the mid-Cantabrian Sea border populations. The two manipulative experiments were done to determine the independent and interactive effects among physical stressors. We first simulated low tide stress conditions (harsh versus mild summer conditions of air temperature, irradiation, humidity, and wind velocity) and examined the differences in response between marginal and central populations. We expected to find significant interactions among stressors and an overall lower performance of marginal populations under acute stress. Finally, we tested the interaction among physical stressors operating during both immersion and emersion. However, a directional hypothesis of expected interactions could not be proposed due to the absence of previous experimental research (but see Lotze and Worm 2002).

Materials and methods

Mensurative field experiment

In the first experiment, we analysed the ecophysiological responses of *Fucus serratus* adults from five populations at the species' southernmost European distribution limit to in situ low-tide conditions. Two populations were "central" populations within this species' distribution in the Iberian Peninsula (San Pedro: 43°37′N, 7°20′W and Peizás: 43°35′N, 7°16′W, Lugo, Galicia, Spain), as they have high temporal persistence (Fischer-Piette 1957; Viejo et al. 2011). The other three were at two margins of the species' distribution: two populations from the mid-Cantabrian Sea about 100 km east of the central populations (Novellana: 43°34′N, 6°10′W and Oleiros: 43°34′N, 6°12′W, Asturias, Spain) and the southernmost population in N Portugal about 220 km south of the central localities (Amorosa: 41°38′N, 8°49′W). The experiment was carried out in summer (August and September 2007 in Spain and Portugal, respectively) to achieve stress conditions. Each population was sampled on one day, except Peizás and Amorosa,

which were each visited twice. We expected that individuals from marginal populations, in particular the dwarf morphs occurring in Novellana and Oleiros (Viejo et al. 2011), would show reduced photosynthetic performance compared to individuals from the central locations.

To characterise the environmental conditions at each locality, PAR was measured at substrate level for two 30 min periods (at the beginning and end of low tide) using a spherical quantum scalar sensor (QSL-2100, Biospherical Instruments Inc., San Diego, CA, USA). Air temperature and relative humidity were continuously recorded during the whole low-tide period using temperature–humidity data loggers (MicroLog EC650, Fourier Systems Ltd., Barrington, RI, USA). Field populations were visited on days with prevailing sunny conditions, except for Peizás, where foggy conditions were dominant (Table 1). Particularly high temperatures and solar irradiance together with low humidity were recorded on the day we examined the San Pedro population. Marginal Spanish populations were visited under sunny conditions with alternating clouds. When Amorosa (Portugal) was surveyed, low tide was earlier in the morning, so harsh conditions were restricted to the end of the emersion period (Table 1).

In situ chlorophyll fluorescence associated with photosystem II (PAM 2000 and MINIPAM, Heinz Walz GmbH, Effeltrich, Germany) was measured to characterise the photosynthetic status of the algae in response to emersion. Maximum quantum yield (F_v/F_m) was used as an indicator of physiological stress according to Maxwell and Johnson (2000). Algae were dark adapted for a minimum of 15 min to allow the complete relaxation or oxidation of reaction centres in order to determine basal fluorescence (F_o). F_o values after the 15 min dark periods were similar to those obtained after 30 min periods combined with far-red light pulses, and thus the former were considered adequate (unpublished data). Then a saturation pulse was applied to determine the maximal fluorescence (F_m), with $F_v = F_m - F_o$ (Maxwell and Johnson 2000). F_v/F_m was determined in seven individuals three times throughout the low-tide

Table 1 Environmental conditions during the mensurative field experiment (first)

Population	Relative position	Date	Initial time	Final time	PAR ± SE (max–min)	Temp. ± SE	Humidity ± SE
San Pedro	Central	1 Sep	12:10	15:27	2,256 ± 1 (2,542–352)	35 ± 0.1	35 ± 0.1
Peizás	Central	28 Aug	10:03	12:38	390 ± 1 (718–98)	25 ± 0.1	70 ± 0.1
		2 Sep	13:29	16:37	330 ± 1 (762–99)	20 ± 0.0	81 ± 0.0
Novellana	Marginal (N Spain)	30 Aug	11:17	13:53	1,318 ± 5.1 (3,015–97)	24 ± 0.2	56 ± 0.2
Oleiros	Marginal (N Spain)	31 Aug	11:50	15:08	1,648 ± 4.3 (2,938–98)	28 ± 0.0	41 ± 0.0
Amorosa	Marginal (Portugal)	26 Sep	08:12	11:27	1,673 ± 2.4 (2,112–562)	24 ± 0.4	42 ± 0.4
		27 Sep	08:40	11:37	1,730 ± 1.8 (2,064–1,030)	21 ± 0.2	45 ± 0.2

Relative position refers to the location of the populations within the geographical distribution of *Fucus serratus* in the Iberian Peninsula. Irradiance of PAR (μmol photons $m^{-2} s^{-1}$), air temperature (°C), humidity (%), and GMT time are specified

period. First, measurements were taken at the beginning of emersion (during the first 20 min of emersion, "initial") and then 130 min later, just before the plants were re-submerged ("final"). Finally, other fronds were immersed inside a tide pool after the emersion period and allowed to fully rehydrate for 15 min, and measurements were taken to detect possible fast recovery responses ("recovery"). The temperature of the surface of the algae (except for those that were immersed) was measured using a thermocouple (Mod. Easyview 15, Extech Instruments Corp., Waltham, MA, USA). In addition, desiccation state (as percentage fresh weight loss, % FW) was estimated by weighing five tagged fronds at the beginning and end of low tide, except at Novellana, where strong wind conditions prevented us from measuring frond weight with enough precision. The results in this study report values of fronds exposed to full solar radiation. The variation in the responses at different times during low tide or on different days was used as the background level relative to geographic variation.

Low-tide stress experiment

Vegetative fronds of *F. serratus* were collected during low tide at San Pedro and Amorosa (central versus marginal populations in the Iberian Peninsula) on two different dates at the beginning of October 2008 to perform a second (manipulative) experiment consisting of two consecutive trials. Fronds were immediately transported to the laboratory in a cool icebox in darkness. For each trial, 128 vegetative fronds of the same size (2.7 ± 0.4 g FW, mean $\pm$ SD, $n = 256$) that were from the same population were randomly tagged, attached to a square plastic frame with lines, and submerged in seawater to a depth of about 8 cm in a 300 L shaded tank outdoors. PAR conditions at the surface of the water were monitored for several days during incubation, and the daily curve reached maximum irradiance values of about 1,400 µmol photons m^{-2} s^{-1}. The temperature of the seawater was controlled by a water refrigerator (18.03 ± 0.024 °C, mean $\pm$ SE, $n = 5,997$). To avoid nutrient limitation, the seawater was enriched every two days by adding inorganic N (NaNO$_3$) and P (NaH$_3$PO$_4$) to a final concentration of over 50 µM N and 5 µM P, respectively (i.e. enrichment plus naturally occurring background levels). This nutrient enrichment protocol had been shown to enhance algae growth in previous cultivation trials. Algae were allowed to acclimate to these conditions for one week.

In order to analyse which environmental factors were more relevant in the generation of physiological stress in *F. serratus* during emersion, fronds from the San Pedro and Amorosa populations were exposed to 16 orthogonal combinations of light, air temperature, humidity, and wind on the 3rd and 9th October 2008. As we hypothesised that these environmental physical factors could be potential stressors of *F. serratus*, we aimed to analyse the independent and interactive effects of these factors and differences that were attributable to geographic pattern. These environmental scenarios were recreated inside white plastic containers sealed with UV-transparent methacrylate covers (Plexiglas GS2458, Evonik Röhm GmbH, Darmstadt, Germany). Two replicated containers per environmental condition (i.e. 32 chambers) were set with four fronds of different algae. Simulations were carried out on the roof of the laboratory building around noon on sunny days to assure high solar irradiance and temperature and low atmospheric humidity. As the confinement of the fronds inside the containers intensified the effect of the natural environmental conditions, we used shorter exposure periods (90 min) than the length of low tides in their natural area.

Mild light was achieved by reducing the incident light (full sun) by 65 % $\pm$ 5.6 (mean $\pm$ SD, $n = 6$) using two layers of neutral fibreglass mesh. Air temperature was reduced by approximately 30 % using portable air conditioner units (average temperature was 34 °C in the harsh treatments versus 24 °C in the cooled chambers: $F_{1,2} = 98.83$, $p < 0.01$, with differences between test dates: $F_{2,48} = 30.29$, $p < 0.01$, but no other significant effects). In the case of humidity, mild conditions were created by increasing ambient humidity with ultrasonic humidifiers (Saivod Humi 5L, Spain) connected to the appropriate chambers. The mean increase in relative humidity in harsh treatments was around 18 % (26 ± 1.8 %, mean $\pm$ SE versus 44 ± 3.4 % in mild treatments, $n = 16$, $F_{1,16} = 25.19$, $p < 0.001$), irrespective of the other environmental conditions (not shown). An electric fan was fixed inside each chamber to create mean wind speeds of 2.8 m s^{-2} (measured with an anemometer, Extech Instruments Corp.). These wind speeds were similar to summer field values measured close to the substrate (authors' pers. obs.). The effect of the fans on water loss was also tested in a trial experiment using wet sponges (24.2 ± 1.6 % water loss after 90 min with the fan versus 15.9 ± 0.7 %, mean $\pm$ SE, $n = 6$, significant differences: $F_{1,4} = 9.68$, $p < 0.05$). These experimental conditions achieved the range of values reported during the field experiment (see "Results").

The temperature of each frond was measured after the first 70 min of emersion using thermocouples. Fronds were weighed before emersion and immediately after the emersion period to determine their desiccation state. The algae were then re-submersed in a seawater tank under the same conditions as those in the acclimation tank but in darkness, and allowed to fully rehydrate for 15 min before F_v/F_m was measured. F_v/F_m was measured again the following morning (18 h overnight recovery period). Predawn F_v/F_m measurements were also taken prior to the experiments (around 5:30 am GMT).

Immersion and emersion stress experiment

For the third experiment, vegetative fronds of *F. serratus* of about 1.29 ± 0.003 g FW (mean $\pm$ SE, $n = 128$) were collected during low tide in mid-August 2009 in Amorosa (marginal population in Portugal). Algae were transported to the laboratory and acclimated to the same conditions as those used for the previous experiments (see above). After six days, half of the algae were transferred to 16 outdoor shaded tanks of 20 L set at 22.1 ± 0.12 °C, representing maximum summer values of the mid-Cantabrian Sea, and the other half to 16 tanks set at 16.5 ± 0.08 °C (mean $\pm$ SE, $n = 81$), representing common conditions in the NW Iberian Peninsula (seasonal sea surface temperature—SST—patterns in Gómez-Gesteira et al. 2008). Algae were incubated inside these tanks for two weeks. During this incubation period, they were transferred to an emersion experimental setup similar to that used in the second experiment but without the wind treatment every two days, i.e. a total of seven times, for 30 min. Thus, 16 orthogonal combinations of two levels of light, humidity, air temperature, and water temperature were used. This setup included two water tanks and incubation chambers per treatment (tank + chamber as a nested factor in ANOVAs), and four frond replicates in each. The main goal was to seek independent and interactive effects between ocean temperature and low tide stressors in growth responses. The FW of fully hydrated fronds was weighed to the nearest 0.1 g the day before the algae were transferred to the tanks (initial FW), and then weekly (i.e. twice more). F_v/F_m of the fronds was measured (after 15 min and 18 h) following the protocols described above.

Statistical analysis

To determine if physiological responses differed consistently among the field populations (first experiment), we first compared *F. serratus* performance for the localities with two visits using analysis of variance (ANOVA, Underwood 1997), including date as a random factor nested in population. We then used multiple regressions to examine the relationship between the environmental conditions of the different populations and dates, and the physiological response of the seaweeds (F_v/F_m, temperature and frond desiccation). Relevant physical predictors were selected with the hierarchical partitioning (HP) procedure using specific software (the "hier.part" package) for the public domain package R (Mac Nally 2002).

The effects of treatment on growth, F_v/F_m (after 15 min and 18 h), algae temperature, and desiccation state were analysed using ANOVAs. For the low-tide stress experiment (second), an analysis was done by pooling data from both experimental trials. Light, humidity, air temperature, wind, and population were included as fixed factors and chamber as a random factor nested in the interaction of the previous five factors. For the immersion and emersion stress experiment (third), light, humidity, air temperature, and water temperature were included as fixed factors and chamber as a random factor nested in the interaction of the previous four factors. Student Newman–Keuls tests (SNK) were performed after significant ANOVA interactions (Underwood 1997) and are shown in the figures as lowercase letters when applicable. Homogeneity of variance was tested using Cochran tests and by visual inspection of the residuals (Underwood 1997). Analyses were done with Statistica 9.0 (StatSoft Inc., Tulsa, OK, USA).

We adopted 0.01 as the threshold α value when testing for treatment effects because we had detected (in this and other studies) significant differences in the response variables between the algae assigned to the different treatments before performing the experiments (e.g. effect of air temperature on predawn F_v/F_m values for Amorosa algae: $F_{1,16} = 4.699$, $p = 0.046$). Although these differences were statistically significant at $\alpha = 0.05$, their magnitude was too limited to be indicative of distinct physiological performance.

Results

F_v/F_m, temperature, and desiccation state in the mensurative field experiment

F_v/F_m values during the first 20 min (initial) and when algae were allowed to recover for 15 min after low tide (recovery) were similar, showing a fast recovery response of the algae, even after the marked F_v/F_m decrease observed during the low tides of harsh conditions (final, Fig. 1a). Initial and final F_v/F_m values were the lowest in algae from the central population sampled on the warmest dry day (San Pedro). In contrast, algae growing just a few km away in Peizás showed the opposite trend in response to mild environmental conditions—they had the highest F_v/F_m values with no signs of dynamic photoinhibition (Fig. 1a, Table 1). Intermediate F_v/F_m values were recorded in response to intermediate weather conditions (marginal populations). Algae temperature and final desiccation state were also closely related to physical conditions (Fig. 1b, c). Physiological variation attributed to environmental changes during low tide or in response to weather conditions was higher than the observed interpopulation or geographic variation.

Statistical analysis for the localities that were sampled twice (Peizás and Amorosa) supports the visual trend. None of the five functional variables measured (F_v/F_m and temperatures before and after emersion stress, as well as
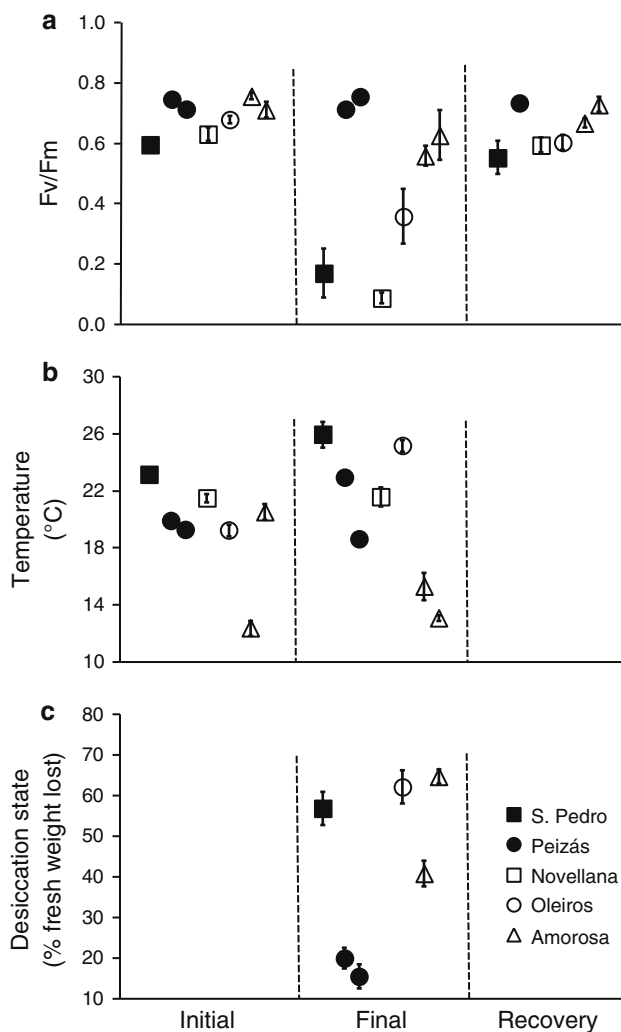
Fig. 1 Mensurative field experiment: **a** F_v/F_m, **b** temperature, and **c** desiccation state of algae (mean ± SE, $n = 7$–10). *Filled symbols* represent data from central populations in the Iberian Peninsula (San Pedro and Peizás in Galicia), and *open symbols* represent data from marginal populations (Novellana and Olerios in Asturias, N Spain, and Amorosa in N Portugal). Measurements were taken at the beginning of the emersion period (*Initial*), 130 min later (*Final*), and after rehydration within 15 min after low tide (*Recovery*)

Using a multiple regression approach combined with the hierarchical partitioning procedure to select relevant predictors, we found that environmental variables explained a significant portion of the measured physiological responses (Table 2). The temperature of fronds before and after the emersion period was mostly explained by air temperature. Air humidity was also a significant predictor of final frond temperature, and particularly of desiccation state. Multiple regression models using these significant predictors explained over 40 % of total variability in the response variables (Table 2). With regard to the photosynthetic performance of fronds, all environmental variables were selected as significant predictors for F_v/F_m immediately after emersion (initial). However, final and recovery F_v/F_m values were better predicted by incident light than by the other variables. These two models had a lower proportion of explained variance (Table 2) as a result of the high variability between replicates.

F_v/F_m, temperature, and desiccation state in the manipulative low-tide stress experiment

In the low-tide emersion experiment (second), harsh levels of irradiance, humidity, and air temperature were associated with a reduction in F_v/F_m, and no distinct trends were observed for marginal (Amorosa) and central (San Pedro) populations when algae were allowed to recover for 15 min (Table 3; Fig. 2a, c, e). After the overnight recovery period, the algae did not fully recover from inhibiting irradiance (Table 3; Fig. 2b). Low humidity had a greater inhibitory effect at high air temperatures and for algae from Amorosa (SNK tests, Fig. 2d, f). Wind did not produce significant effects in any case. The lowest F_v/F_m values attributed to a single stressor (others pooled) were measured at high-irradiance conditions (0.22 ± 0.01 after 15 min, mean ± SE, $n = 128$, Fig. 2a). Full recovery to predawn values was observed for fronds subjected to ameliorated conditions of all factors. Predawn values were high, suggesting that acclimation conditions before the experiment were adequate (0.78 ± 0.002, mean ± SE, $n = 256$).

Harsh air temperature, irradiance, and humidity conditions had a significant effect on frond temperature during the low-tide stress experiment (irradiance: $F_{1,32} = 142.517$, $p < 0.001$; humidity: $F_{1,32} = 73.438$, $p < 0.001$; air temperature: $F_{1,32} = 32.037$, $p < 0.001$), with no significant population effect ($F_{1,32} = 0.358$, $p = 0.554$) or interactions (not shown). Final frond temperature increased by 9.6 and 4.2 °C in response to high air temperatures and high irradiance, respectively, while mild humidity was associated with a 3.5 °C decrease (other factors pooled). Wind did not have a significant effect on frond temperature in any case ($F_{1,32} = 1.716$, $p = 0.199$). Mean temperatures ranged from 17.5 ± 0.4 (mild conditions of all factors) to 34.8 ± 0.4 (harsh conditions of all factors, mean ± SE, $n = 32$).

desiccation state) showed any differences among populations (ANOVAs, factor population: F_v/F_m before stress: $F_{1,2} = 0.01$, $p = 0.93$; F_v/F_m after stress: $F_{1,2} = 12.7$, $p = 0.08$; temperature before stress $F_{1,2} = 0.6$, $p = 0.52$; temperature after stress $F_{1,2} = 7.48$, $p = 0.11$; desiccation state after stress $F_{1,2} = 4.9$, $p = 0.15$). However, differences within populations were found between dates for three of the physiological variables (frond temperature before and after emersion stress, and final desiccation state: ANOVAs, factor date (population): temperature before stress $F_{2,24} = 115$, $p < 0.001$; temperature after stress $F_{2,24} = 21.8$, $p < 0.001$; desiccation state $F_{2,16} = 12.6$, $p < 0.001$).

Table 2 Relationships between environmental variables (first column) and the physiological responses of *Fucus serratus* (second line) in the mensurative field experiment (first)

Hierarchical partitioning

Dependent variables	Initial temp.	Final temp.	Desiccation state	F_v/F_m initial	F_v/F_m final	F_v/F_m recovery
	% I	% I	% I	% I	% I	% I
Predictors						
Temp. (air)	**73.6*****	**58.7*****	18.1	**40.7*****	26.2	8.5
Humidity	11.9	**39.8*****	**55.9*****	**26.6*****	21.4	**36.7****
Irradiance (PAR)	**14.3***	1.45	**25.9***	**32.5*****	**52.4*****	**54.6*****
Linear model						
Adjusted R^2	**0.49*****	**0.46*****	**0.43*****	**0.52*****	**0.12****	**0.16****

A hierarchical partitioning procedure was carried out to select significant predictors; and linear multiple regression including the significant predictors for calculating adjusted R^2

% I indicates the percentage of independent contribution to the model-explained variance. *Temp.* indicates temperature. Significant predictors are indicated in bold. * *P* values <0.05, ** *P* values <0.01, *** *P* values <0.001

Maximum mean desiccation (as FW loss) was 77.3 ± 0.6 % (mean ± SE, *n* = 4, algae from Amorosa under conditions of high irradiance and temperature, and low humidity and wind in one of the two replicated chambers). ANOVAs showed that desiccation was related to all physical variables (data not shown). Most treatments reached a similar desiccation state (around 70 %), suggesting that the rate of water loss slows down after an initial period with a duration that depended on the strength of the treatment (authors' pers. obs.). However, our data suggested a similar final desiccation state of algae in the different treatments. This may explain why the ANOVA included high-order significant interactions that were difficult to interpret (not shown).

Growth and F_v/F_m in the combined immersion and emersion stress experiment

All of the stressors in the combined immersion and emersion stress experiment (third) exerted a growth inhibition effect on Amorosa (Portugal) algae (Table 4; Fig. 3). The high-irradiance treatment was associated with a 49 % decrease in weight compared to the mean final weight of algae under mild light conditions (other factors pooled, Fig. 3a). High water temperature was associated with a 38 % decrease in the mean final weight of algae after two weeks (Fig. 3b). No significant interaction was found between these two stressors and the others, suggesting major additive inhibition effects (Table 4). Humidity and air temperature showed an antagonistic response: when high humidity was combined with high air temperature, growth was not significantly lower than in the low-temperature treatments (SNK test, Fig. 3c). Combined harsh conditions of these two factors reduced mean growth by about 89 % (other factors pooled). This reduction in growth was

slightly lower than that caused by low humidity plus air thermal stress (data not shown). Individuals subjected to the treatment combining the four stressors showed signs of necrosis and eventually died. However, a single stressor may also drive algae to death, if harsh enough (unpublished data). On the other hand, fronds subjected to ameliorated stressors experienced the greatest increase in growth (about 0.64 g FW ± 0.04 in two weeks, for a final size of 1.94 g FW ± 0.04, mean ± SE, *n* = 8).

In the third experiment, irradiance was consistently associated with a reduction in F_v/F_m after low-tide simulation periods, suggesting that this stressor makes a major contribution to photosynthetic potential. However, the magnitude of F_v/F_m decay was lower than in previous experiments, probably because we used shorter emersion periods (30 min instead of 130 and 90) to avoid frond mortality before the end of the experiment. Moreover, the response of F_v/F_m to other stressors apart from light was minor (significant effects were detected only a few times). Although F_v/F_m conveniently detected acute stress situations (involving different stressors) in the previous experiments, it showed a reduced response in comparison to growth in this experiment (Tables 4, 5), so it was less reliable for detecting cumulative stress conditions.

Discussion

Our results suggest that the stressful conditions created by high solar radiation, high water and atmospheric temperatures, and low humidity inhibited the growth and physiological performance of *Fucus serratus*, and that these factors had an additive effect. High solar irradiance was associated with consistent growth and photosynthetic inhibition and damage. No meaningful interactive effects with

Table 3 ANOVAs of the effect of irradiance (PAR), humidity, air temperature, and wind treatments on the F_v/F_m of algae collected in San Pedro (Galicia, central) and Amorosa (Portugal, marginal) populations after the manipulative low-tide stress experiment (second, 90 min)

	df	15 min recovery			18 h recovery		
		MS	F	p	MS	F	p
Irradiance (E)	**1**	**5.675**	**142.52**	**<0.001**	**1.468**	**49.63**	**<0.001**
Humidity (H)	**1**	**2.924**	**73.44**	**<0.001**	**2.511**	**84.88**	**<0.001**
Air temperature (T_a)	**1**	**1.276**	**32.04**	**<0.001**	**1.497**	**50.62**	**<0.001**
Wind (W)	1	0.068	1.72	0.200	0.090	3.04	0.091
Population (P)	1	0.014	0.36	0.554	0.206	6.97	0.013
Chamber C ($T_a \times H \times W \times E$)	**32**	**0.040**	**5.91**	**<0.001**	**0.030**	**6.05**	**<0.001**
$E \times H$	1	0.044	1.11	0.300	0.106	3.57	0.068
$E \times T_a$	1	0.005	0.13	0.723	0.173	5.86	0.021
$H \times T_a$	**1**	0.082	2.06	0.161	**0.572**	**19.33**	**<0.001**
$E \times W$	1	0.001	0.01	0.918	0.007	0.23	0.633
$H \times W$	1	0.010	0.25	0.622	0.051	1.71	0.200
$T_a \times W$	1	0.035	0.87	0.358	0.036	1.22	0.277
$E \times P$	1	0.056	1.41	0.244	0.033	1.12	0.297
$H \times P$	**1**	0.150	3.76	0.062	**0.497**	**16.80**	**<0.001**
$T_a \times P$	1	0.008	0.19	0.667	0.045	1.51	0.228
$W \times P$	1	0.001	0.03	0.862	0.009	0.31	0.582
$E \times H \times T_a$	1	0.038	0.96	0.334	0.002	0.06	0.816
$E \times H \times W$	1	0.013	0.32	0.573	0.001	0.01	0.907
$E \times T_a \times W$	1	0.009	0.23	0.634	0.042	1.44	0.240
$H \times T_a \times W$	1	0.069	1.73	0.198	0.019	0.64	0.430
$E \times H \times P$	1	0.017	0.44	0.513	0.007	0.22	0.641
$E \times T_a \times P$	1	0.030	0.77	0.388	0.014	0.46	0.503
$H \times T_a \times P$	1	0.020	0.51	0.479	0.057	1.94	0.173
$E \times W \times P$	1	0.011	0.28	0.603	0.005	0.16	0.694
$H \times W \times P$	1	0.001	0.00	0.972	0.006	0.19	0.667
$T_a \times W \times P$	1	0.001	0.01	0.914	0.009	0.30	0.589
$E \times H \times T_a \times W$	1	0.038	0.96	0.335	0.011	0.36	0.552
$E \times H \times T_a \times P$	1	0.006	0.16	0.691	0.006	0.20	0.655
$E \times H \times W \times P$	1	0.003	0.08	0.782	0.011	0.37	0.548
$E \times T_a \times W \times P$	1	0.059	1.48	0.233	0.003	0.10	0.759
$H \times T_a \times W \times P$	1	0.001	0.00	0.954	0.009	0.30	0.586
$E \times H \times T_a \times W \times P$	1	0.016	0.41	0.528	0.001	0.00	0.989
Residual	192*	0.007			0.005		

F_v/F_m was measured after recovery periods of 15 min and 18 h (overnight)

Significant differences at $\alpha < 0.01$ are shown in bold. To achieve homogeneity of variances in the 18 h recovery data, three outliers were replaced by the mean of the group ($df = 189$)

other stressors were evident. Photoinhibitory damage from macroalgae (photosynthetic organisms) involves the oxidation or denaturalization of pigments and proteins, particularly at the PS II, sometimes with the suppression of the repair mechanisms that protect reaction centres (reviewed in Takahashi and Badger 2011; Nishiyama et al. 2011). Thus, it is not surprising that solar irradiance, in both the in situ and ex situ experiments, was the main factor driving the variation in F_v/F_m, as reported by other authors for different seaweeds, including brown algae (Häder and Figueroa 1997; Gómez et al. 2004; Abdala-Díaz et al. 2006). F_v/F_m was, however, less reliable than growth in its response to nonacute cumulative stress from irradiance and other stressors. This seems to indicate that F_v/F_m is more

strongly linked to maximum photosynthetic efficiency than to photosynthetic production (see Schreiber et al. 1995), but more research is needed to clarify this response.

Excessive radiation also induced overheating and desiccation, triggering the production of oxidative radicals by impairing metabolic activities (Nishiyama et al. 2011). Other potential injuries from oxidative stress are damage to DNA and lipids, as described for fucoids by Collén and Davison (1999, 2001) and for higher plants and animals by Dat et al. (2000) and Lushchak (2011). Such direct damage and/or resource tradeoffs, which are known to compensate for physiological stress in macroalgae and plants (Alonso-Alvarez et al. 2004; Dethier et al. 2005), ultimately compromise growth, thus potentially influencing the geographic

Fig. 2 Low-tide stress experiment: F_v/F_m (mean $\pm$ SE) of the algae collected in San Pedro and Amorosa (pooled) after recovery periods of (**a**, **c**, **e**) 15 min and (**b**, **d**, **f**) 18 h. *Lower-case letters* in **d** and **f** denote significant differences in F_v/F_m after SNK tests (see "Materials and methods") from different combinations of humidity with the air temperature and the population of origin, respectively
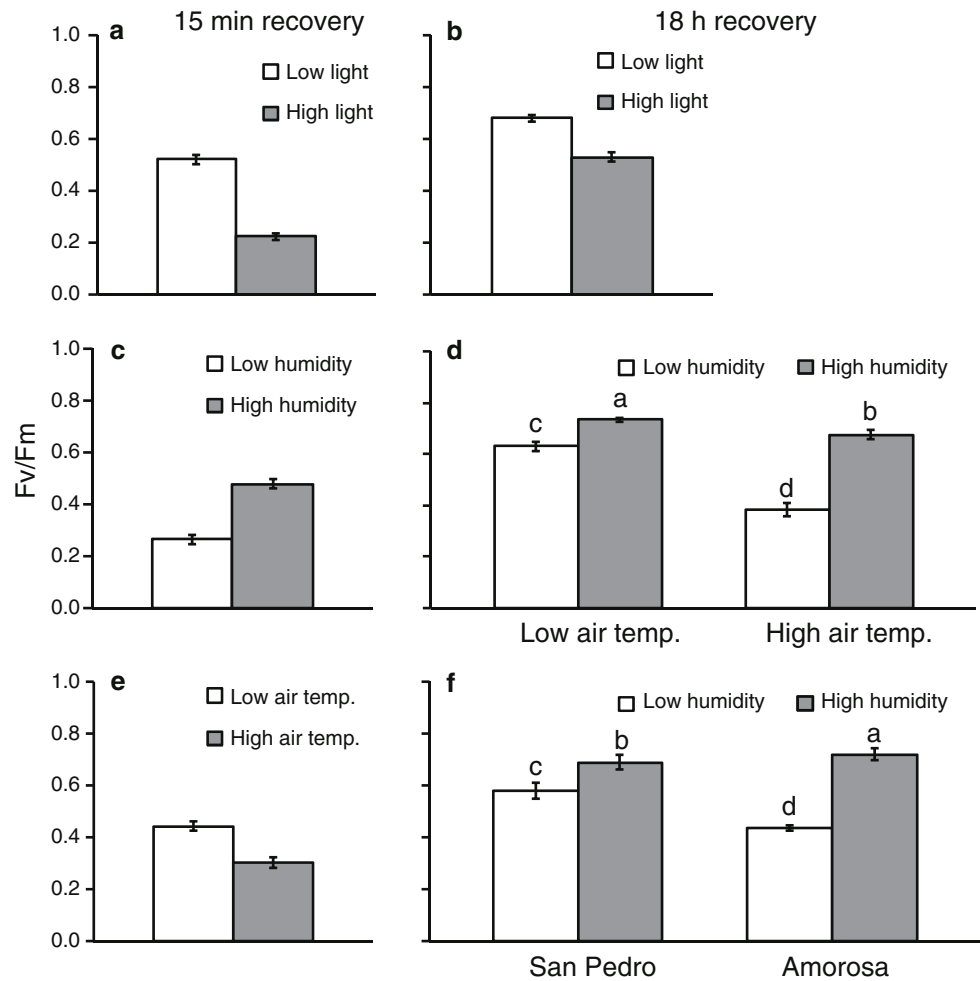

Table 4 ANOVAs of the effect of irradiance (PAR), humidity, air temperature, and water temperature treatments on the FW (g) of the algae after the first and second weeks of the combined immersion and emersion stress experiment (third)

Initial FW was constant across treatments

Significant differences at $\alpha < 0.01$ are shown in bold. Variances were homogeneous

	df	First week			Second week		
		MS	F	p	MS	F	p
Irradiance (E)	**1**	**0.219**	**33.84**	**<0.001**	**1.445**	**32.79**	**<0.001**
Humidity (H)	**1**	**0.096**	**14.76**	**0.001**	**1.015**	**23.04**	**<0.001**
Air temperature (T_a)	**1**	**0.203**	**31.34**	**<0.001**	**1.620**	**36.77**	**<0.001**
Water temperature (T_w)	**1**	**0.107**	**16.49**	**0.001**	**0.845**	**19.18**	**<0.001**
Tank + chamber (C)	16	0.006	0.87	0.607	0.044	2.12	0.013
$E \times T_a$	1	0.004	0.59	0.453	0.125	2.84	0.112
$E \times H$	1	0.041	6.37	0.023	0.340	7.72	0.013
$T_a \times H$	**1**	**0.131**	**20.25**	**<0.001**	**0.813**	**18.45**	**0.001**
$E \times T_w$	1	0.018	2.71	0.119	0.001	0.03	0.868
$T_a \times T_w$	1	0.018	2.71	0.119	0.080	1.82	0.197
$H \times T_w$	1	0.006	0.98	0.338	0.038	0.86	0.368
$E \times T_w \times H$	1	0.049	7.53	0.014	0.165	3.75	0.071
$E \times T_w \times T_w$	1	0.041	6.37	0.023	0.361	8.20	0.011
$E \times H \times T_w$	1	0.000	0.01	0.914	0.070	1.60	0.225
$T_a \times H \times T_w$	1	0.004	0.59	0.453	0.053	1.20	0.290
$E \times T_a \times H \times T_w$	1	0.009	1.46	0.245	0.003	0.06	0.804
Residual	96	0.007			0.021		

Fig. 3 Immersion and emersion stress experiment: growth (the difference between the initial and final fresh weights, FW, in g) of algae from Amorosa after two weeks in response to **a** light, **b** water temperature, and **c** combined humidity and air temperature conditions (other factors were pooled). *Lowercase letters* denote significant differences after SNK tests (see "Materials and methods")
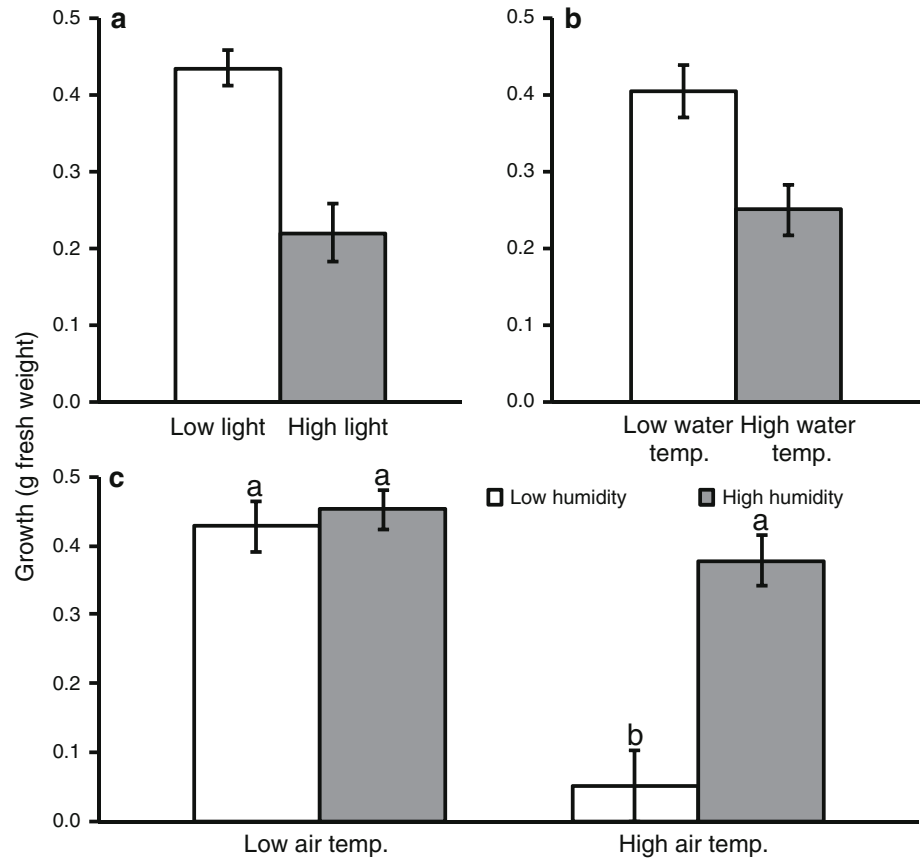

Table 5 ANOVAs of the effect of irradiance (PAR), humidity, air temperature, and water temperature treatments on the F_v/F_m of the algae after seven emersion simulation periods (30 min) in the combined immersion and emersion stress experiment (third)

Date	F_v/F_m mean ± SE	% Red.	E	T_a	H	T_w	Interactions
15 min recovery							
25 Aug	0.37 ± 0.116	45.7	***				
27 Aug	0.32 ± 0.142	51.1	***				
29 Aug	0.22 ± 0.133	69.3	***	***			
31 Aug	0.47 ± 0.080	40.0	***	***	**	**	$E \times T_a$**, $E \times T_a \times T_w$**
2 Sep	0.49 ± 0.135	27.3	***				
4 Sep	0.41 ± 0.067	37.6	***				
6 Sep	0.48 ± 0.057	38.9	***				
18 h overnight recovery							
25 Aug	0.78 ± 0.042	4.5	**				
27 Aug	0.76 ± 0.019	3.2	***				
29 Aug	0.76 ± 0.041	3.5					
31 Aug	0.74 ± 0.021	5.3	***		***	**	$E \times T_a$**
2 Sep	0.76 ± 0.029	5.4	***				$E \times T_a \times H$**
4 Sep	0.78 ± 0.040	5.1					
6 Sep	0.77 ± 0.035	3.6	***				

F_v/F_m was measured after recovery periods of 15 min and 18 h. F_v/F_m *mean* shows the mean value ($n = 8$) of the most stressful treatment, i.e. the combination of the four stressors. *% Rev* shows the degree of the corresponding F_v/F_m reduction in reference to values obtained with mild conditions for all factors. Abbreviations as in Tables 3 and 4

** $p < 0.01$, *** $p < 0.001$. Outliers were replaced with the mean of the corresponding group, and the degrees of freedom were corrected as necessary

distribution of the species (e.g. Wernberg et al. 2010). Solar irradiance is currently considered a nonclimatic physical factor that has a major influence in plant biogeography (see Austin and Van Niel 2011). Irradiance in the southern distribution of *F. serratus* is mainly driven by regional differences in cloudiness, which are minimal at the N Portuguese limit (e.g. http://meteonorm.com/?id=32).

With regard to growth, *F. serratus* was also vulnerable to thermal stress while submerged, but did not show interactive effects with other factors. Thermal conditions in the sea directly drive algal temperature. Overheating affects virtually all physiological responses of living organisms, such as protein and membrane stability and enzymatic reaction rates (Lobban and Harrison 1997), leading to unbalanced metabolic states during increased oxidative stress (see above). We observed that the thermal conditions in the sea had an inhibitory effect at temperatures below the 25 °C lethal threshold (at around 22 °C) reported for this species (Lüning 1990). Remarkably, based on our experiments, mid-Cantabrian Sea temperatures are reaching inhibitory levels for *F. serratus* (e.g. maximum SST in August 2003 of 22.3 °C in Gijón, 43°32′N, 5°42′W, from AVHRR satellite images provided by the Spanish Ministry of Defence). Likewise, ocean warming has been suggested to be a major factor in the ongoing decline in abundance of *F. serratus*, and in the historical westward displacements of this species' border in this area (see "Introduction"). At the Portuguese limit, summer upwellings prevent August ocean temperatures from reaching mid-Cantabrian Sea temperatures (Gómez-Gesteira et al. 2008). In this area, the distribution of *F. serratus* does not seem to be retracting, and border populations have been historically more persistent (see "Introduction"). Regional differences in ocean temperature may explain these two different scenarios. The balance between latitudinal change in temperature and the regional and local thermal regime has a crucial influence on species' distributions (Huey 1991; Helmuth et al. 2006; Jump et al. 2006). However, variation at this scale has not been included in traditional models that relate intertidal species distribution to August and February oceanic isotherms (reviewed in Lüning 1990).

We also found that humidity and air temperature interacted in our experiments. Higher humidity levels compensated for atmospheric thermal stress, ameliorating growth inhibition. Changes in the frond temperature at different levels of hydration, as reported for other macroalgal species (Dromgoole 1980; Bell 1995), may explain this response. Indeed, air humidity was inversely related to frond temperature and directly related to desiccation state in our mensurative (in situ) and manipulative (in culture) experiments. When emerged, macroalgae may lose water without any active retention mechanisms such as the stomata or impermeable cuticles of plants, resulting in passive cooling

(Dromgoole 1980; Lüning 1990; Bell 1995; Chapman 1995). This was evident at experimental humidity levels which were above those measured in situ in summer low tides with intermediate conditions (e.g. alternating clouds). Terrestrial organisms also lower their temperature by active transpiration, increasing their tolerance to mild drought and thermal stress (Schmidt-Nielsen 2001). A similar response was evident in photosynthetic potential: algae at high humidity levels showed a faster overnight recovery (18 h). In this study, humidity inhibition was also evident at ameliorated levels of the other factors. This is most likely due to physiological damage related to cellular water loss resulting from low external vapour pressure (Dromgoole 1980; Lobban and Harrison 1997). This response has rarely been analysed in previous studies, which were limited by the lack of treatments of variable ambient humidity (but see Dromgoole 1980). As observed for solar irradiance and air temperature, humidity is driven in the NW Iberian Peninsula by regional differences in cloudiness, i.e. maximum values in the NW corner. This results in harsher low-tide conditions at the N Portuguese limit of *F. serratus* than at northern localities. However, this has not been related to the current decline in this species or other dominant fucoids (authors' pers. obs). Thus, we can conclude that ocean warming—more so than low tide stress—has triggered the recent decline of cold-temperate *F. serratus* in S Europe.

The responses of the dwarf specimens that survived at the Cantabrian Sea edge (see "Introduction") and the responses of individuals from the marginal Portuguese populations were not different from those of individuals from the central locations. Environmental variations during low tide on days with harsh weather conditions yielded the greatest physiological variations in field individuals. Photochemical efficiency values were the lowest and temperature and desiccation values were the highest in algae from a central population in the Iberian Peninsula (San Pedro) after low tide on a sunny summer day. Huppertz et al. (1990) also found that photosynthesis in *F. serratus* was dependent on the light conditions during low tide. Other measured physiological parameters (phenolic, C, N, chlorophylls *a* and *c*, tocopherol, fucoxanthin, violaxanthin, antheraxanthin, zeaxanthin, pheophytin, and *β*-carotene contents; authors' unpublished data) did not suggest any geographic pattern. In agreement with the mensurative field experiment, the manipulative low-tide experiment found that the response of algae to acute stress was not significantly different between the marginal and central populations of the Atlantic coast. However, it did find a minor trend for marginal Portuguese algae to recover at a slower rate under low-humidity conditions. Thus, our results rule out the possibility of differential adaptive responses of *F. serratus* to withstand the extreme stress conditions at its distributional limits

(but see Pearson et al. 2009). Furthermore, potential adaptive life-trait responses, such as lower minimum reproduction size in dwarfs from marginal populations, have also proven insufficient to avoid drastic reductions in the reproductive capacities of these populations under the ongoing warming cycle in the Cantabrian Sea (see Viejo et al. 2011).

The occurrence of an interaction between stressors remains unexplored in biogeographical studies, thereby limiting ecologists' capacity to predict the effects of global climate change (reviewed in Darling and Côté 2008). Our study suggests that three additive variables (solar irradiation and ocean and air temperatures) are relevant for forecasting *F. serratus* distributional shifts, except under mildly humid conditions that ameliorated atmospheric thermal stress (i.e. two additive variables). Previous data on intertidal species suggested the importance of synergistic deleterious effects, which were not evident in this study (Lotze and Worm 2002; Przeslawski et al. 2005; Helmuth et al. 2006). Indeed, predictive models that only consider additive effects of physical stressors have been successfully used in large geographic areas across different ecological systems and for different biological taxa (Darling and Côté 2008 and references therein). To increase the confidence in forecasted trends, regional variations in physical factors—namely ocean temperature and cloudiness along the NW Iberian Peninsula—should also be considered. Climatic and nonclimatic physical factors operating at regional to local scales also proved meaningful for setting the distributional limits of the target macroalgae, as recently stated for plants and animals (reviewed in Austin and Van Niel 2011). The model species showed limited potential to withstand increased stress in response to global change. In particular, according to this study, temperatures in the Cantabrian Sea due to global warming are reaching levels that can physiologically inhibit this cold-temperate fucoid. We also expect a secondary contribution from low tide stress. Rapid distributional shifts driven by extreme conditions involving a combination of several physical factors have been described (e.g. Paine et al. 1998; Denny et al. 2009; Harley and Paine 2009), as shown for example in the heat wave of 2003 in Europe (e.g. Wegner et al. 2008). Dispersal limitations and other biological responses, which were important factors in determining *F. serratus* boundaries during periods of favourable conditions (Arrontes 2002, 2005), will probably become less relevant in future climate scenarios (see review in Beniston et al. 2007). Mechanistic approaches such as the one adopted in this study, based on combined mensurative and manipulative experiments to analyse the additive and interactive effects of stressors that are relevant to species biogeography, are the first step towards improving the confidence in forecasted range shifts in future climate scenarios (see Denny et al. 2009; Kearney and Porter 2009).

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