

Intraspecific phenotypic variation in two estuarine rhodophytes across their intertidal zonation

Raquel Sánchez de Pedro¹  · Ulf Karsten² · F. X. Niell¹ · Raquel Carmona¹

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Abstract The intraspecific variability in the physiology and biochemical composition of the rhodophytes *Bostrychia scorpioides* (Hudson) and *Catenella caespitosa* (Withering) was investigated in relation to the environmental gradients across their 30-cm intertidal vertical zonation in the Palmones estuary (Southern Spain). Both species are spatially segregated but overlap at intermediate tidal levels, with *B. scorpioides* dominating at intermediate to upper parts and *C. caespitosa* at lower to intermediate ones. Photosynthesis–irradiance (PE) curves, nutrient uptake rates, pigment content, elemental composition and organic osmolytes were compared between specimens collected from their upper and lower zonation limits. Photosynthetic maximum capacity (P_{max}) and efficiency (α), and the content of liposoluble pigments increased from upper to lower individuals of *B. scorpioides*, reflecting its photoacclimation to a broad range of irradiance. Osmotic acclimation via increased concentrations of organic osmolytes (D-dulcitol and D-sorbitol) was only detected in upper specimens of *B. scorpioides*. In *C. caespitosa*, ammonium and phosphate uptake, total P content and phycobiliprotein content were lower in upper specimens. Decreasing turnover rates

of internal nutrients towards upper tidal positions support the ability of *B. scorpioides* to cope with reduced nutrient availability due to longer emersion. This study underlines the occurrence of phenotypic variations in a small-scale intertidal zonation. How such changes may influence the outcome of interspecific interactions and shape this intertidal community is discussed.

Introduction

Macroalgal zonation is the most distinctive feature of intertidal environments. Many studies have managed to understand the observed distribution patterns by examining physiological responses to resource availability (i.e. light, water, nutrients, space), regulating factors (i.e. temperature, salinity, pH, water motion) and the interactions with co-occurring species (i.e. herbivory, competition, facilitation) (Carpenter 1990; Lüning 1990; Hurd et al. 2014). Intertidal seaweeds face long emersion, high exposure to irradiance and temperature, and reduced nutrient availability, which intensify and fluctuate more drastically towards the upper zonation limits (Thomas et al. 1987b; Hurd and Dring 1991; Tomanek and Helmuth 2002; Skene 2004; Kim et al. 2013). Though high thermal and desiccation tolerance seem to be a prerequisite to inhabit such challenging environment (Zaneveld 1969; Davison and Pearson 1996), its energetic costs should be balanced by the potential benefits in a way that ensures survival (Johnson et al. 1998; Hunt and Denny 2008; Karsten 2012).

Physiological acclimation and adaptation to environmental gradients allow macroalgae to modify their resource acquisition, leading to the phenotypic and/or genotypic differentiation at inter- and intraspecific levels (Wattier and Maggs 2001; Bischof et al. 2006; Hays 2007; Zardi et al.

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✉ Raquel Sánchez de Pedro
rsdpc@uma.es

¹ Departamento de Ecología, Facultad de Ciencias, Universidad de Málaga (UMA), Campus de Teatinos, s/n, 29071 Málaga, Spain

² Institute of Biological Sciences, Applied Ecology and Phycology, University of Rostock, Albert-Einstein-Strasse 3, 18057 Rostock, Germany

2011; Krueger-Hadfield et al. 2013). Physiological changes in pigment content and photosynthetic activity enable their growth under different light environments (Bjorkman and Holmgren 1963; Coutinho and Yoneshigue 1988; Beach and Smith 1996a, b; Fairhead and Cheshire 2004). Adjustments in their internal nutrient content, nutrient uptake and turnover rates (internal nutrient recycling) can occur in response to different nutrient availability, due to variable nutrient concentrations or differential inputs, exclusively via submersion (Thomas et al. 1987a; Phillips and Hurd 2003; Kim et al. 2008; Young et al. 2009). Also, desiccation tolerance can be enhanced when intertidal seaweeds are exposed to harsher abiotic conditions (Hunt and Denny 2008; Schagerl and Möstl 2011; Delebecq et al. 2013). On the other hand, genotypic differentiation has been recently identified among conspecifics located at different tidal elevations or from different intertidal microhabitats, where tidal action and emersion-related variables act as the main selective pressures (Hays 2007; Zardi et al. 2011; Krueger-Hadfield et al. 2013; Clark et al. 2013; Mota et al. 2015; Muangmai et al. 2016).

In community ecology, there is an increasing interest on how intraspecific variability can modify the outcome of ecological interactions (Bolnick et al. 2011). For benthic organisms, Edwards and Connell (2012) suggested that the strength and outcome of the interspecific interactions may depend on their ability to optimize the resource acquisition along the tidal gradient. Interspecific interactions gain relevance at low and intermediate tidal levels where environmental stability increases (Lewis 1964; Olson and Lubchenco 1990), although harshness does not necessarily exclude them (Chesson and Huntly 1997). Competition for resource availability (i.e. space, light, nutrients) among seaweeds is a dynamic and complex process (Miller 1967; Dudgeon et al. 1999; Underwood 2000), and it is more frequent in nutrient-enriched environments where herbivory is low or absent (Fong and Paul 2010). Despite estuaries fulfil these habitat conditions, interspecific interactions among estuarine macroalgae have been poorly studied, probably due to the low number of species (Davey and Woelkerling 1985), but clear intertidal zonation patterns have been described in them (Coppejans and Gallin 1989; Phillips et al. 1996; Peña-Salamanca et al. 1999).

In the Natural Area of Palmones estuary, a shallow mesotidal temperate estuary located in Algeciras Bay (Southern Spain), *Bostrychia scorpioides* (Hudson) and *Catenella caespitosa* (Withering) grow attached to the basal stems of the halophytic salt-marsh vegetation located at the fringe of the tidal channels. Species from these genera possess a high phenotypic variability and genetic diversity (Karsten et al. 1994; Zuccarello et al. 2011; Muangmai et al. 2016), and can grow under a broad variety of light,

salinity and emersion conditions (Almodóvar and Biebl 1962; Davey and Woelkerling 1985; Peña-Salamanca et al. 1999; Karsten 2012). In Palmones estuary, *B. scorpioides* and *C. caespitosa* are spatially segregated within a narrow intertidal zonation with a vertical extent of 30 cm, but they overlap at intermediate tidal levels. *Catenella caespitosa* occupies the lower to intermediate intertidal zone (1.05–1.20 m above lowest astronomical tide (LAT)), whereas the intermediate to uppermost one is dominated by *B. scorpioides* (1.15–1.30 m above LAT) (Sánchez de Pedro et al., 2013, 2014). In an earlier study (Sánchez de Pedro et al. 2014), the potential for photoacclimation of these species was evaluated under laboratory conditions, showing that: (1) *C. caespitosa* had a greater ability than *B. scorpioides* to grow and acclimate to shaded conditions; (2) both species performed similarly at the irradiance measured at their overlap zone; and (3) other factors distinct than high irradiance prevent *C. caespitosa* from growing at the upper parts of the intertidal. A plausible explanation for the zonation of these species was proposed based on their differences in nutrient uptake abilities and internal nutrient turnover rates (Sánchez de Pedro et al. 2013).

The present study aims to identify the intraspecific variability in the physiology and biochemical composition of *B. scorpioides* and *C. caespitosa*, in relation to their zonation pattern. We hypothesize that each species would be physiologically constrained by a different resource (i.e. light, nutrients) or regulating factor (i.e. temperature, desiccation) towards their respective distribution limits, as none of them dominates the overall resource supply. To test this, we assessed photosynthesis, nutrient uptake and biochemical composition of specimens collected from the lowest and highest tidal elevations of occurrence. The possible types of interspecific interactions are ultimately discussed considering the phenotypic variability in the use of shared resources.

Materials and methods

Sampling site, algal collection and experimental conditions

Basal stems of *Sarcocornia perennis* (Mill.) A. J. Scott. (Chenopodiaceae) homogeneously covered by thalli of *Bostrychia scorpioides* and *Catenella caespitosa* were collected from the intertidal zone of the Palmones estuary (36° 10' 13.4076" N, 5°26' 27.5742" W), at neap low tide, in March 2013. This estuary has semi-diurnal tides, and the amplitude of tidal movements ranges from 0.6 to 2 m (Clavero et al. 1997). Tidal data were obtained from the nearest tide gauge (Algeciras, 5.40° W, 36.18° N, <http://www.puertoes.es/en-us/oceanografia/Pages/portus.aspx>).

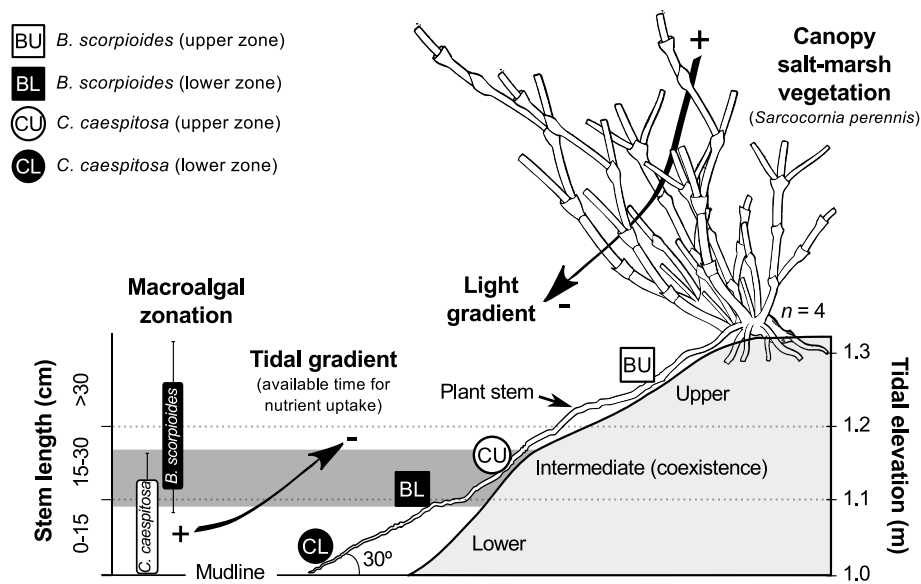


Fig. 1 Estuarine intertidal zone in Palmones estuary (Southern Spain) showing the distribution of the rhodophytes *Bostrychia scorpioides* and *Catenella caespitosa* on a basal stem of the halophyte *Sarcocornia perennis*. The dark grey area indicates the 2-species coexistence area at intermediate tidal levels. Thalli for the experiments were collected from four independent stems, at the upper and lower distri-

bution limits for each species. Algal zonation along the stem length relative to mudline and tidal elevation above the lowest astronomical tide (LAT) are represented. The original illustration of *Sarcocornia perennis* was taken from Britton and Brown (1913), available online from <https://commons.wikimedia.org> with permission of Public Domain (USDA)

Table 1 Mean environmental conditions at different tidal levels of the macroalgal zonation of *Bostrychia scorpioides* and *Catenella caespitosa* in the Palmones river estuary, between February and March 2013

	Tidal position (m above LAT)	Irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$)	Air temperature ($^{\circ}\text{C}$)	Total submersion time per diurnal tidal cycle (h)
<i>B. scorpioides</i>				
Upper	1.25	230	15.1 ± 0.7	5
Lower	1.10	20–30	13.1 ± 0.1	7.5
<i>C. caespitosa</i>				
Upper	1.15	70	13.7 ± 0.3	7.5
Lower	1.05	5–10	11.9 ± 0.1	10

Lowest astronomical tide (LAT)

Figure 1 illustrates how basal woody stems of *S. perennis* were prostrated in the estuarine intertidal zone, forming a 30–40° angle with the mudline. Distribution of each species was measured relative to the stem length, from the mudline (0 cm, 1 m above LAT) upwards (up to 45–50 cm) (Fig. 1). In spite of the narrow vertical extent of the macroalgal zonation, irradiance, air temperature and submersion hours per tidal cycle differ substantially among tidal levels (Table 1). Irradiance values for thalli located at each tidal position were obtained from the light attenuation curves beneath the *S. perennis* canopy obtained by Sánchez de Pedro et al. (2014). Air temperature during the sampling increased from 11.9 ± 0.1 to 15.1 ± 0.7 °C from lower to upper tidal positions (Table 1). For winter months, mean water temperature was 13 ± 1.1 °C and mean relative humidity was

79.6 ± 11.4 %. Over a diurnal tidal cycle intertidal zone is submerged from 5 to 10 h (Table 1). Nutritional conditions during late winter were $81 \mu\text{M NH}_4^+$, $23 \mu\text{M NO}_3^-$ and $4.1 \mu\text{M PO}_4^{3-}$, with a water-column DIN:PO₄³⁻ ratio of 25. Water samples were analysed in an automated continuous flow analyser (QuAatroAQ2AACE, Seal Analytical), using standard methods for ammonium (Slawyk and MacIsaac 1972), nitrate (Shinn 1941; Wood et al. 1967) and phosphate (Murphy and Riley 1962).

The algae-covered stems were transported in plastic bags to the laboratory, where vegetative thalli of each species were carefully removed from their respective upper and lower limits of distribution (Fig. 1). Fresh material was gently cleaned from sediment and epiphytes and rinsed in nutrient-free artificial sea water (ASW) prior to the experiments.

Macroalgal measurements in the laboratory started at the same time as the high tide in the field (15:50 GMT), in order to simulate the re-submersion. Photosynthesis and nutrient uptake experiments were performed in parallel with four independent replicates of each species at 15 ± 1 °C and at a photon fluence rate of $45 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ of white light (cool daylight, F-18 W/54-765 Syl-vania) in a 12:12-h light/dark cycle. These conditions are reflective of what these species experience in the estuary at intermediate tidal levels (Sánchez de Pedro et al. 2014). All irradiances provided in this study were measured using a spherical sensor (US-SQS/L, Walz, Germany) connected to a radiometer (LI-250A Quantum, Radiometer, Photometer, LI-COR Biosciences, Lincoln, Nebraska, USA). Initial samples were taken for the analyses specified in the subsection “biochemical composition”.

Oxygen evolution rate vs irradiance curves (P – E curves)

Photosynthesis was determined by oxygen evolution using a Clark-type oxygen electrode in a 2.5 ml DW1/AD chamber (Oxygraph systems, Liquid-Phase Oxygen Electrode Chamber, Hansatech Instruments), thermostated by a F25-ME Refrigerated/Heating Circulator (JULABO USA, Inc.) connected to the water jacket of the electrode. About 25 mg of alga was held in the reaction vessel containing 1.5 ml of filtered natural sea water (NSW) (Whatman GF/C, Maidstone, UK), pH 8.2 and salinity of 36. Light was provided by a halogen lamp (KL 1500 compact Schott). Respiration (R_d) was measured in darkness before switching on the light. Oxygen evolution was recorded for 5–10 min at twelve different photon fluence rates ranging from 5 to $650 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, which were obtained using neutral density filters. Photosynthetic rates were expressed on fresh weight (FW), area (SA) and chlorophyll *a* (chl *a*) basis. To normalize the photosynthetic parameters on a surface area basis, a relationship of 107 and 43 g FW cm^{-2} for the fresh weight per unit surface area ratio (FW:SA) was used for *B. scorpioides* and *C. caespitosa*, respectively. This relationship was estimated by determining the surface area of samples previously weighed ($n = 40$), following the procedure described by Rosenberg and Ramus (1984). Flat images were obtained from the thalli by scanning them in a CanoScan LiDE 25 (Canon USA, Melville, New York, USA), and thallus area was quantified using ImageJ (Image Processing and Analysis in Java, National Institutes of Health, Bethesda, Maryland, USA).

To test for interspecific differences, net photosynthetic rates (NPR) at the mean irradiance at each tidal elevation (E_z) were compared. P – E parameters were obtained by fitting the data to nonlinear least-squares regression analysis,

following the equation of Harrison and Platt (1986), based on a previous procedure of model selection, which is described in the subsection “Statistical analysis”. The equation is:

$$P = P_{\max} \left[1 - \exp \left(-\alpha \times \frac{E}{P_{\max}} \right) \right] \quad (1)$$

where P_{\max} is the maximal photosynthetic rate at saturating irradiance, α is the photosynthetic efficiency or the ascending slope at limiting irradiance and E is the irradiance. The light saturation parameter (E_k) was estimated as the ratio P_{\max}/α (Henley 1993), and the light compensation point (E_c) as the ratio between dark respiration rates and the photosynthetic efficiency (R_d/α) (Enriquez et al. 1996).

Biochemical composition

To determine chl *a* and total carotenoids, about 0.25 g FW was extracted in *N,N*-dimethylformamide (DMF) and their concentrations were calculated spectrophotometrically (Wellburn 1994). The same amount of algae was used to extract phycobiliproteins (PBP) in 0.1 M phosphate buffer (pH 6.8), homogenized at 4 °C using a mixer mill (model MM400, Retsch UK Ltd, Whitford, UK) and then centrifuged at $11,000 \times g$ for 15 min. R-phycoerythrin (R-PE) and R-phycoerythrin (R-PC) concentrations were determined in the supernatant spectrophotometrically using the chromatic equations of Beer and Eshel (1985). Total soluble proteins (TSP) were also determined from the same extract as for PBP, following the bicinchoninic acid method (Smith et al. 1985).

Thalli for elemental composition (C, N, P) and organic osmolytes analysis were dried at 60 °C overnight and then powdered with a mixer mill. Elemental C and N content was determined using a CHN elemental auto-analyser (model 2400C; PerkinElmer Analytical Instruments, Waltham, Massachusetts, USA) by DOI method (Kristensen and Andersen 1987). Total P content was analysed using the procedure of Pardo et al. (1999) and then determined by the green malachite method (Fernandez et al. 1985). Total C, N and P content of algae was expressed as percentage DW, and tissue C:N and N:P values as molar ratios. Organic osmolytes were extracted from 10 to 15 mg dry algal powder. The heteroside digeneaside (α -D-mannopyranosyl-(1-2)-glycerate) and the sugar alcohols D-sorbitol and D-dulcitol in *B. scorpioides* (Karsten et al. 1990, 2007) and the heteroside floridoside (α -D-galactopyranosyl-(1-2)-glycerol) in *C. caespitosa* (Karsten et al. 1995) were quantified following the analytical HPLC methods described by Karsten et al. (1991, 2005). Final contents were expressed as mmol kg^{-1} DW. For all biochemical component analyses, four independent samples were used.

Nutrient uptake and turnover rates

About 100 mg FW of algal biomass was incubated in 25 mL of nutrient-enriched ASW (40 μM NH_4Cl , 5 μM NaNO_3 , 2 μM Na_2HPO_4), pH 8.2 and salinity of 36, over 30 min, to determine the uptake rates of ammonium, nitrate and phosphate. Nutrient concentrations and nutrient ratios were chosen accordingly to the most frequent values found in the estuary (Sánchez de Pedro et al. 2013). Initial rates between 0 and 15 min were not considered to avoid over-estimations in the uptake capacities related to surge uptake mechanisms (Pedersen 1994; Sánchez de Pedro et al. 2013). Incubation tubes were placed on an orbital shaker (SSL1STUART®, Camlab, UK) at $2 \times g$, to prevent boundary layer formation around thalli. Nutrient uptake was followed by taking 3 mL aliquots of the incubation medium at 15 and 30 min that were frozen for later nutrient concentration analyses by the methods indicated above.

Nutrient uptake rates ($\mu\text{mol nutrient g}^{-1} \text{DW h}^{-1}$) were calculated from depletion of the nutrient concentration in the incubation medium over time using the equation:

$$V = \frac{(S_0 - S_t) \times V_0}{B \times t} \quad (2)$$

where V is the net uptake rate, S_0 and S_t are the nutrient concentrations (μM) at the initial and at the end of the interval, V_0 is the volume during the incubation time interval, t is the time elapsed between two successive samplings (h), and B is the dry weight biomass (g DW).

Total N and P uptake per tidal cycle was estimated by multiplying nutrient uptake rates per daily submersion times (5 and 10 h for *B. scorpioides* and *C. caespitosa*, respectively). Uptake was assumed to be equal in light and in darkness. Turnover rates for N and P (d^{-1}) were estimated by the following formula:

$$\text{Turnover rate} = \frac{N, P \text{ uptake} \times m(N, P)}{\%N, P \times 10^4} \quad (3)$$

where N, P uptake is the total nitrogen or phosphorous amount taken up over a 24-h tidal cycle ($\mu\text{mol N, P g}^{-1} \text{DW d}^{-1}$), $m(N, P)$ is the molecular mass ($\text{mol g}^{-1} \text{DW}$), and $\%N, P$ is the total content of N or P per DW. Turnover time (d) was calculated as the inverse of turnover rates (d^{-1}).

Statistical analysis

For each species, significant differences in the ecophysiological responses between thalli at intertidal high and low zones were tested using independent sample t tests, using the data analysis software system STATISTICA version 7 (StatSoft, Inc. 2004). The same t tests were used to analyse the interspecific differences of all measured variables between *B.*

scorpioides at its lower limit and *C. caespitosa* at its uppermost position, which are the ones in the coexistence area. To select the model that was more likely to have generated the P–E relationships, the rectangular hyperbolic model of Harrison and Platt (1986) and the photoinhibition model of Platt et al. (1980) were compared based on the corrected Akaike's information criterion (AICc, Akaike (1973)) using GraphPad Prism 6.01 (GraphPad Software Inc.). This index based on information theory is an alternative tool for model selection in ecological studies (Hobbs and Hilborn 2006; Beninger et al. 2012). The model of Harrison and Platt (1986) was the preferred model for the 71 % of the P–E curves obtained in the experiment, with a mean probability to be correct of 93.2 %. According to this, this model was applied to fit all the P–E data. The significance level was set at $\alpha = 0.05$ for all statistical analyses in the study.

Results

Photosynthetic responses (P–E curves)

Maximum photosynthetic capacity (P_{max}) and efficiency (α) was 1.7 and 1.5 times higher for specimens of *Bostrychia scorpioides* collected at their lower intertidal limits than at the upper ones (Table 2; Fig. 2). However, these differences were not evident when expressed on a chl a basis (Tables 2 and 3). On the other hand, there were no differences in the photosynthetic parameters between upper and lower thalli of *Catenella caespitosa*.

Net photosynthetic rates measured at the mean irradiance values found at each tidal position (NPR at E_z) decreased from upper to lower specimens of each species (Tables 2 and 3), and no interspecific differences were found. At intermediate irradiances (45 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), *B. scorpioides* (lower) had 1.5 times greater NPR than *C. caespitosa* (upper) on a FW basis (t test, $df = 5$, $t = 4.15$, $P = 0.009$, Fig. 2b), while no differences occurred on a chl a basis. High and low specimens of both species displayed the same saturation irradiances (E_k), light compensation points (E_c) and dark respiration rates (R_d) (Tables 2 and 3). On the other hand, interspecific differences were only observed for P_{max} on a FW and surface area basis and E_k (Table 2), *B. scorpioides* having 1.7-fold higher E_k and 2.3-fold higher P_{max} values than *C. caespitosa* (Tables 2 and 3).

Biochemical composition

Specimens of *B. scorpioides* from its lower intertidal level had 1.5-fold greater content of chlorophyll a (chl a) and total carotenoids than specimens from the higher level, but they did not differ in their phycobiliprotein content (Table 3; Fig. 3). On the contrary, liposoluble pigments

Table 2 Photosynthetic parameters derived from the P–E curves obtained for *Bostrychia scorpioides* and *Catenella caespitosa* collected from their upper and lower limits of occurrence

	NPR at E_z ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$)	$P_{\text{max}}(\text{FW})$ ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$)	$P_{\text{max}}(\text{chl } a)$ ($\mu\text{mol O}_2 \text{ mg}^{-1} \text{ chl } a \text{ h}^{-1}$)	$P_{\text{max}}(\text{SA})$ ($\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$)	α (FW) ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$)	α (pho-) ($\mu\text{mol pho-} \text{ tons m}^{-2} \text{ s}^{-1}$)	E_k ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	E_c ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$)	R_d ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ FW h}^{-1}$)	R^2
<i>B. scorpioides</i>										
Upper	40.4 ± 6.2**	44.7 ± 7.9**	64.6 ± 19	1.51 ± 0.27**	0.54 ± 0.04*	84.3 ± 19.3	3.6 ± 2.4	2.0 ± 1.3	0.99	
Lower	19.9 ± 4.3**	67.2 ± 2.8**	64.4 ± 6.4	2.27 ± 0.09**	0.88 ± 0.17*	78.5 ± 14.3	2.6 ± 2.1	2.0 ± 1.3	0.99	
<i>C. caespitosa</i>										
Upper	21.4 ± 3.0***	32.9 ± 3.6	61.3 ± 15.1	0.87 ± 0.10	0.59 ± 0.12	57.2 ± 11.6	1.8 ± 1.1	1.1 ± 0.7	0.99	
Lower	5.13 ± 3.0***	38.7 ± 5.6	63.6 ± 11.5	1.03 ± 0.15	0.68 ± 0.25	61.9 ± 20.4	3.0 ± 2.5	1.5 ± 1.0	0.99	

Net photosynthetic rates at acclimation irradiances (NPR at E_z), maximum photosynthesis (P_{max}), photosynthetic efficiency (α), saturation irradiance (E_k), light compensation point (E_c) and dark respiration rates (R_d). Values are mean ± SD ($n = 3-4$). Asterisks denote significant intraspecific differences between tidal levels (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$). Bold indicates significant interspecific differences at the coexistence tidal level (t test, $df = 4-6$, $P < 0.05$)

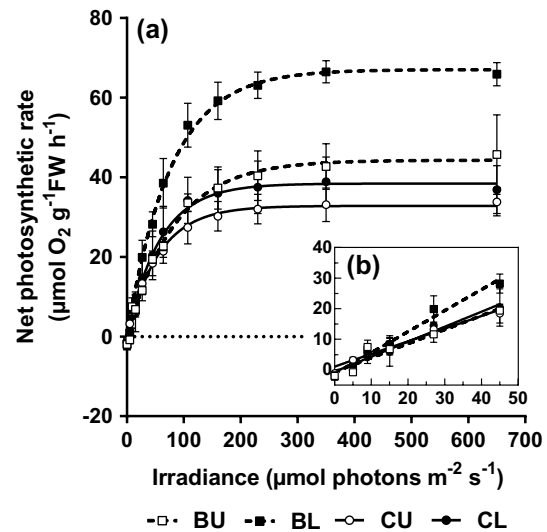


Fig. 2 Photosynthesis–irradiance (P – E) curves of *Bostrychia scorpioides* (B) and *Catenella caespitosa* (C) collected at the upper (U) and lower (L) limits of their intertidal zonation (a). Net photosynthetic rates at the linear portion of the P – E curves at low irradiances (b). Values are mean ± SD ($n = 3-4$)

in *C. caespitosa* did not vary between tidal levels, while R-PE and R-PC were 1.7 and 2 times higher, respectively, in specimens from the low intertidal zone (Table 3; Fig. 3). At intermediate tidal positions both species showed similar pigment composition, except for the 1.5-fold greater content in total carotenoids of lower thalli of *B. scorpioides* (Table 3; Fig. 3). Total soluble proteins were unaffected by position in the intertidal zone and did not differ between species, with a mean TSP content of $130 \pm 25 \text{ mg g}^{-1} \text{ DW}$ (Table 3).

Total C and N contents and C:N ratio were not affected by tidal elevation in none of the species (Tables 3 and 4). Only P content of *C. caespitosa* significantly decreased by 20 % at upper intertidal position, while no significant differences were found for this nutrient in *B. scorpioides* (Tables 3 and 4). Despite N and P did not vary between lower and upper specimens of *B. scorpioides*, N:P molar ratio significantly decreased in upper ones (Tables 3 and 4). Conversely, specimens of *C. caespitosa* from its upper limits had 1.2 times greater values of N:P, according to their lower P content (Table 4). Taken together, both species differed in their elemental composition and major differences were obtained for C and C:N, with higher values for *B. scorpioides* (Tables 3 and 4).

Changes in organic osmolytes with tidal elevation were only observed for the sugar alcohols in *B. scorpioides* (Fig. 4). Upper thalli of *B. scorpioides* had 1.3 and 1.75 times higher concentration of D-dulcitol and D-sorbitol, respectively, than those collected from its lower distributional limits (Fig. 4; Table 3). Concentrations of the

heteroside digeneaside in *B. scorpioides* and floridoside in *C. caespitosa* did not present intraspecific variations (Fig. 4; Table 3).

Nutrient uptake and turnover rates

Uptake rates of ammonium in *B. scorpioides* and of nitrate in both species were unaffected by tidal level, and high variability was obtained among replicates (Table 3; Fig. 5). Ammonium uptake rate of *C. caespitosa* was reduced by 84 % in specimens collected from the upper intertidal limits (Table 3; Fig. 5). On the other hand, intra- and interspecific differences were observed in phosphate uptake rates (Table 3; Fig. 5). Capacity of upper thalli of *B. scorpioides* and *C. caespitosa* to take up P declined by 58 and 88 %, respectively, when compared to the P uptake rates of specimens from their lower intertidal limits (Fig. 5). Consequently, *B. scorpioides* had 3.7-fold greater NH_4^+ and 4.4-fold higher phosphate uptake rates than *C. caespitosa* at intermediate tidal levels where they overlap (Table 3; Fig. 5).

Total N and P uptake over a 24-h tidal cycle and turnover rates were estimated for each species and tidal level (Table 5). Despite having similar internal N, upper specimens of *B. scorpioides* took up 45 % less N than the lower ones. Consequently, thalli located towards its upper limits presented lower turnover rates, requiring 33 ± 14 days to recycle their internal N storage vs. 17 ± 1 days in the lower thalli (Table 5). Likewise, total P uptake was fivefold and 8.7-fold higher in lower thalli of *B. scorpioides* and *C. caespitosa*, respectively (Table 5). Therefore, renewal times of internal P reserves were 7 and 10 times greater in the upper thalli than in the lower ones in *B. scorpioides* (28 ± 23 days vs 4 ± 1) and *C. caespitosa* (20 ± 11 days vs 2 ± 1).

Interspecific comparison indicated that at intermediate tidal levels, *B. scorpioides* took up twofold more N and fourfold more P than *C. caespitosa* over a tidal cycle, thus having faster turnover rates (Tables 3 and 5). Despite the fact that upper specimens of each species were located at different tidal positions (higher for *B. scorpioides*), they exhibited similar nutrient turnover rates (Tables 3 and 5).

Discussion

Our results reveal that there is phenotypic variability in the physiological responses and biochemical composition of *Bostrychia scorpioides* and *Catenella caespitosa*. In addition, interspecific differences were substantial in the region where both species overlap and were the result of intraspecific variations in their light and nutrient responses across the tidal gradient.

The greater variability found in the photophysiology, and pigment content of *B. scorpioides* was confirmed

by the broader range of habitat photon fluence rates ($30\text{--}230 \mu\text{mol m}^{-2} \text{s}^{-1}$) compared to those experienced by *C. caespitosa* ($0\text{--}70 \mu\text{mol m}^{-2} \text{s}^{-1}$). P–E curves showed that lower specimens of *B. scorpioides* exposed to reduced photon fluence rates had greater photosynthetic capacity and efficiency than the upper ones. These changes were encompassed to an increased content in liposoluble pigments, since these differences were not detected when the results were chl *a*-normalized. This fact evidenced the common photoacclimative response of macroalgae to light gradients (Lüning 1981; Gantt 1990).

Catenella caespitosa inhabits the intertidal parts where the vegetation canopy attenuates 80–98 % of incident irradiance. Under these conditions, upper and lower specimens had the same photosynthetic capacity and efficiency and only presented different phycobiliprotein contents. This result contrasted with the active photoacclimation response of *C. caespitosa* when grown at 10 and $70 \mu\text{mol m}^{-2} \text{s}^{-1}$ in a 3-day light experiment (Sánchez de Pedro et al. 2014). Reductions in nutrient availability and increasing emersion from the lower to mid-intertidal positions may have somehow masked the potential photoacclimation in this species in their natural habitat. For instance, the lower PBP content observed in upper specimens of *C. caespitosa* may be attributed to the higher irradiance and/or reduced nutrient availability due to its tidal position. The positive effect of increased N availability on these pigments is widely documented (i.e. Bird et al. 1982; Vergara et al. 1993; Carmona et al. 2006), and similar changes along tidal gradients have been observed in other intertidal rhodophytes (Kim et al. 2008).

Despite nutrient availability is reduced towards the upper parts of the zonation, there were no intraspecific differences in the total N content in the species of this study. However, ammonium uptake declined in upper specimens of *C. caespitosa*, while no differences were found for *B. scorpioides* or in the nitrate uptake in both species. Relationship between nitrogen uptake rates and zonation varies greatly among intertidal habitats. For instance, Phillips and Hurd (2003, 2004) found higher tissue N and N uptake rates in species occupying higher shore positions such as *Bostrychia arbuscula* W.H. Harvey (= *Stictosiphonia arbuscula*), whereas others have not found a clear trend (Thomas et al. 1987a; Thomas and Harrison 1987). High- and low-shore specimens of *B. arbuscula* exposed to similar submersion time than *B. scorpioides* from our study did not show differences in N content nor uptake rates, suggesting that *Bostrychia* species are well adapted to the potential N-limitation experienced at high-shore positions.

Turnover rates integrate total nutrient amount taken up over a tidal cycle and internal nutrient content of algae, providing an estimation of the rate of internal nutrient recycling (Teichberg et al. 2007). The higher turnover time for N found in upper thalli of *B. scorpioides* supports the

Table 3 Results of *t* tests examining the intra- and interspecific differences in photosynthetic parameters, nutrient uptake and biochemical composition of *Bostrychia scorpioides* and *Catenella caespitosa*

Variable	Intraspecific					
	<i>B. scorpioides</i>		<i>C. caespitosa</i>		Interspecific	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
<i>Photosynthesis</i>						
NPR at E _z	4.704	0.010**	7.748	0.000**	0.550	0.606
P _{max} (FW)	4.658	0.010**	1.739	0.133	13.45	0.000***
P _{max} (chl _a)	0.023	0.982	0.234	0.823	0.319	0.763
P _{max} (SA)	4.639	0.010**	1.732	0.134	19.15	0.000***
α	3.459	0.026*	0.628	0.553	2.612	0.048*
E _k	0.422	0.695	0.402	0.702	2.185	0.081
E _c	0.553	0.610	0.857	0.424	0.682	0.525
R _d	0.064	0.952	0.615	0.561	1.202	0.283
<i>Biochemical composition</i>						
Chl <i>a</i>	4.111	0.015*	0.616	0.560	0.064	0.951
Total carotenoids	3.670	0.021*	0.740	0.487	3.110	0.027*
R-PE	2.134	0.077	4.320	0.008**	1.568	0.168
R-PC	1.764	0.128	3.429	0.019*	1.413	0.207
TSP	1.659	0.148	1.255	0.265	0.152	0.885
<i>Elemental composition</i>						
C	1.002	0.355	0.173	0.869	10.402	0.000***
N	1.109	0.310	0.233	0.823	3.733	0.010*
P	2.160	0.074	3.022	0.023*	5.564	0.001**
C:N	0.809	0.450	0.200	0.848	13.809	0.000***
N:P	3.421	0.014*	3.376	0.015*	4.865	0.003**
<i>Osmolytes</i>						
D-dulcitol	4.587	0.006**	–	–	–	–
D-sorbitol	6.614	0.001**	–	–	–	–
Digeneaside	2.038	0.097	–	–	–	–
Floridoside	–	–	1.168	0.287	–	–
<i>Nutrient uptake</i>						
NH ₄ ⁺	1.416	0.230	3.206	0.024*	3.706	0.014*
NO ₃ ⁻	0.607	0.571	0.143	0.891	0.747	0.489
PO ₄ ³⁻	5.997	0.004**	3.873	0.012*	7.149	0.001**
Total N	3.238	0.032*	1.864	0.112	3.539	0.017*
Total P	10.18	0.001**	3.348	0.029*	6.766	0.002**
<i>Turnover rates</i>						
Turnover N	3.100	0.036*	1.806	0.121	3.424	0.019*
Turnover P	12.25	0.000***	2.698	0.046*	5.843	0.004**

Asterisks denote significant differences (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$)

hypothesis of Sánchez de Pedro et al. (2013) that specimens located at higher tidal positions, even at an intraspecific scale, can retain longer internal N, not depending on the external nutrient supply of this nutrient. On the contrary, upper and lower specimens of *C. caespitosa* had similar turnover times for N regardless of the different tidal position, suggesting that this species needs more frequent submersion to meet its nutrient requirements. Nevertheless, C:N ratio and tissue N showed that *C. caespitosa* is not N-limited in the field.

The capacity of both species to take up P was reduced towards their upper zonation limits, opposite to the trend observed in furoids by Hurd and Dring (1990). The reductions in P availability and uptake rates also decreased the total P of upper thalli of *C. caespitosa*. These specimens presented turnover times much higher than the lower ones (20 vs 2 day), which suggest that over prolonged emersion periods (up to 10–20 day), these specimens may become P-limited. In fact, N:P values above 40 obtained for these thalli are indicative of

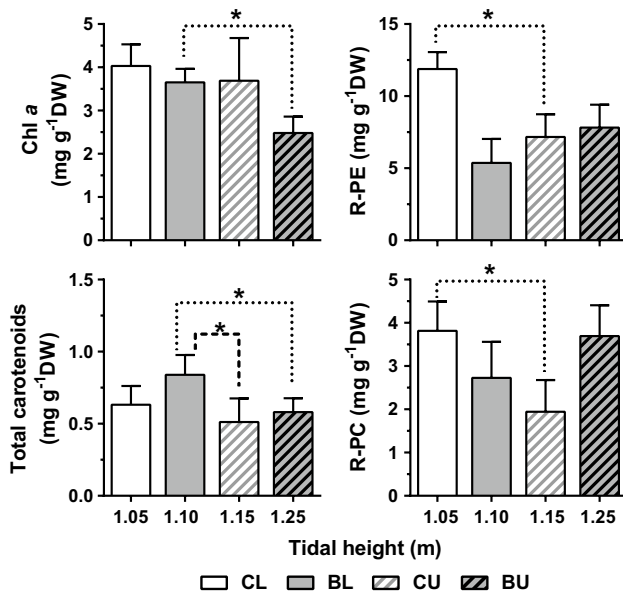


Fig. 3 Chlorophyll *a* (chl *a*), total carotenoids, R-phycoerythrin (R-PE) and R-phyocyanin (R-PC) contents of *Bostrychia scorpioides* (B) and *Catenella caespitosa* (C) collected at the upper (U) and lower (L) limits of their intertidal zonation. Values are mean ± SD (*n* = 3–4). Asterisks (*) denote significant differences at intraspecific (dotted lines) and interspecific (dashed lines) level (*P* < 0.05)

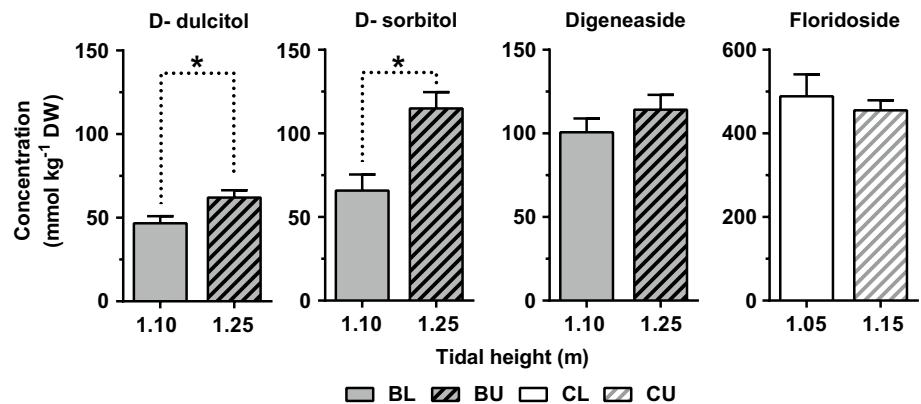
Table 4 Total C, N and P contents (%DW) and C:N and N:P (molar ratios) of thalli of *Bostrychia scorpioides* and *Catenella caespitosa* from the upper and lower limits of their intertidal zonation

	C	N	P	C:N	N:P
<i>B. scorpioides</i>					
Upper	32.3 ± 0.8	4.82 ± 0.16	0.32 ± 0.01	7.82 ± 0.07	32.9 ± 1.22*
Lower	32.8 ± 0.6	4.92 ± 0.09	0.30 ± 0.01	7.78 ± 0.06	35.9 ± 1.22*
<i>C. caespitosa</i>					
Upper	28.8 ± 0.4	4.71 ± 0.07	0.25 ± 0.01*	7.14 ± 0.07	42.2 ± 2.3*
Lower	28.8 ± 0.5	4.69 ± 0.19	0.31 ± 0.04*	7.17 ± 0.26	34.2 ± 4.11*

Values are mean ± SD (*n* = 4)

Asterisks denote significant intraspecific differences between tidal levels (*P* < 0.05). Bold indicates significant interspecific differences at the coexistence tidal level (*t* test, *df* = 4–6, *P* < 0.05)

Fig. 4 Organic osmolytes contents of *Bostrychia scorpioides* (B) and *Catenella caespitosa* (C) collected at the upper (U) and lower (L) limits of their intertidal zonation. Values are mean ± SD (*n* = 3–4). Asterisks (*) denote significant differences between tidal levels for each organic osmolyte (*P* < 0.05)



P-limitation for seaweeds (Wheeler and Björnsäter 1992; Flores-Moya et al. 1997).

Macroalgae can host epiphytic microbial communities or endosymbionts (Olson and Kellogg 2010; Trias et al. 2012), and complex seaweed-microbial interactions can occur (Singh and Reddy 2014). In our experiments, algae were cleaned of epiphytes but not treated with antibiotics, so the uptake responses should be attributed to the possible algal-bacterial consortia. However, despite it has been suggested by some studies (Wheeler 1979; Stewart 2015), it is still unclear how these interactions may interfere in macroalgal nutrient acquisition.

Emersion-related variables (i.e. desiccation, salinity and temperature) can mediate the nutrient uptake responses, for example by enhancing N uptake rates following re-submersion (Thomas et al. 1987b; Hurd and Dring 1991; Datta and Datta 1999). This effect gains importance after long emersion periods when N concentrations are very low in the water column. However, during late winter–early spring, species from this study were not N-limited and reached their maximum seasonal growth (pers. obs.). Further emersion and recovery experiments would be required to test these interactive effects.

Higher contents of D-dulcitol and D-sorbitol found in upper specimens of *B. scorpioides* from our study

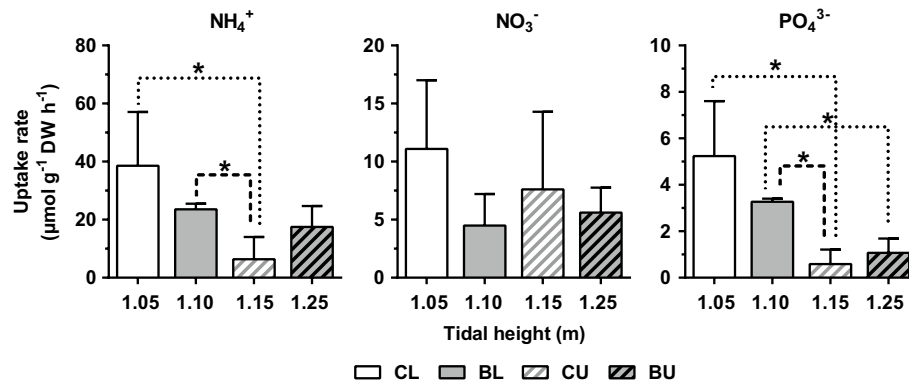


Fig. 5 Uptake rates of ammonium, nitrate and phosphate (15–30 min) in thalli of *Bostrychia scorpioides* (B) and *Catenella caespitosa* (C) collected at the upper (U) and lower (L) limits of their intertidal zonation. Note the different scale of the axis for the uptake

rates of each nutrient. Values are mean \pm SD ($n = 3\text{--}4$). Asterisks (*) denote significant differences at intraspecific (dotted lines) and interspecific (dashed lines) level ($P < 0.05$)

Table 5 Estimations of total N and P taken up ($\mu\text{mol nutrient g}^{-1}\text{DW d}^{-1}$) over a tidal cycle and turnover rates (d^{-1}) of *Bostrychia scorpioides* and *Catenella caespitosa* from the upper and lower parts of their intertidal zonation

	Total N uptake	Total P Uptake	N turnover rate (d^{-1})	P turnover rate (d^{-1})
<i>B. scorpioides</i>				
Upper	116 \pm 48*	5 \pm 3**	0.03 \pm 0.01*	0.05 \pm 0.03***
Lower	211 \pm 14*	25 \pm 1**	0.06 \pm 0.00*	0.26 \pm 0.01***
<i>C. caespitosa</i>				
Upper	105 \pm 49	6 \pm 5*	0.03 \pm 0.01	0.07 \pm 0.05*
Lower	372 \pm 283	52 \pm 24*	0.11 \pm 0.09	0.55 \pm 0.30*

Similar submersion time per tidal cycle was considered in the calculations for the lower specimens of *B. scorpioides* and the upper ones of *C. caespitosa*

Asterisks denote significant intraspecific differences between tidal levels (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$). Bold indicates significant interspecific differences at the coexistence tidal level (t test, $df = 4\text{--}6$, $P < 0.05$)

suggest that the capacity to withstand longer emersion periods increases with tidal elevation. These compatible solutes represent a major fraction of the internal osmotic potential of *B. scorpioides* (Karsten and Kirst 1989). The involvement of rather unusual sugar alcohols in the osmoprotective mechanism has been associated with the broad range of salinity and desiccation tolerance in similar red algal species (Mann and Steinke 1988; Kirst 1990; Karsten 2012). In this regard, the higher sugar alcohol content may be associated with the transient increases in salinity during emersion or with the osmotic changes induced by water loss. Karsten and Kirst (1989) reported increasing concentrations of these osmolytes with rising salinities in specimens of *B. scorpioides* from another temperate saltmarsh (Brittany, France). However, these authors found that this species is not able to fully regulate the turgor pressure at high salinities, probably associated with high desiccation. Therefore, although increasing salinities and desiccation are comparable stresses, they differ in their effects at physiological level (Karsten

2012; Kumar et al. 2014), and their combined effects should be investigated.

Our results indicate the existence of physiological and biochemical differences in relation to the tidal zonation. Phenotypic variations have led to the differentiation of ecotypes (Karsten et al. 1994), haplotypes (Zuccarello et al. 1999, 2001) and even cryptic species within some populations of similar species (Zuccarello and West 2003; Muangmai et al. 2016). Recent studies have also documented genetic differentiation among specimens located at different tidal positions or microhabitats in response to selective pressure of emersion stressors (Zardi et al. 2011; Krueger-Hadfield et al. 2013; Mota et al. 2015). In this regard, it cannot be ruled out that the phenotypic variations observed in our study may be also linked to genetic variability.

From an ecological perspective, similar species sharing common resources are likely to compete, but there is also evidence that they can coexist (Dudgeon et al. 1999; Muangmai et al. 2016). In the present study, differences in light and nutrient use of *B. scorpioides* and *C.*

caespitosa in the overlapping zone may contribute to their coexistence. This aspect would reduce or prevent species competition, together with the differential effect of abiotic stress and/or disturbances on algal productivity, as suggested for other seaweed assemblages (Duggins and Dethier 1985; Dudgeon et al. 1999; Vaz-Pinto et al. 2014; Muangmai et al. 2015). On the other hand, the coexistence of both species might also result in positive interactions in terms of vertical distribution through amelioration of emersion stress, as suggested for other intertidal habitats (Bertness et al. 1997; Hunt and Denny 2008) and plant communities (Kéfi et al. 2016). For instance, if *B. scorpioides* is absent at the upper boundary of their coexistence zone, it is likely that *C. caespitosa* would experience harsher abiotic pressures, since biomass of *B. scorpioides* may contribute to maintain humidity and reduce light. While *C. caespitosa* might grow overshadowed by thalli of *B. scorpioides* due to its low light requirements, the opposite would be very unlikely to occur.

Bostrychia scorpioides and *C. caespitosa* also grow segregated beyond the overlap zone. Towards the upper parts, time for nutrient uptake and active photosynthesis is reduced despite light becomes more available. The slower nutrient turnover time benefits *B. scorpioides*, which is also the best competitor for light. This allows this species to dominate the overall resource supply and supports its uppermost intertidal distribution. On the other hand, at the lower intertidal zone, *C. caespitosa* would be a better competitor for light, since it possesses low light requirements for growth, lower respiration rates and high thallus-specific carbon (Pedroche et al. 1995; Batelli 2004; Sánchez de Pedro et al. 2014). In addition, its lower capacity to adjust its N turnover rates and increasing P-limitation towards higher tidal positions can explain its dominance at the lower intertidal parts.

Further research is essential to unravel how the intra- and interspecific responses to environmental gradients shape the distribution and abundance of intertidal macroalgal communities (Olson and Lubchenco 1990; Davison and Pearson 1996; Bolnick et al. 2011; Edwards and Connell 2012), and how these patterns can be altered by future scenarios of eutrophication or climate change (Underwood 2000; Brauer et al. 2012).

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Compliance with ethical standards

Conflict of interest All authors declare that there is no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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