

Additive effects of emersion stressors on the ecophysiological performance of two intertidal seaweeds

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ABSTRACT: The latitudinal distribution of species is primarily driven by physical factors, particularly temperature. However, several studies suggest the importance of non-climatic physical stressors, such as irradiance, which may interact synergistically with temperature as unexpected deleterious effects or, alternatively, as additive factors. We performed 1 field and 1 mesocosm experiment to evaluate the potential interactive effects of physical stressors operating at the southern distributional ranges of *Fucus serratus* and *F. spiralis*, 2 intertidal macroalgae with contrasting geographic distributions. The first experiment analysed field response to low tide stress by measuring temperature increase, desiccation rate and photoinhibition in fronds exposed to air and fronds covered by other fronds. The second experiment compared the response of these 2 furoid species by recreating harsh vs. mild conditions of major intertidal stressors. Results suggest that the additive effects of climatic factors, namely air temperature, and non-climatic physical variables, namely irradiance and air humidity, partially explain and can help to forecast distributional shifts of intertidal macroalgae. *F. spiralis* was better adapted than its congener *F. serratus* with lower inhibition and faster recovery. This agrees with its low latitude affinity and higher position in the intertidal. The only major interactive effect observed was the antagonistic amelioration of atmospheric thermal stress by mid to high humidity conditions, particularly when irradiance was not excessive. These intertidal seaweeds retain ambient humidity and shade themselves, lowering physiological damage through self-cover with their fronds. We observed overall additive effects of stressors and higher physiological resilience of covered algae to low tide stressors than expected by their additive effects.

KEY WORDS: Biogeography · Climate change · Ecophysiology · *Fucus serratus* · *Fucus spiralis* · Seaweed · Stress

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INTRODUCTION

The relationship between latitudinal species distribution and climate is a central concept in biogeography (Hutchins 1947, Araújo & Luoto 2007), and unambiguous evidence indicates that climate change is altering the distribution of many species worldwide (Beardall et al. 1998, Parmesan & Yohe 2003). In

the last decade, numerous studies have reported a relationship between global warming and shifts in species distribution in marine systems (e.g. Lima et al. 2007, Wernberg et al. 2011, Harley et al. 2012). However, less attention has been paid to the relevance of non-climatic physical factors; those environmental conditions that show large regional variation, as for example with incoming irradiation (but see

Austin & Van Niel 2011, Martínez et al. 2012a); and the potential interactive effects of these factors and climatic forces (Crain et al. 2008, Darling & Côté 2008). This limits our understanding of the processes underpinning species distribution and our ability to predict future shifts. Better mechanistic knowledge is needed to reduce the uncertainty of predicted contractions and expansions of distributional ranges under climate change (e.g. Buckley et al. 2010).

Intertidal organisms living at the boundary between land and sea experience consecutive periods of emersion and immersion that may take them close to their physiological tolerance thresholds. During low tide, seaweeds undergo periods of high air temperature combined with non-climatic physical stress such as high solar radiation, low humidity and windy conditions. These physical conditions typically drive the rapid overheating and desiccation of macroalgae, causing oxidative stress due to metabolic impairment, inhibition and damage to photosynthetic machinery and other cellular components (Chapman 1995, Collén & Davison 1999, Hoffman et al. 2003). The modular structure and high density of these algae can protect them against desiccation or heat stress by accumulating water and preventing photodamage through self-covering during low tide (Scrosati & De Wreede 1997, 1998), increasing collective survival and growth (Ang & De Wreede 1992, Scrosati 2000). However, little is known about the interactions among these physical factors, which may operate synergistically causing unforeseen damage (Darling & Côté 2008) or additively in a more predictable way (Martínez et al. 2012a, Ferreira et al. 2014). Moreover, the combined effects of climatic and non-climatic physical stressors have been poorly related to species' geographic distributions and shifts in response to climate change, particularly in marine organisms (but see Martínez et al. 2012a,b, Ferreira et al. 2014).

Ocean temperature, solar irradiation and air temperature during low tide seem to be key predictors of the current distribution limit of the cold-temperate furoid *Fucus serratus* in southern Europe (Martínez et al. 2012a). In the Biscay Bay (northern Spain), ocean warming has been associated with the recent decline of this species (Viejo et al. 2011, Duarte et al. 2013) as well as the decline and disappearance of other foundation kelps and furoids (Fernández 2011, Díez et al. 2012, Lamela-Silvarrey et al. 2012). Using correlative approaches based on field observations, Díez et al. (2012) concluded that other physical factors apart from temperature may have contributed to the decline of seaweeds in the eastern Cantabrian

Sea and that synergistic effects between them may be relevant. However, using an experimental manipulative approach, Martínez et al. (2012a) found few signs of interactive effects among various climatic and non-climatic physical stressors in *F. serratus* and recently in *F. spiralis* and *F. vesiculosus* (Ferreira et al. 2014). Thus, the interactive or additive effects of physical stressors in macroalgae remain largely unknown. In northern Portugal, where the southernmost European populations of *F. serratus* occur, persistent coastal upwelling events keep sea temperature low, particularly in summer (Gómez-Gesteira et al. 2008). In this area, water temperature seems to be less relevant in controlling this species' distribution limit than in the Cantabrian Sea, and emersion stress during low tide seems to be more relevant (see Martínez et al. 2012a), making it an ideal system to test the joint effect of temperature and other relevant non-climatic physical stressors operating in the intertidal.

To explore the additive contribution of major climatic and non-climatic low tide physical stressors, we compared the physiological tolerance of *F. serratus* and its congener *F. spiralis*. *F. spiralis* extends further south along the eastern Atlantic coast, reaching Morocco, and occupies higher levels in the intertidal (Margalet et al. 1993). Both species have a similar life cycle, but *F. spiralis* shows greater tolerance to low tide stressors (Chapman 1995) such as temperature and UV radiation (e.g. Altamirano et al. 2003, Ferreira et al. 2014). To date, few changes in this species' distribution or abundance have been reported in the Iberian Peninsula (Lamela-Silvarrey et al. 2012), and it seems to have higher resilience to the current warming scenario than *F. serratus*. Consequently, *F. serratus* is expected to show lower physiological performance in response to acute low tide stress. This would compromise its survival in northern Portugal, thus revealing the nature of this distributional limit. Evidence suggests that *F. serratus* has little adaptation potential to harsher conditions that may eventually occur at its distribution limit. We thus hypothesized that individuals from marginal populations would show lower physiological performance than central populations (see Pearson et al. 2009).

We performed 1 mensurative field experiment (*in situ*) and 1 manipulative (in culture) experiment to simultaneously analyse the effects of the major physical stressors acting during low tide in these 2 species. Temperature increase, desiccation and photosynthetic response of individuals were monitored during the low tide cycle in the 2 southernmost pop-

ulations of *F. serratus* in the mid-southern distributional range of *F. spiralis*, the unique localities in Portugal where both species coexist. In the intertidal zone, organisms should benefit from living in dense intraspecific stands that buffer a harsh physical environment (Bertness & Leonard 1997, Stachowicz 2001, Bruno et al. 2003). Thus, in the field experiment, we compared stress conditions between fronds exposed to full sun and air stress and those covered by other fronds to explore the role of conspecific self-covering as a stress-ameliorating mechanism. We considered self-covering to be a mechanism of low tide stress attenuation because algal morphology allows them to be protected under others of the same species. Previous studies in other brown algae have reported positive effects of crowding due to particular environmental constraints and species morphology (Schiel 1985, Lazo & Chapman 1998). These stress-buffering mechanisms have frequently been ignored by ecologists when examining structuring drivers along rocky shores (Bertness & Leonard 1997) and, like other biotic interactions, have been mostly neglected in relation to species distributions (Araújo & Louto 2007). The manipulative experiments combined harsh vs. mild levels of irradiance, atmospheric temperature, humidity and wind velocity, following an orthogonal design (modified from Martínez et al. 2012a). We expected to find overall additive effects of these climatic and non-climatic physical factors as previously found in fucoids (Martínez et al. 2012a, Ferreira et al. 2014), suggesting a certain generality for the stressors examined in macroalgae. We interpreted the results in light of the geographic distributions and responses of the species to climate change. The target species form canopies which provide shelter and substrate and serve as a nursery for other species of invertebrates, fish or algae (e.g. Viejo et al. 2011). These 2 fucoids are of key ecological importance to the coastal system as foundation species and autogenic engineers (Jones et al. 1997).

MATERIALS AND METHODS

Field experiment

We analysed the ecophysiological responses of *Fucus serratus* and *F. spiralis* adults during low tide in 2 populations in northern Portugal: Amorosa (41°38'30" N, 8°49'18" W) and Viana do Castelo (41°41'48" N, 08°51'11" W, hereafter Viana). These are the 2 southernmost populations of *F. serratus* in Europe (Araújo et al. 2011) and the unique localities

in Portugal where both species coexist. The experiment was carried out in November 2008 and March and September 2009 to test the temporal consistency under varying environmental conditions. Each population was monitored once in the above-mentioned months for a total of 6 monitoring surveys.

To characterize environmental conditions during the field experiment, air temperature and relative humidity were continuously recorded using 3 temperature–humidity data loggers (MicroLog EC650, Fourier Systems). Photosynthetically active radiation (PAR) was measured at the substrate level during low tide in March using a spherical quantum scalar sensor (QSL-2100, Biospherical Instruments). Additionally, daily radiant exposure, i.e. the sum of the irradiance expressed in joules per square metre, was obtained from the nearby meteorological station Amorosa (Instituto Português do Mar e da Atmosfera).

Variation in algae temperature and desiccation state in the 2 species was estimated by 6 successive measures of surface temperature and weight of 6 fronds exposed to aerial conditions vs. 6 fronds covered by other fronds of the same individual or self-covered (hereafter exposed and covered, respectively) during low tide.

To characterise the photosynthetic performance of the algae in response to emersion *in situ*, we measured maximum quantum yield of photosynthesis (F_v/F_m) of dark-adapted material using a MiniPAM (Heinz Walz) as an indicator of *in vivo* physiological stress following the method of Maxwell & Johnson (2000). F_v/F_m was determined in 6 exposed and 6 covered fronds of each species 2 times during low tide. Initial measurements were taken in the first 5 min after emersion (hereafter initial F_v/F_m), at the end of the emersion period (between 133 and 217 min of duration) and 15 min after re-immersion in a tide pool in darkness (hereafter recovery F_v/F_m). The responses of 288 individuals were assayed in the field (48 individuals per low tide on 6 monitoring dates).

Manipulative low tide stress experiment

To carry out winter and summer trials, 96 vegetative fronds of both *F. serratus* and *F. spiralis* were collected at Amorosa during low tide in November 2008 and August 2009. After collection, the fronds were immediately transported to the laboratory in a cool icebox in darkness and kept in an outdoor shaded 360 l tank at 16°C for 2 to 3 d to allow for acclimation.

To avoid nutrient limitation, seawater was enriched every 2 d by adding inorganic nitrogen (NaNO_3) and phosphorus (NaH_2PO_4) to a final concentration of over 50 μM nitrogen and 5 μM phosphorus. Initial frond weight was similar in both trials (1.5 ± 0.02 g fresh wt, mean \pm SD, $n = 384$).

Fronds were randomly assigned to 16 orthogonal combinations of irradiance, air temperature, humidity and wind using a modified version of the experimental design of Martínez et al. (2012a). Two levels of stress were included in each experiment for each factor to represent harsh vs. mild ameliorated conditions. Thus, irradiance levels were full incident light and reduced light conditions, the latter of which was simulated using 2 layers of neutral fibreglass mesh, which decreased incident light by approximately $65 \pm 5.6\%$ (mean \pm SD, $n = 6$). Harsh irradiation conditions were used in summer and less harsh conditions were used in winter because natural incoming irradiation is lower in winter than in summer (approximately $20.1 \pm 0.3\%$ lower, $n = 26774$). Ambient temperature represented ameliorated conditions in the winter trial, and a higher temperature obtained using heaters represented harsh conditions. In the summer trial, ambient temperature represented harsh conditions, and ameliorated conditions were obtained using portable air conditioner units. This resulted in a decrease in air temperature in the summer trial of approximately 30% (average temperature of 34°C in the harsh treatment vs. 24°C in the cooled chambers: $F_{1,2} = 98.83$, $p < 0.01$, with differences only between test date: $F_{2,48} = 30.29$, $p < 0.01$). Regarding humidity, mild conditions were created by increasing ambient humidity around 18% in the summer trial (26 ± 1.8 vs. $44 \pm 3.4\%$ in mild treatments, $n = 16$, $F_{1,16} = 25.19$, $p < 0.001$) using ultrasonic humidifiers (Saivod Humi 5L). Finally, harsh wind conditions were created using fans fixed inside the corresponding chambers, creating mean wind speeds of 2.8 m s^{-2} (measured with an anemometer, Extech Instruments). The effect of the fans on water loss was also tested in a trial experiment using wet sponges ($24.2 \pm 1.6\%$ water loss after 90 min with the fan vs. $15.9 \pm 0.7\%$, $n = 6$, significant differences: $F_{1,4} = 9.68$, $p < 0.05$).

These environmental scenarios were recreated inside white plastic chambers (20 l, length \times width \times height: $48 \times 30 \times 14$ cm) covered (not hermetically closed) with UV transparent Plexiglass covers (Plexiglass GS2458, Rohre). These chambers had in one lateral an intake of air with a little fan connected either to the portable air conditioner unit in the mild temperature treatments or to the open air in the

harsh temperature treatments in the summer trial. In November, the setup was slightly modified, and harsh conditions were created connecting air heaters. Additionally, 2 apertures in the other chamber end allowed the exit of the air. We used 2 replicated chambers for each combination of environmental treatments, for a total of 32 chambers (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m536p135_supp.pdf). Three fronds of each species were placed in each chamber for 90 min. Simulations were done outdoors on sunny days to ensure high solar irradiance and temperature and low atmospheric humidity.

The temperature of each frond was measured 10 min before the end of the emersion period using thermocouples. Before and immediately after emersion, fronds were weighed to determine their final desiccation state. The algae were then re-submersed in a seawater tank under the same conditions as in the acclimation tank but in darkness and left to rehydrate for 15 min before measuring F_v/F_m . The next morning (i.e. after an overnight recovery period of 20 h), F_v/F_m was remeasured.

Statistical analyses

The field responses of *F. serratus* and *F. spiralis* to emersion stress were analysed using several statistical approaches. To characterize the temporal change of temperature and desiccation state of fronds during the emersion periods, i.e. desiccation rate, we used linear and quadratic polynomial regressions, when significant (Sokal & Rohlf 2012).

To explore the relationship between frond temperature ($^\circ\text{C}$), desiccation state (% fresh wt lost) and *in situ* environmental conditions, we constructed linear mixed effects models (Crawley 2007) using data from the field study. These data were *in situ* solar irradiation, air temperature and humidity (from November and March 2008 at Amorosa and from March 2009 at Viana). Therefore, the predictors included in the model were solar irradiation, air temperature, air humidity and period of emersion. The factors frond and day of the mensurative study were added as random effects in the model. Since we performed repeated measures of the fronds, we included a correlation term in the models after examining the autocorrelation structure of the residuals (Crawley 2007). Backwards stepwise selection procedures were used to obtain the minimum adequate model. We started with the full model including all predictors and then removed fixed effect terms as long as model fit did

not significantly decrease, as assessed by ANOVA. All analyses were done in R 3.0.0 (R Development Core Team 2013).

Initial and recovery F_v/F_m values were analysed using ANOVA (Underwood 1997). The terms species (*F. serratus* vs. *F. spiralis*), population of origin (Amorosa vs. Viana) and exposure (exposed vs. covered) were treated as fixed factors, and date was a random factor in an orthogonal design. We performed one ANOVA with initial F_v/F_m data and a second analysis with recovery F_v/F_m data.

In the manipulative experiment, the effects of treatment on algal temperature, final desiccation state and F_v/F_m (after 15 min and 20 h) were analysed using ANOVAs. Data from both trials (winter and summer) were used in the same analysis by including the factor trial. Thus, irradiance (PAR), air temperature, humidity, wind, species and trial were included as fixed factors with 2 levels, and chamber was included as a random factor nested in the interaction of irradiance, air temperature, humidity, wind and trial.

Student-Newman-Keuls tests after significant ANOVA interactions (Underwood 1997) are shown in lower-case letters in the corresponding figures (i.e. Figs. 2 & 3). Homogeneity of variances was tested using the Cochran test (Underwood 1997). Analyses were carried out with Statistica 10 (StatSoft). We adopted 0.01 as the α threshold 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 chamber on predawn F_v/F_m values for the summer trial of the manipulative experiment: $F_{1,16} = 2.25$, $p = 0.027$). Although these differences were statistically significant at $\alpha = 0.05$, their magnitude was too limited to be indicative of distinct physiological performance.

RESULTS

Temperature, desiccation state and F_v/F_m in the field experiment

Environmental conditions at the seashore during the *in situ* experiment showed the expected seasonal patterns, with high levels of incident light and air temperature in September (summer) and low levels in November (autumn) (Table 1). In early spring (March), high levels of incident light and intermediate values of temperature were recorded. Maximum levels of air humidity were recorded in November, and intermediate levels were found in September at both localities and in March at Viana. The minimum average humidity was found in Amorosa in March (37%, Table 1).

As expected, emerged fronds from both species lost more water when exposed to full aerial conditions than when covered (Fig. 1, Table 2). In most cases, desiccation kinetics were linear, suggesting a passive process. However, on dates with high air temperature and low ambient humidity (in March and September at Amorosa and in September at Viana, Table 1), the desiccation of exposed fronds reached a maximum water loss threshold which ranged between 60 and 80 % fresh wt loss (Fig. 1c,e). Linearity was lost at the end of the emersion period, and clear saturation-type responses were evident. Both species followed this pattern but with different frequencies (3 times for *Fucus serratus* vs. once for *F. spiralis*) and magnitudes (lower water loss in *F. spiralis* than in *F. serratus*, Fig. 1c,e, Table 2, Table S1 in the Supplement at www.int-res.com/articles/suppl/m536p135_supp.pdf). Under covered conditions, most regressions were linear with low slope estimates, except for 2 significant quadratic polynomials that did not show a clear saturation plateau phase (Fig. 1d) and in one case where water loss was not

Table 1. Environmental conditions during the mensurative field experiment (first). Values of irradiance of radiant exposure measured at a nearby meteorological station on the day of the experiment, photosynthetically active radiation (PAR), air temperature, humidity and initial and final times of low tide are specified. na: not applicable

Population	Date	Radiant exposure (kJ m ⁻²)	PAR ± SE (max., min.) (µmol photons m ⁻² s ⁻¹)	Temperature ± SE (°C)	Humidity ± SE (%)	Initial time low tide (h GMT)	Final time low tide (h GMT)
Amorosa	18 Nov 2008	8021	1381 ± 1 (4144, 583)	15 ± 0.1	69 ± 0.5	09:38	13:50
	24 Mar 2009	22330	1894 ± 1 (2212, 943)	21 ± 0.3	37 ± 0.5	08:01	13:28
	7 Sep 2009	23518	na	28 ± 0.2	53 ± 0.5	09:00	17:52
Viana	19 Nov 2008	10762	na	13 ± 0.1	77 ± 0.6	10:05	14:15
	25 Mar 2009	22258	1839 ± 1 (2020, 1522)	17 ± 0.3	58 ± 1.1	07:44	12:18
	8 Sep 2009	21530	na	27 ± 0.3	51 ± 0.9	09:02	17:53

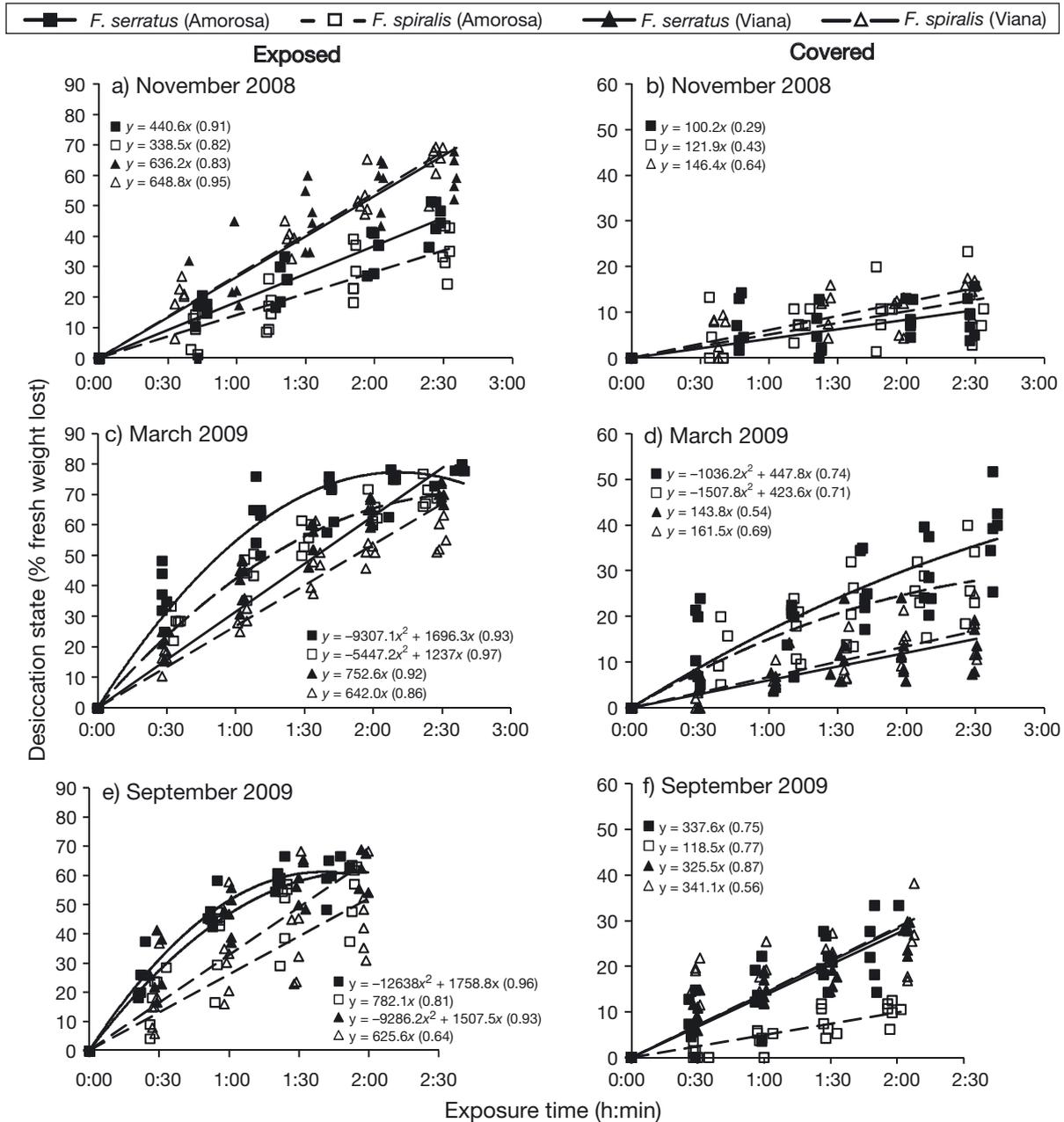


Fig. 1. Desiccation state (% fresh wt lost) for the field experiments on Amorosa (squares) and Viana (triangles) furoid algae populations on 3 dates. Filled symbols represent *Fucus serratus*, and open symbols represent *F. spiralis*. Measurements were taken in fronds exposed to aerial conditions and in fronds covered by other fronds during low tide. The equations of the linear or quadratic regression are shown. Values in brackets correspond to R^2

detected (*F. serratus* at Viana in November, Fig. 1b, regression line not shown). Even though the linearity of this response was lost in some cases, thus violating assumptions for ANOVA tests, analyses of the final values of desiccation state (Table S1 in the Supplement) were related to the interaction date \times species ($F_{2,120} = 10.44$, $p < 0.001$) and population \times date \times exposure conditions ($F_{2,120} = 25.06$, $p < 0.001$). Frond temperature was generally constant, but some varia-

tion was observed around the mean at the beginning of the mensurative experiment (not shown). Temperature was higher in exposed fronds in both species, thus reaching a higher desiccation percentage, as shown by averaged values of the last measurements taken (Table 2). ANOVA of the final temperature values (Table S1 in the Supplement) showed 2 statistically significant interactions, population \times date \times species ($F_{2,120} = 44.10$, $p < 0.001$) and population \times date \times

Table 2. Summary of the mean \pm SD frond temperature and desiccation percentages for each treatment during the 3 field mensurative experiments and summary of the results of the mixed linear models predicting temperature and desiccation rate of *Fucus serratus* and *F. spiralis* fronds fully exposed to air conditions (Exposed) or self-covered by other fronds (Covered) from the environmental parameters emersion time, solar irradiation, air humidity, air temperature and the intercept. Data were measured during the field mensurative experiments at 2 localities in northern Portugal, Amorosa and Viana in March and Amorosa in November. Results of linear mixed models: coefficient values for each predictor, significance level (ns: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) and Akaike's information criterion (AIC) for the minimum adequate model (MAM) using backwards stepwise selection of significant predictors ($n = 36$)

Species	Treatment	Amorosa Mar	Viana Mar	Amorosa Nov	Overall	Summary of linear model					
						Emersion time	Solar irradiation	Air humidity	Air temperature	Intercept	MAM AIC
Frond temperature		°C	°C	°C	°C						
<i>F. serratus</i>	Exposed	27.1 \pm 0.6	24.6 \pm 0.4	16.2 \pm 0.4	22.6 \pm 0.5	0.02***	ns	ns	0.71***	5.11*	393.7
	Covered	23.4 \pm 0.2	22.3 \pm 0.4	15.7 \pm 0.1	20.5 \pm 0.2	0.01***	ns	ns	0.45***	8.88***	352.3
<i>F. spiralis</i>	Exposed	28.7 \pm 0.5	23.2 \pm 0.9	14.7 \pm 0.1	22.2 \pm 0.5	-0.02*	0.01***	-0.47***	-0.27*	32.3***	414.5
	Covered	25.2 \pm 0.4	23.0 \pm 0.8	14.5 \pm 0.1	20.9 \pm 0.4	0.02***	ns	-0.11***	0.63***	12.6**	384.9
Frond desiccation		%	%	%	%						
<i>F. serratus</i>	Exposed	77.5 \pm 1.0	69.6 \pm 2.4	45.8 \pm 5.8	64.3 \pm 3.1	0.32***	0.004*	ns	3.4***	-336.2***	722.6
	Covered	38.9 \pm 3.6	16.2 \pm 6.0	10.2 \pm 1.4	21.8 \pm 3.7	0.13***	ns	ns	ns	ns	677
<i>F. spiralis</i>	Exposed	70.2 \pm 1.8	59.4 \pm 8.7	35.1 \pm 3.0	54.9 \pm 4.5	0.31***	ns	-0.63***	1.41**	ns	673.9
	Covered	28.3 \pm 3.6	16.0 \pm 2.3	12.6 \pm 2.3	19.0 \pm 2.7	0.08***	ns	ns	1.27***	-17.8***	549.2

exposure conditions ($F_{2,120} = 19.59$, $p < 0.001$). Note that for the September period, both populations reached the same temperature. Results from the mixed linear models fitted with data available for *in situ* physical predictors are shown in Table 2. Upon emergence during low tide, fronds gradually became affected by physical conditions. Thus, as expected, emersion time was a significant predictor for both frond temperature and frond desiccation, with higher linear coefficients in the latter (Table 2). Except in the case of the desiccation rate of covered *F. serratus* fronds, air temperature was a significant predictor of frond condition, suggesting the relevance of this physical factor even when fronds are covered by other fronds. Higher air temperatures generally resulted in higher frond temperatures and desiccation rates. Air humidity was not a significant predictor of *F. serratus* performance, but it was a significant predictor 3 out of 4 times in the case of *F. spiralis*. Furthermore, it did not follow the rigid rule of 0.01 in the case of desiccation rates of covered *F. spiralis* fronds. The sign of the coefficients suggested that higher humidity levels reduce frond temperature and desiccation rates. Irradiance was only significant in 2 cases, both in exposed fronds (Table 2). All of these physical predictors are inter-related, making it very difficult to completely separate their effects.

Regarding F_v/F_m , initial values were similar between populations, species and exposure conditions but not between dates. This factor was statistically significant ($F_{2,120} = 5.51$, $p = 0.005$) in the ANOVA

performed with initial F_v/F_m data (Table S1 in the Supplement). Initial F_v/F_m values were high (0.731 ± 0.005 in November, 0.716 ± 0.004 in March and 0.692 ± 0.005 in September; mean \pm SE, $n = 144$), suggesting optimal photosynthetic performance of both species at the beginning of low tide. The ANOVA performed with recovery F_v/F_m data (after emersion and rehydration periods) showed significant population \times date ($F_{2,120} = 19.66$, $p < 0.001$) and date \times species interactions ($F_{2,120} = 9.22$, $p < 0.001$, other sources of variation not shown, Table S1 in the Supplement). The F_v/F_m of fronds exposed to aerial conditions decreased greatly on dates with higher environmental stress in March and September 2009 (Fig. 2a). In September, this decrease was only observed in *F. serratus*, suggesting lower tolerance of this species, and a decrease was also evident in March (Fig. 2a). Fronds that remained covered by other fronds showed no marked photosynthetic inhibition, as indicated by the relatively high F_v/F_m values, although values were somewhat lower for both species on the dates with the harshest weather conditions (Fig. 2b).

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

The manipulative experiments simulating low tide conditions showed that frond temperature differed between both trials ($F_{1,256} = 240.55$, $p < 0.001$, Table S2 in the Supplement at www.int-res.com/articles/

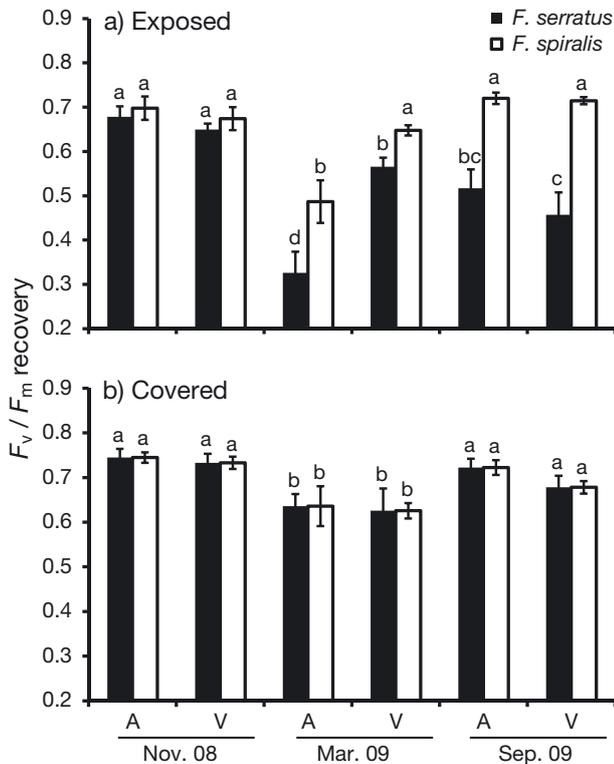


Fig. 2. Averaged maximum quantum yield of photosynthesis (F_v/F_m) (mean \pm SE, $n = 35$) for the mensurative field experiment on Amorosa (A) and Viana (V) fucoid algae populations on 3 dates. Filled bars: *Fucus serratus*; open bars: *F. spiralis*. Measurements were taken in (a) fronds exposed to aerial conditions and (b) fronds covered by other fronds during low tide. Means with a common letter do not differ significantly based on Student-Newman-Keuls tests at $\alpha = 0.05$

[suppl/m536p135_supp.pdf](#)). This frond temperature was clearly affected by the additive effect of harsh air temperature (reaching an average temperature of 23.0 ± 0.3 and $35.6 \pm 0.3^\circ\text{C}$ for the high or 17.4 ± 0.2 and $28.3 \pm 0.2^\circ\text{C}$ [mean \pm SE, $n = 96$] for the low air temperature treatments in the winter and summer trials, respectively), irradiance (21.8 ± 0.3 and $34.0 \pm 0.3^\circ\text{C}$ for the harsh or 18.6 ± 0.2 and $29.9 \pm 0.3^\circ\text{C}$ [$n = 96$] to mild irradiance treatments in the winter and summer trials, respectively) and humidity conditions (22.1 ± 0.3 and $32.8 \pm 0.3^\circ\text{C}$ for the low or 18.3 ± 0.2 and $31.0 \pm 0.2^\circ\text{C}$ [$n = 96$] for the high humidity treatments in the winter and summer trials, respectively), with a mean temperature increase of 6.4, 3.7 and 2.8°C for the high air temperature, full irradiance and low humidity treatments, respectively. The significance of the factor and the interaction including chamber suggested some environmental heterogeneity of the experimental prototypes, but no major interactive effects were found between stressors in

frond temperature. Wind condition was not associated with significant effects (Table S2 in the Supplement).

As mentioned before, the non-linear nature of water loss with time impeded a proper comparison of desiccation state using ANOVAs. When the threshold of maximum water loss was reached, final desiccation state values were similar between the different treatments. However, desiccation kinetics were probably not the same among treatments and species, as suggested by the field experiment. Nevertheless, a tentative ANOVA (Table S2) related desiccation state to species ($F_{1,32} = 13.33$, $p < 0.001$) and an irradiance \times humidity interaction ($F_{1,32} = 8.95$, $p = 0.005$). These results may suggest that desiccation state is a response which is dependent on the interactive effect of environmental stressors, but because of the constraint of the statistical approach, caution should be taken in assuming the generality of this response.

F_v/F_m results showed that harsh levels of irradiance, air temperature and humidity were associated with decreases in F_v/F_m after the 15 min recovery, whereas wind did not produce a significant effect. No significant evidence of synergistic effects was found. The interaction irradiance \times air temperature \times humidity had a significant effect on recovery F_v/F_m , suggesting an antagonistic stress-ameliorating response driven by humidity. Thus, high humidity conditions compensated for thermal stress when irradiance was also low, resulting in high F_v/F_m values (similar to those in the corresponding low temperature treatment, see lower-case letters in Fig. 3a). When other factors were pooled, recovery F_v/F_m values decreased as a result of high irradiance (by approximately 50 and 41% in *F. serratus* and *F. spiralis*, respectively), low humidity (by 22 and 14% in *F. serratus* and *F. spiralis*, respectively) and high air temperature (by 5.9 and 3.7% in *F. serratus* and *F. spiralis*, respectively). The magnitude of inhibition was greater in *F. serratus* in all combined treatments (significant species effect in Table S2, Fig. 3a).

After the 20 h immersion period under optimal conditions, the algae were able to recover F_v/F_m from the stress, but previous values were only reached in the less stressful treatments. Differences in F_v/F_m values between the 2 species were greater than after the 15 min recovery period, suggesting that *F. serratus* has a slower recovery rate compared to *F. spiralis* (Fig. 3b). The interaction irradiance \times air temperature \times humidity \times species was significant. Inhibiting effects of harsh conditions were additive except for the attenuating effect of humidity on thermal stress

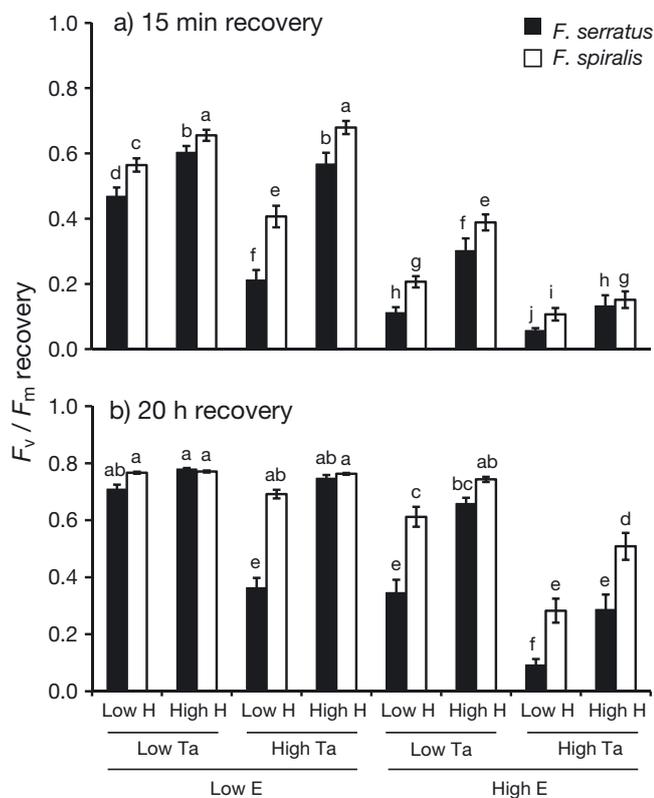


Fig. 3. Averaged maximum quantum yield of photosynthesis (F_v/F_m) (mean \pm SE, $n = 24$) for the manipulative low tide stress experiment (data pooled from November 2008 and August 2009 trials) as a function of low or high humidity (H), air temperature (Ta) and irradiance (E) treatments. Filled bars: *Fucus serratus*; open bars: *Fucus spiralis*. Measurements were taken after rehydration within (a) 15 min or (b) 20 h recovery periods after the experiments. Means with a common letter do not differ significantly based on Student-Newman-Keuls tests at the $p = 0.05$ level

under shaded conditions. The different recovery rate of the 2 species justifies the inclusion of the corresponding term in the interactions. In the field, dehydration and temperature were only positively related to air humidity in *F. spiralis*. Wind conditions did not have a significant effect, and other interactions including chamber or date reflected some environmental variability in the experimental units and trials (Table S2 in the Supplement).

DISCUSSION

Results showed that, in general, the main physical factors that drive stress during low tide in the intertidal have an additive effect on the physiological responses of fucoid algae. During emersion, stressful conditions created by high air temperature, in addi-

tion to the non-climatic conditions of high solar radiation and low humidity, reduced the physiological performance of *Fucus serratus* and *F. spiralis* at the southern distribution of the first species. The temperature of the fronds exposed to aerial conditions increased rapidly, and water content decreased gradually until a maximum desiccation threshold was reached, i.e. a saturation-type functional response, with few signs of major synergistic effects between the physical stressors in driving these responses. We found no signs of harmful interactive effects in this study or in previous research (Martínez et al. 2012a, Ferreira et al. 2014). However, we did detect an antagonist effect on photosynthetic performance (expressed as F_v/F_m) where thermal stress was compensated by high ambient humidity under shade conditions. This was ultimately responsible for the greater growth of *F. serratus*, as shown in Martínez et al. (2012a). Intertidal seaweeds often form dense populations of individuals with many fronds arising from a holdfast or rhizoids. Such high density of populations of modular individuals provides protection against harsh conditions during emersion periods, functioning like an intraspecific facilitation mechanism (Lazo & Chapman 1998, Viejo & Åberg 2001, Bruno et al. 2003). Conspecific self-covering helps to retain humidity and lowers incoming light by self-shading, effectively protecting the algae from overheating, desiccation and photoinhibition (Johnson & Scheibling 1987, Scrosati & De Wree 1997, 1998, Lazo & Chapman 1998), a mechanism similar to mechanisms recorded in other stressful habitats (Fajardo & McIntire 2011).

Although the 2 species responded similarly to stress, *F. serratus* showed lower stress tolerance and a slower recovery rate. This differential tolerance, which was consistent across the experiments, is in agreement with the northern geographic distribution of *F. serratus* and its lower position in the intertidal zone relative to *F. spiralis*. It also agrees with the observed decline of *F. serratus* in the last decade in the Cantabrian Sea (northern Spain), whereas a persistent decline of *F. spiralis* has not been observed to date in this area (Viejo et al. 2011, Lamela-Silvarrey et al. 2012, Martínez et al. 2012a,b). Ferreira et al. (2014) also found that *F. spiralis* showed higher tolerance to summer emersion stress and recovered levels of photosynthetic performance more efficiently than *F. vesiculosus*. The latter species and other dominant species of the low intertidal rocky shore are also in retraction in areas adjacent to Portugal.

Regarding the ecophysiological mechanisms behind responses, our results showed that light had

deleterious effects on photoinhibition, overheating and desiccation of the algae. F_v/F_m was the physiological response most tightly linked to PAR intensity because it is a direct measure of the *in vivo* performance of Photosystem II (PSII) (Maxwell & Johnson, 2000). Photosynthesis inhibition was followed by slow recovery under optimum immersion conditions, which was proportional to the magnitude of the stress previously suffered by the algae i.e. full recovery after 20 h under benign conditions but not under harsh conditions (Ferreira et al. 2014). Excessive light energy absorbed by the photosynthetic apparatus may lead to direct inhibition and damage (Häder & Figueroa 1997). Excess light may also cause oxidative stress by increasing the production of reactive oxygen species, preventing the synthesis of proteins *de novo*, which is essential for the repair of PSII machinery (Nishiyama et al. 2011). Our results suggest that both species are able to synthesize proteins *de novo*, developing a dynamic or transient inhibition and recovery response, but this recovery process may not necessarily be complete before the onset of the next low tide, particularly in *F. serratus* (Takahashi & Murata 2008). However, the same fronds are unlikely to lie on the algae surface in consecutive tides. Moreover, inhibition was reversed by the self-shading of algae in the field and by lowering incoming PAR 65% with neutral filters in the experiments. Irradiance values measured under the cover of fronds in adult plants were below the detection limit of a spherical PAR sensor and thus much lower than those used in our experiments. In dense populations with adult plants, a significant portion of the fronds remain covered by the top layer of fronds during low tide. In stressful habitats, facilitation may create positive density effects in plant populations (Bertness & Callaway 1994). In seaweed assemblages, intraspecific facilitation has been reported in areas with wave exposure (Andrew & Viejo 1998), grazing (Chapman 1995) or desiccation stress (Vadas et al. 1992). However, ecophysiological studies have mostly neglected stress-ameliorating mechanisms.

The environmental condition that generated the highest overheating was air thermal stress, which is also associated with oxidative stress (Lobban & Harrison 1997, Helmuth et al. 2006) as well as with the denaturalization of protein and nucleic acids, the instability of cell membranes and the impairment of enzymatic reactions (Lobban & Harrison 1997). Thermal stress may exceed the buffering capacity of cell recovery mechanisms (Collén & Davison 1999, Eggert 2012). Air temperature also had a relevant effect on frond desiccation rate during the field experi-

ments and reduced photosynthetic performance, i.e. low F_v/F_m in the experimental simulations of emersion stress. Overall, our results suggest that temperature makes an additive contribution to stress during low tide.

Air humidity was inversely proportional to desiccation rate, directly proportional to photosynthetic potential and inversely proportional to frond temperature (in order of importance in the manipulative experiment). Low ambient humidity values were thus related to the inhibition of algae. We may expect direct physiological damage due to cellular water loss resulting from low external vapour pressure (Dromgoole 1980, Lobban & Harrison 1997). Furthermore, overheating due to low humidity could trigger damage induced by thermal stress, for example oxidative stress (see previous paragraph). Changes in frond temperature at different levels of hydration have been reported for several macroalgae (Dromgoole 1980, Bell 1995, Martínez et al. 2012a). Again, frond cover on the algae surface was an effective mechanism in ameliorating stress due to low humidity (Johnson & Scheibling 1987, Lobban & Harrison 1997). This mechanism is similar to that observed in the field experiment with self-shading or the decrease of irradiation due to increased cloudiness. The most surprising effect of humidity, and the only interactive response detected in this and a previous study, was the compensation of thermal stress under shade conditions, resulting in greater F_v/F_m values. In a previous study, this was linked to enhanced growth in *F. serratus* and was observed under high light conditions (Martínez et al. 2012a). The same response was also found in *F. vesiculosus* in a previous study (Martínez et al. 2012b). Consequently, the algae were in better physiological condition than expected considering ambient temperature during low tide. To our knowledge this response, which was consistent across the experiments, has not been described previously. We believe that the lack of a clear distinction between ambient humidity and desiccation in previous research, the former an environmental condition and the latter an organism's response, may have obscured the important role of atmospheric humidity in algae and plants in marine and terrestrial systems (but see Dromgoole 1980).

With regard to the geographic distribution of *F. serratus*, our results support the significant contribution of low tide stress to this species' distribution in the north of the Iberian Peninsula, as previously stated in Viejo et al. (2011) and Martínez et al. (2012a,b). As we move south along the Atlantic coast of northern Portugal, where this species' southernmost limit oc-

curs, incoming irradiance and maximum air temperature (in August) show a clear increase. This pattern is magnified by the lower cloud cover in this area compared to further north in the very northwestern corner of the Iberian Peninsula (Sanchez-Lorenzo et al. 2013). This results in harsh low tide conditions at the Portuguese limit, which could be a key factor in shaping the distributional limit of *F. serratus* in this and previous studies (Martínez et al. 2012a). However, it should be highlighted that lack of available substrate may also play a key role, since long rocky shores are not abundant further south in this area (Boaventura et al. 2002). This reinforces the idea that low tide stress is comparatively more important than environmental conditions of the water in shaping the Portuguese limits of this and probably other cold-temperate macroalgae. Although border populations of most fucoids in this area have been historically stable (e.g. Araújo et al. 2011), contractions have been observed in adjacent areas, like Morocco, northern Spain and the Bay of Biscay (Lamela-Silvarrey et al. 2012, Nicastro et al. 2013). The abundance of *F. serratus* populations also seems to have decreased in the last decade in northern Portugal (F. Arenas pers. obs.). While it is well known that the contraction of *F. serratus* in northeastern Spain is mainly due to an increase in ocean temperature (Marsh et al. 2008, Jueterbock et al. 2013), the distribution of *F. serratus* in northwestern Portugal responds to different combinations of factors (Martínez et al. 2012a). We hypothesized that the future contraction of *F. serratus* in this area would be due to increased air temperature. Regarding *F. spiralis*, we expect this species to have higher resilience to physical stress in this area due to its higher physiological tolerance than *F. serratus* (this study and Ferreira et al. 2014). The mechanisms driving this response are probably similar to those argued for *F. serratus* but at higher stress levels.

Thus, results confirm that *F. serratus* has lower physiological tolerance to environmental conditions in southern Europe than other fucoids, even though they are all considered cold-temperate species (e.g. Lüning 1990) and have a close phylogenetic position (Serrão et al. 1999). This species is predicted to decline more than other dominant intertidal fucoids in southern Europe. Although the competitive dominance of *F. serratus* over *F. vesiculosus* in the mid-Cantabrian Sea was suggested in the 1990s (Arrontes 2002), the opposite may occur, and *F. serratus* may continue to decline under future scenarios of warmer conditions. This predicted decline would be particularly intense in border populations, where smaller

dwarf morphs are dominant (Viejo et al. 2011) and thus the effect of conspecific ameliorating mechanisms would be lower. Moreover, maladaptation to stress in edge populations has been reported (Pearson et al. 2009). Our results, together with those obtained by Martínez et al. (2012a) and Ferreira et al. (2014), suggest that the additive effects of climatic factors and non-climatic physical variables may provide insight into the mechanisms governing macroalgae distribution and help to forecast responses under future scenarios of global warming. Evidence of addictiveness has also been found by other authors in marine ecosystems (see review by Crain et al. 2008), suggesting that additive and antagonistic effects may be as common as synergies (Sandman et al. 2008).

CONCLUSIONS

Intertidal systems are natural laboratories where species occur close to their physiological tolerance thresholds, and shifts driven by climate change have already become clear (Fernández 2011, Díez et al. 2012, Lamela-Silvarrey et al. 2012, Duarte et al. 2013). The clumping growth of many seaweeds creates dense populations of fronds in the intertidal, and the fronds on the top partially protect a significant percentage of fronds in the population against emersion stress. Thus, seaweeds retain ambient humidity and shade themselves, lowering overheating, desiccation and photoinhibition, resulting in less physiological damage. Subtidal algae tend to have single large canopies to absorb maximum incoming light (Wahl et al. 2011), a trait that is not crucial in intertidal systems. Contrary to potential unexpected damage, our results suggest higher resilience of algae to low tide stressors than expected by their additive effects. This could be an important consideration when predicting shifts to climate change. Indeed, predictive models that only consider additive effects of climatic factors, and recently those that also include non-climatic physical predictors, have been successfully used in terrestrial systems (Darling & Côté 2008 and references therein, reviewed in Austin & Van Niel 2011). However, variation in non-climatic factors has not been included in traditional biogeographical models of oceanic organisms based on isotherms (reviewed in Lüning 1990). Physiological knowledge from a mechanistic perspective can help to explain current species distributions and increase the accuracy of climate change predictions (see Denny et al. 2009, Buckley et al. 2011).

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