

Context-dependent interaction between an intertidal sponge and a green macroalga in a variable temperate Patagonian bay

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ABSTRACT: The stress gradient hypothesis states that positive interactions become more important as physical stress increases. Organisms living in Patagonian intertidal ecosystems are subjected to high desiccation stress due to elevated temperatures and strong winds. In coastal environments, sponges and macroalgae are common benthic organisms, and they occur from the subtidal to intertidal zones. As human pressure has increased in these systems, both sponges and macroalgae have become more abundant, which has led to more frequent interactions between them. To evaluate the interaction between *Hymeniacidon perlevis* and *Ulva lactuca* in 2 contrasting environments, we sampled their abundance and conducted field factorial experiments consisting of sponge transplants and manipulation of algal presence. Our results show that in harsher environments, *U. lactuca* facilitates *H. perlevis* likely through the reduction of direct solar radiation or high substrate temperature beneath its canopy, whereas in milder environments, *U. lactuca* negatively affects *H. perlevis* throughout other physical or chemical mechanisms (e.g. abrasion, feeding interference through changing current dynamics, depletion of oxygen, allelochemical compound production).

KEY WORDS: Stress gradient hypothesis · *Hymeniacidon perlevis* · *Ulva lactuca* · Intertidal · Shallow subtidal · Temperate Patagonia

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INTRODUCTION

The stress gradient hypothesis (SGH) states that the relative importance of processes structuring a community depends on the biotic and abiotic environmental conditions. The SGH predicts that negative biotic effects (e.g. competition) are more important under milder environments, whereas positive interactions (e.g. facilitation) increase in importance as environmental stress increases (Bertness & Call-

away 1994, Bertness et al. 1999, Bruno et al. 2003). Since its formulation, the SGH has been tested in many environments (see He et al. 2013), and huge empirical evidence supports its predictions. For example, plant interactions shift from facilitative to competitive as stress decreases along a precipitation gradient (e.g. Dohn et al. 2013, Moustakas et al. 2013), and facilitation among macroalgae and other intertidal organisms increases with increasing environmental stress along the intertidal zone of coastal

marshes (e.g. Bertness & Ewanchuk 2002, Molina-Montenegro et al. 2005, Watt & Scrosati 2013a). However, other empirical and observational studies do not support SGH predictions and propose that other factors, such as the harshness of the stress gradient (Michalet et al. 2006, Maestre et al. 2009), its nature (Kawai & Tokeshi 2007) or density-dependent effects (Bulleri et al. 2011), have to be included in an extension of the SGH.

Canopy-forming macroalgae are classic examples of ecosystem engineers (Jones et al. 1994) that, by means of their physical structure, may have strong positive to neutral or negative effects on other organisms, depending on environmental stress (Bertness et al. 1999, Watt & Scrosati 2013a,b). For example, macroalgae can ameliorate physical stress for understory organisms by preventing extreme levels of radiation and temperature at more stressful intertidal heights (Watt & Scrosati 2013a,b). At more benign heights, in contrast, macroalgae may negatively affect understory organisms by competing for nutrients, light or space (Bertness et al. 1999). One important group of organisms that inhabit the intertidal together with algae and may be affected by algae are sponges. Sponges are conspicuous and functionally important members of benthic communities (Bell 2008a), and great attention has focussed on the factors affecting their abundance. Furthermore, eutrophication events have promoted rising abundances of both sponges and macroalgae in coastal environments, leading to increased interactions between them (Valiela et al. 1997, Bell 2008b). Some studies suggest that macroalgae can benefit sponges through provision of structural support and alleviation of desiccation stress, thereby expanding their distribution (Palumbi 1985, Carballo et al. 2006, Ávila et al. 2013). In other cases, they may negatively affect sponge survivorship by releasing allelopathic compounds, shading or enhancing sedimentation (Maldonado et al. 2008, Freeman & Thacker 2011, González-Rivero et al. 2012). The result of this interaction may have major implications since sponges effectively filter seawater during feeding (Reiswig 1974), retaining dissolved and particulate carbon, nitrogen and phosphorus as well as pathogen microbes (Fu et al. 2006, 2007), with an important role in both benthic–pelagic coupling (Gili & Coma 1998) and nutrient cycling (see Maldonado et al. 2012). Due to this remarkable role, small changes in the abundance of sponges may have profound effects on ecosystem functions and the services they provide. Despite growing interest in the direction, extent and involved mechanisms in the interaction between both taxa (Ávila et al. 2010, Cár-

denas et al. 2012, Easson et al. 2014), few studies have tested changes in the outcome of the interaction under contrasting environmental stress levels (but see Palumbi 1985).

In this work, we studied the interaction between the fast-growing sponge *Hymeniacidon perlevis* and the green macroalga *Ulva lactuca* at low intertidal and shallow subtidal areas of Bahía San Antonio (BSA), northern Patagonia, to investigate how the interaction changes across different environmental stress levels and the mechanisms involved. A 2 yr survey of both species showed that *H. perlevis* is more abundant in the absence of *U. lactuca*, irrespective of the tidal height considered, showing strong opposite seasonal patterns of abundance (Gastaldi et al. 2016). Specifically, in autumn we observed the highest abundance of *H. perlevis* and the lowest abundance of *U. lactuca*, whereas in spring this was the opposite, i.e. highest abundance of *U. lactuca* and the lowest abundance of *H. perlevis*. Sponges are considered successful competitors for space (Bell 2008a,b, Wulff 2012). However, our previous results suggest strong competitive displacement of *H. perlevis* in the presence of *U. lactuca* (Gastaldi et al. 2016). Thus, our hypotheses are that (1) irrespective of the level of abiotic stress, there is a strong negative effect of *U. lactuca* on *H. perlevis* abundance; (2) the presence of *U. lactuca* negatively affects *H. perlevis* growth at both tidal levels; and (3) the mechanism underlying the negative effect of *U. lactuca* on *H. perlevis* is related to shading, since *H. perlevis* probably harbours photosynthetic symbionts (see Alex et al. 2012). In this context, we predict to find higher *H. perlevis* cover in patches without *U. lactuca* (prediction 1); higher growth of *H. perlevis* transplants in plots without *U. lactuca* (prediction 2); and lower growth of *H. perlevis* transplants in shaded plots, at both tidal levels surveyed.

MATERIALS AND METHODS

Study site

BSA is a temperate bay (80 km²) located in northern Patagonia (40° 46' S, 64° 54' W), in the most arid region of Argentina (Cabrera 1976). Its climate is characterized by strong westerly winds, which are strongest and most variable during spring and summer (Paruelo et al. 1998, Lucas et al. 2005). There is a strong seasonal pattern of precipitation, wind and temperature (Paruelo et al. 1998) as well as high maximum daily thermal amplitude (28.33 ± 8.10°C

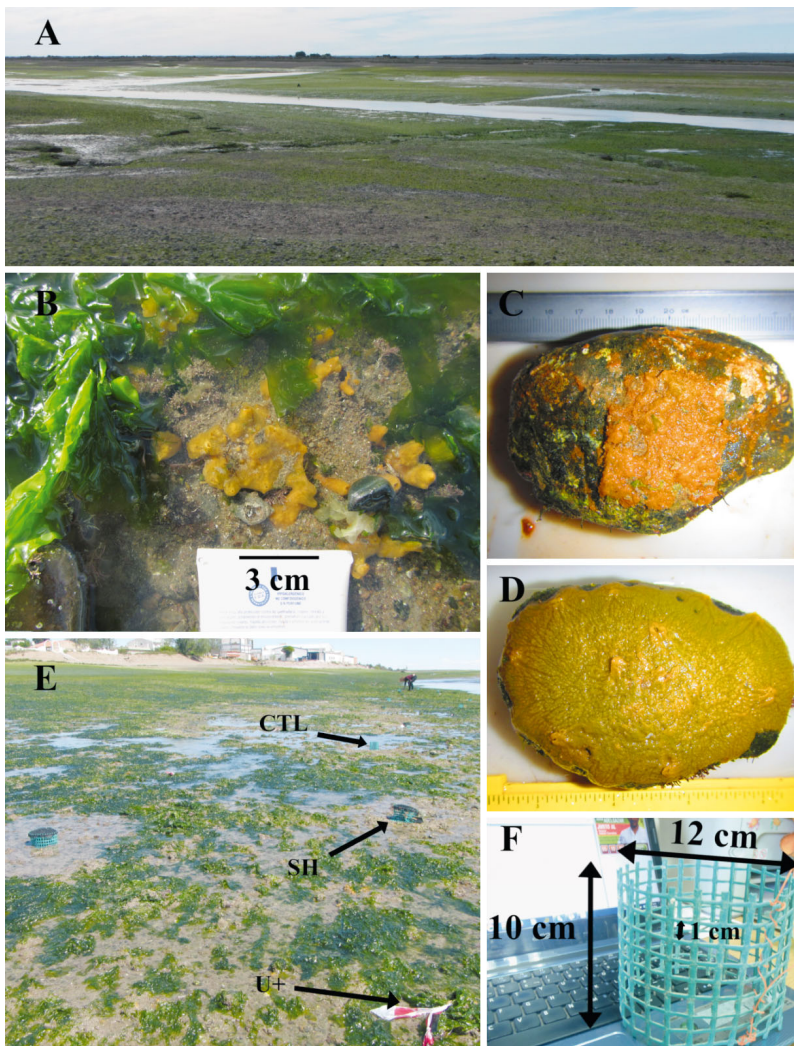


Fig. 1. (A) Intertidal zone of Bahía San Antonio, northern Patagonia. (B) The sponge *Hymeniacidon perlevis* and the green alga *Ulva lactuca* occur at both the intertidal and the shallow subtidal. (C) Experiments performed with sponges (transplants) attached to cobbles and cut to a known initial size. (D) Sponges recovered for surface area measurements. (E) Experiments were performed *in situ* (CTL: procedural control; SH: artificial shading treatment; U+: with *U. lactuca*). (F) In the *U. lactuca* shading experiment, artificial shading and procedural controls were performed with plastic mesh cages

[mean \pm SD]; Canepuccia et al. 2013). The bay is dominated by tidal currents (Schnack et al. 1996), with a semidiurnal macrotidal regime (up to 9 m tidal amplitude; Aliotta et al. 2000) and little wave action (Isla et al. 2001). Tidal currents are weak in the inner sector of the bay ($<0.5 \text{ m s}^{-1}$), but they increase near the mouth (2 m s^{-1} ; Schnack et al. 1996). The sediment in the intertidal zone is composed of a wide range of grain sizes (silt, sand and gravel, including granules, pebbles and cobbles [Salas et al. 2016]; Fig. 1A), and the low intertidal zone remains exposed

up to 6 h at low tide (M. Gastaldi pers. obs.). Due to the low average precipitation (250 mm yr^{-1}), BSA is a hypersaline system. Mean annual atmospheric temperature is 15.1°C , with extreme records in July and February (winter: -7.7°C ; summer: 41.4°C); the mean annual humidity is $57 \pm 13\%$, and wind speed can reach up to 64 km h^{-1} (Lucas et al. 2005, Genchi et al. 2010, Gastaldi et al. 2016). The bay is subjected to high anthropogenic nitrogen loading as a result of freshwater input, enriched with nitrogen, introduced via the septic system from the nearby city of San Antonio Oeste (Teichberg et al. 2010).

Samplings and experiments were performed in austral springs 2013 and 2014 in the inner sector of the main channel, where *Hymeniacidon perlevis* and *Ulva lactuca* inhabit the low intertidal and the shallow subtidal with apparent contrasting temporal and spatial distribution patterns (Gastaldi et al. 2016; Fig. 1B). The upper distribution of *H. perlevis* at the intertidal height is $\sim 0.5 \text{ m}$ from mean low tide level (MLTL), while the upper distribution of *U. lactuca* is up to $\sim 2 \text{ m}$ from MLTL (M. Gastaldi pers. obs.). We considered the low intertidal and shallow subtidal heights as contrasting environments (see Gastaldi et al. 2016), since at the subtidal (up to 0.5 m under MLTL), the sponges are permanently submerged and not exposed to desiccation or thermal stress, being able to feed and breathe during the entire tidal cycle. Conversely, the lower intertidal height

(0 to 1 m above MLTL) is exposed $\sim 12 \text{ h d}^{-1}$ (considering both low tides). During emersion, intertidal sponges are subjected to loss of oxygen and food supply, increased salinity, exposure to direct solar radiation and, eventually, loss of cellular water upon draining and drying of the internal cavity and aquiferous system (Rützler 1995). At our study site, *H. perlevis* distribution is quite uniform, while *U. lactuca* shows a patchy distribution; however, no apparent low-scale differences in physical conditions can be observed.

Distribution of *H. perlevis* and *U. lactuca*

To evaluate the relationship between the abundances of *H. perlevis* and *U. lactuca*, we sampled the percent cover of both species at the beginning and end of spring (early October and middle December 2013) at both tidal heights. We randomly placed 10 quadrats (0.25 m²) on the substrate at the low intertidal and shallow subtidal heights (3 m wide area adjacent to the waterline) where both species were present, and we took 2 photographs of each quadrat, one for *U. lactuca* canopy and the other to record *H. perlevis* after carefully removing *U. lactuca*. In addition, to assess the abundance of *H. perlevis* in the absence of *U. lactuca*, we sampled 10 quadrats (0.25 m²) in patches similar to the previous ones but where *U. lactuca* was absent. We analyzed the photographs with ImageJ free software (<http://rsb.info.nih.gov/ij/download.html>) by manually drawing the surface area to obtain percent cover of both species.

To evaluate if the effect of the presence of *U. lactuca* on the abundance of *H. perlevis* is related to tidal height, we included both *U. lactuca* presence and Tidal height in the analysis as main factors (significant interaction between Tidal height and *U. lactuca* presence may indicate that the response of *H. perlevis* to the presence of *U. lactuca* depends on Tidal height; see Zar 1999). In this model, we also included Tidal height as a random factor to reflect the blocked nature of the model (i.e. Time and *U. lactuca* presence were blocked in Tidal height; see Zuur et al. 2009). When Tidal height × *U. lactuca* presence was significant, and to further evaluate the differences within each tidal height, we tested for differences in the percent cover of *H. perlevis* with full factorial ANOVA, with *U. lactuca* presence and Time as fixed factors. To compare the percent cover of *U. lactuca* between the intertidal and subtidal heights, we included Tidal height as a random factor and Time as a fixed factor, as described in this paragraph. We performed the analyses using the `lme()` function from the `nlme` package (Pinheiro et al. 2016), the `ghtl()` function from the `multcomp` package (Hothorn et al. 2013), the `LSD.test()` function from the `agricolae` package (De Mendiburu & Simon doi:10.7287/peerj.preprints.1404v1) and `aov()` in R3.3.1 (R Core Team 2016).

U. lactuca presence experiment

To evaluate the effect of the presence of *U. lactuca* on the surface growth and biomass of *H. perlevis*, we performed a full factorial experiment at both tidal

heights. The extent of the experiment was limited by climatic conditions and lasted 52 d (given the fast-growing nature of *H. perlevis*, we considered this period long enough to provide reliable conclusions). In spring 2013, we collected *H. perlevis* specimens attached to cobbles from the intertidal. Once in the laboratory, we inspected them for bite marks of predators or evident epithelial damage to evaluate potential predation pressure, but no marks were found. Sponges were maintained in 20 l chambers with unfiltered seawater and gently aerated for 24 h prior the experiment. Since the initial size of the transplants can affect sponge growth during experimentation (Duckworth et al. 1997), we standardized transplant size by cutting sponges into 3 size classes: 2 × 2, 2.5 × 2.5 and 3.5 × 3.5 cm (hereafter small, medium and large sponge transplants, respectively; Fig. 1C). Sponge transplants are widely used in ecological and culture studies due to their capacity to regenerate and recover from wounds (Freeman & Thacker 2011, Duckworth et al. 2012, Easson et al. 2014, Fang et al. 2014); even so, we disturbed *H. perlevis* transplants as little as possible, selecting transplants similar in height and cutting them always from the sides. Then, we photographed and randomly assigned each *H. perlevis* transplant to one of the following treatments: (1) plots with *U. lactuca* (where the alga was naturally present), (2) plots without *U. lactuca* (where the alga was naturally absent), (3) *U. lactuca*-removed plots (where *U. lactuca* was naturally present but removed every 2 d) and (4) procedural control plots (where *U. lactuca* was naturally present but removed only at the beginning of the experiment and then allowed to grow again). This last treatment was implemented to evaluate if thallus removal had an additional effect on transplant growth. We placed a single sponge transplant in the center of each plot (1 × 1 m², marked with steel nails). Each treatment was replicated 10 times for each transplant size category (i.e. small, medium and large) and at each tidal level, giving a total of 240 plots. At the end of the experiment, we collected the transplants, checked them again for predation marks and then photographed (Fig. 1D) and dried them (during 48 h at 65°C) to obtain final surface area and dry biomass, as a surrogate of transplant surface growth and biomass, respectively. Due to the lack of strong correlations to estimate initial dry biomass of *H. perlevis* from surface cover or wet biomass, we chose sponges of similar height and standardized size, considering initial dry biomass to be the same for all the transplants of each size category. Thus, differences in final dry biomass between treatments may reflect differ-

ences in transplant change in biomass. We calculated sponge surface growth as the change in surface area (i.e. final surface area – initial surface area)/initial surface area.

To evaluate the separate and interactive effect of algae presence, initial sponge size and tidal height on the biomass and surface growth of *H. perlevis*, we included *U. lactuca* presence, Size class and Tidal height as fixed factors. We also included Tidal height as a random factor (as detailed in the previous subsection), while Size class and *U. lactuca* presence were blocked in Tidal height. When Tidal height \times *U. lactuca* presence was significant, the differences in transplant biomass and surface growth of *H. perlevis* were tested with full factorial 2-way ANOVA (Zar 1999), with Size class and *U. lactuca* presence as fixed factors.

***U. lactuca* shading experiment**

To test the effect of *U. lactuca* shading on *H. perlevis* growth, we performed a transplant experiment at both tidal heights. The extent of the experiment was limited by climatic conditions and lasted 63 d. In spring 2014, we collected *H. perlevis* attached to cobbles in the intertidal zone. Based on the results of the *U. lactuca* presence experiment, sponges were cut to homogeneous squares of 2 \times 2 cm and maintained in chambers as described in the previous subsection. Then, we photographed and randomly assigned each *H. perlevis* transplant to one of the following treatments (n = 15 per treatment and tidal height): (1) plots with *U. lactuca* (where the alga was naturally present), (2) artificial shading plots and (3) procedural control plots (see Fig. 1E). Artificial shading consisted of cylindrical cages of plastic mesh (1 cm mesh size, 12 cm in diameter and 10 cm height) covered with a plastic cloth on the top, reducing substrate temperature by almost 3°C, direct radiation by approximately 84% and diffuse radiation by approximately 70% (US-SQS/L Submersible Spherical Micro Quantum Sensor). Procedural controls consisted of cylindrical cages without the cloth (Fig. 1F) to test for any cage effect. At both tidal heights, cylindrical cages were attached to the substrate with steel nails, and *U. lactuca* thalli were periodically removed from the artificial shading and control treatments in a 1 \times 1 m area around cages. At the end of the experiment, we collected the transplants, checked for predation marks and photographed and dried them. We used surface area and dry biomass as estimates of transplant surface

growth and biomass as described for the previous experiment (see previous subsection).

To assess whether algal shading ameliorates desiccation stress in the intertidal, we quantified desiccation rates on the first and last day of the experiment by placing weighed water-saturated artificial sponges (3 cm² pieces of plastic foam; see Bertness et al. 2006, Gastaldi et al. 2016) at each experimental plot. After 3 h, we recovered the artificial sponges and reweighed them. Desiccation was expressed as the loss of weight per hour (g h⁻¹).

To evaluate the effect of algal shading and tidal height on the biomass and surface growth of *H. perlevis*, we included Shading and Tidal height as fixed factors. We also included Tidal height as a random factor (as detailed in the previous subsection). When Tidal height \times Shading was significant, the differences in the biomass and surface growth of *H. perlevis* transplants were tested with 1-way ANOVA (Zar 1999). Differences in desiccation between treatments were tested with 2-way ANOVA, with Shading and Time as fixed factors.

RESULTS

Distribution of *Hymeniacidon perlevis* and *Ulva lactuca*

The response of *H. perlevis* to *U. lactuca* presence was contingent on tidal height (significant Tidal height \times *U. lactuca* presence, $\chi^2 = 8.23$, df = 1, p = 0.004). The percent cover of *H. perlevis* varied between (mean \pm SD) 1.31 \pm 1.20 and 7.11 \pm 6.47% in the intertidal and subtidal heights, respectively. In the intertidal, the percent cover of *H. perlevis* was higher in patches with than without *U. lactuca* (*U. lactuca* presence: $F_{1,36} = 10.11$, p = 0.003; Fig. 2A). Nevertheless, neither a time effect nor an interaction between factors was found (Time: $F_{1,36} = 0.54$, p = 0.47; *U. lactuca* presence \times Time: $F_{1,36} = 0.003$, p = 0.95). In the subtidal zone, the percent cover of *H. perlevis* also differed between patches with and without *U. lactuca*; however, the highest *H. perlevis* percent cover was observed in patches without *U. lactuca* (Fig. 2B), in contrast with what occurred in the intertidal height. There was neither a time effect nor an interaction between factors (*U. lactuca* presence: $F_{1,36} = 6.44$, p = 0.02; Time: $F_{1,36} = 0.16$, p = 0.69; *U. lactuca* presence \times Time: $F_{1,36} = 2.42$, p = 0.13).

The percent cover of *U. lactuca* was contingent on tidal height (significant Tidal height \times Time, $\chi^2 = 4.26$, df = 1, p = 0.04) and varied between 87.42 \pm

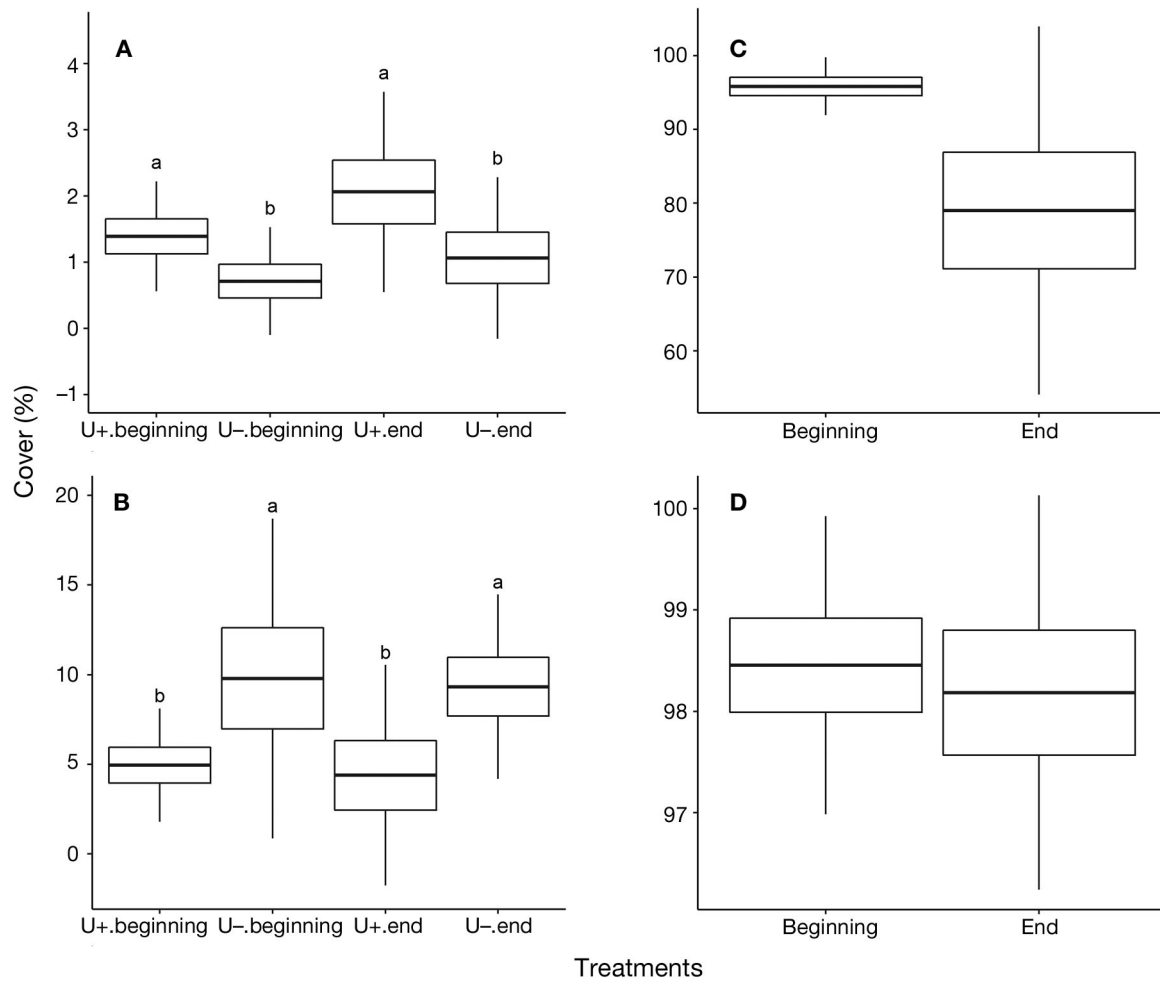


Fig. 2. Percent cover of *Hymeniadidon perlevis* with and without *Ulva lactuca* (U+ and U-, respectively) in the different sampling times (beginning and end, respectively) in the (A) intertidal and (B) subtidal. Percent cover of *U. lactuca* at different sampling times in the (C) intertidal and (D) subtidal. Limits of the boxes represent standard error, whiskers represent standard deviation and lines inside boxes represent means. Different lowercase letters indicate differences between treatments

19.39% in the intertidal and $98.32 \pm 1.68\%$ in the subtidal heights. However, when analyzed separately, the percent cover of *U. lactuca* did not differ in either the intertidal ($F_{1,18} = 3.70$, $p < 0.07$; Fig. 2C) or in the subtidal ($F_{1,18} = 0.123$, $p = 0.73$; Fig. 2D) heights.

U. lactuca presence experiment

The response of biomass and surface growth of transplants (hereafter referred to as sponges) to *U. lactuca* presence was contingent on tidal height (significant Tidal height \times *U. lactuca* presence for both response variables, Biomass: $\chi^2 = 10.55$, $df = 3$, $p = 0.01$, Surface growth: $\chi^2 = 8.18$, $df = 3$, $p = 0.01$).

In the intertidal, the biomass of sponges differed among size classes, without effect of *U. lactuca* presence or their interaction (Size class: $F_{2,60} = 18.71$, $p < 0.0001$, *U. lactuca* presence: $F_{3,60} = 0.48$, $p = 0.70$, Size class \times *U. lactuca* presence: $F_{6,60} = 1.50$, $p = 0.19$). The surface growth of sponges was different among size classes and *U. lactuca* presence treatments but without interaction (Size class: $F_{2,60} = 14.16$, $p < 0.0001$, *U. lactuca* presence: $F_{3,60} = 3.03$, $p = 0.036$, Size class \times *U. lactuca* presence: $F_{6,60} = 0.67$, $p = 0.67$). Although large sponges reached the highest biomass (Fig. 3A), small transplants showed the highest surface growth during the experiment (Fig. 3B). Sponges showed the highest surface growth in plots with *U. lactuca* and in procedural controls, while those in *U. lactuca*-removed plots

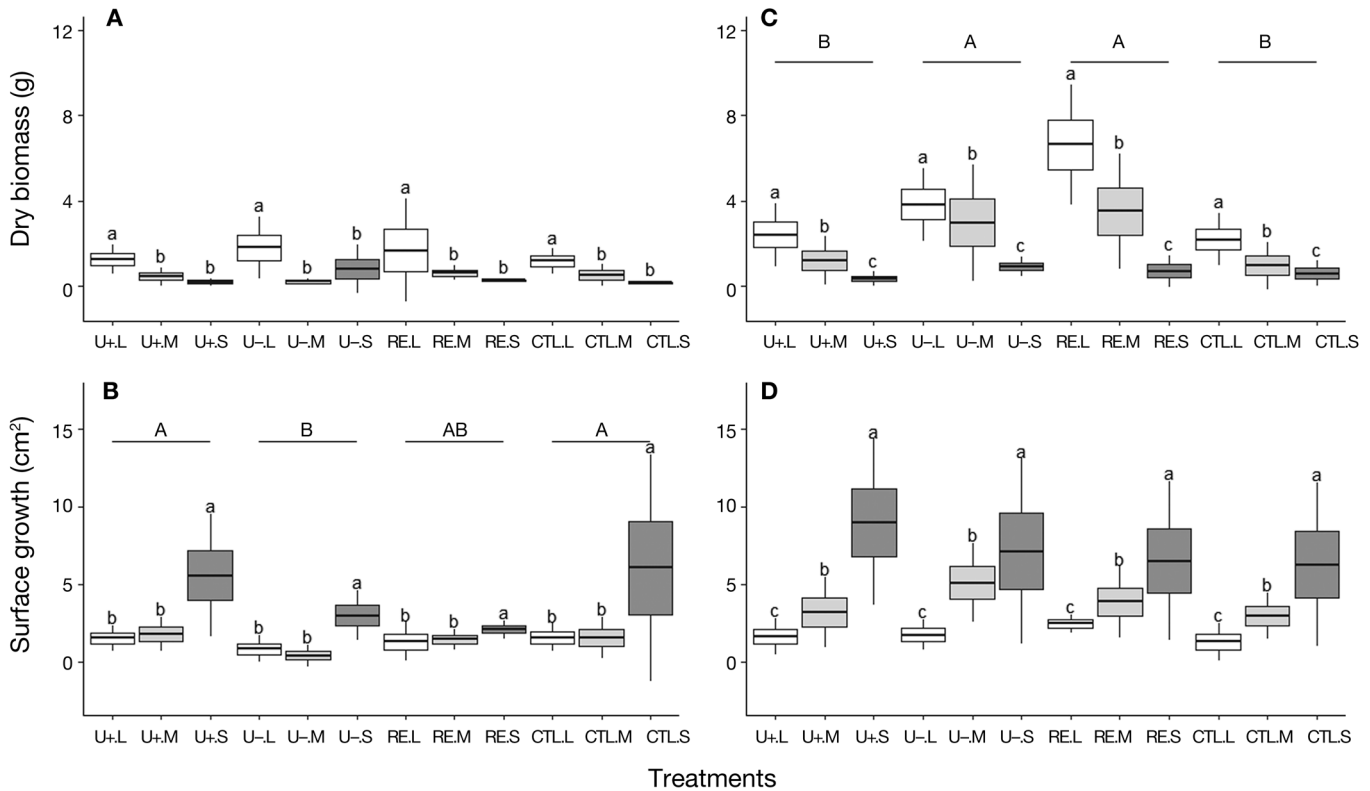


Fig. 3. *Ulva lactuca* presence experiment. Intertidal sponges: (A) dry biomass and (B) surface growth of *Hymeniacidon perlevis* of different initial size classes at different treatments. Subtidal sponges: (C) dry biomass and (D) surface growth of *H. perlevis* of different initial size classes at different treatments. U+: with *U. lactuca*; U-: without *U. lactuca*; RE: *U. lactuca* periodically removed; CTL: procedural control; L: large sponge transplant; M: medium sponge transplant; S: small sponge transplant. Limits of the boxes represent standard error, whiskers represent standard deviation and lines inside boxes represent means. Different letters indicate differences between treatments. Different uppercase letters indicate differences in *U. lactuca* presence factor. Different lowercase letters indicate differences in size class factor

and in plots without *U. lactuca* showed intermediate and the lowest surface growth, respectively (Fig. 3B). In the subtidal, the biomass of sponges differed among size classes and *U. lactuca* presence treatments, without interaction (Size class: $F_{2,60} = 5.29$, $p < 0.01$, *U. lactuca* presence: $F_{3,60} = 3.90$, $p = 0.01$, Size class \times *U. lactuca* presence: $F_{6,60} = 1.11$, $p = 0.37$). Sponges in plots without *U. lactuca* and those in *U. lactuca*-removed plots showed the highest biomass, while sponges in plots with *U. lactuca* and in procedural controls showed the lowest (Fig. 3C). The surface growth of sponges differed among size classes but was not affected by *U. lactuca* presence or its interaction with sponge size class (Size class: $F_{2,60} = 17.97$, $p < 0.0001$, *U. lactuca* presence: $F_{3,60} = 1.13$, $p = 0.35$, Size class \times *U. lactuca* presence: $F_{6,60} = 0.65$, $p = 0.69$). Thus, although large sponges reached the highest biomass (Fig. 3C), small sponges showed the highest surface growth during the experiment (Fig. 3D).

U. lactuca shading experiment

The response of biomass and surface growth of sponges to shading treatments was also contingent on tidal heights (significant Tidal height \times Shading, Biomass: $\chi^2 = 9.01$, $df = 2$, $p = 0.01$, Surface growth: $\chi^2 = 9.31$, $df = 2$, $p < 0.01$). Both biomass and surface growth of intertidal sponges differed among shading treatments (Biomass: $F_{2,21} = 13.33$, $p < 0.001$, Surface growth: $F_{2,21} = 4.99$, $p = 0.017$). Although sponge biomass was higher in artificial shading plots, followed by plots with *U. lactuca* and procedural controls (Fig. 4A), the highest sponge surface growth was observed in plots with *U. lactuca* and artificial shading and the lowest in procedural control plots (Fig. 4B). In the subtidal, the biomass and surface growth of sponges also differed among shading treatments (Biomass: $F_{2,42} = 18.71$, $p < 0.001$, Surface growth: $F_{2,42} = 17$, $p < 0.001$), with the highest biomass and surface growth in plots with artificial shad-

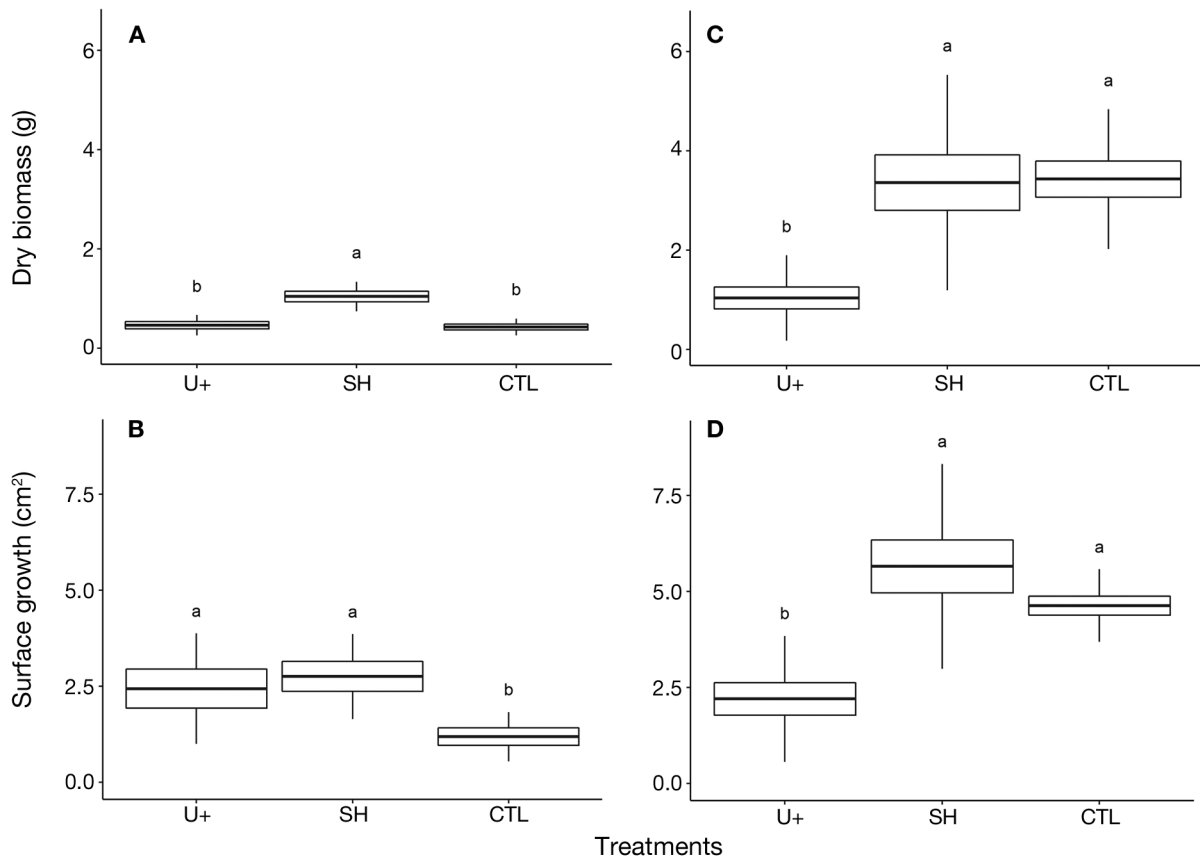


Fig. 4. *Ulva lactuca* shading experiment. Intertidal sponges: (A) dry weight and (B) surface growth of *Hymeniacidon perlevis* at different treatments. Subtidal sponges: (C) dry weight and (D) surface growth of *H. perlevis* at different treatments. U+: with *U. lactuca*; SH: artificial shading treatment; CTL: procedural control. Limits of the boxes represent standard error, whiskers represent standard deviation and lines inside boxes represent means. Different lowercase letters indicate differences in shading factor

ing and procedural controls and the lowest in plots with *U. lactuca* (Fig. 4C,D).

Desiccation differed among shading treatments and time, without interaction (Shading: $F_{2,72} = 14.99$, $p < 0.001$, Time: $F_{1,72} = 29.4$, $p < 0.001$, Shading \times Time: $F_{2,72} = 1.83$, $p = 0.17$). The highest desiccation was observed at artificial shading and procedural control plots and the lowest under the canopy of *U. lactuca* (0.82 ± 0.29 , 0.84 ± 0.14 and 0.58 ± 0.11 g h⁻¹ for artificial shading, procedural controls and plots with *U. lactuca*, respectively, Fig. 5). Desiccation was higher at the beginning of the experiment (1.02 ± 0.30 and 0.75 ± 0.23 g h⁻¹ for the beginning and end of the experiment, respectively; Fig. 5).

DISCUSSION

Our results demonstrate that the interaction between *Hymeniacidon perlevis* and *Ulva lactuca* is context-dependent, varying with the environmental

conditions. In the stressful intertidal, the cover and growth of *H. perlevis* were higher in the presence of *U. lactuca*, while at the milder subtidal height, the cover and growth of *H. perlevis* were higher in the absence of *U. lactuca*. Shading and desiccation experiments further suggest that the positive effect of *U. lactuca* observed in the intertidal may be related to the amelioration of physical stressful conditions beneath the *U. lactuca* canopy. On the other hand, in the subtidal, the negative effect of *U. lactuca* may be related to effects of the algae other than shading, since the highest sponge growth was observed in artificially shaded plots and procedural controls, where *U. lactuca* was absent. Therefore, *U. lactuca* facilitates *H. perlevis* in harsher environments, likely through the reduction of direct solar radiation or substrate temperature beneath its canopy. In contrast, *U. lactuca* negatively affects *H. perlevis* in milder environments, probably through other physical or chemical mechanisms that remain to be tested.

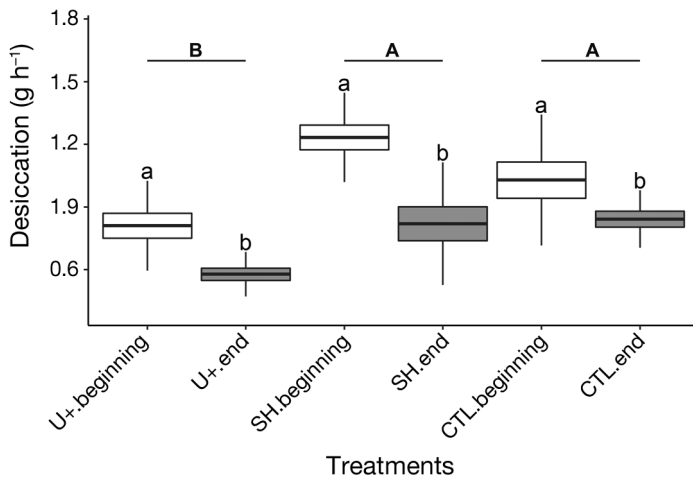


Fig. 5. Facilitative effect of *Ulva lactuca* on reducing desiccation stress at different treatments (U+, SH and CTL, representing plots with *U. lactuca*, artificial shading treatment and procedural control, respectively) and times (beginning and end, corresponding to the beginning and end of the experiment, respectively). Limits of the boxes represent standard error, whiskers represent standard deviation and lines inside boxes represent means. Different lowercase letters indicate differences between experiment times, and different uppercase letters indicate differences between shading treatments

Both positive and negative relationships between sponges and macroalgae have been reported for different habitats (e.g. Palumbi 1985, Cárdenas et al. 2012, Ávila et al. 2013). However, few studies have focused on the changing effects under different biotic or abiotic conditions. Palumbi (1985) showed that the relationship between the sponge *Halichondria panicea* and the alga *Corallina vancouveriensis* in the upper intertidal zone shifts from positive to neutral or negative in the lower intertidal, depending on the presence of a grazer. Here, our samplings showed a positive association between both species in the intertidal height and a negative association in the subtidal. This seems to contradict our previous results from a 2 yr survey which showed a negative association between the 2 species at both tidal heights (Gastaldi et al. 2016). Nonetheless, this previous study reported the association based on seasonal averaged abundances, without considering the intra-seasonal variability. We proposed that the 2 tidal heights considered as contrasting environments were probably not so. However, *H. perlevis* and *U. lactuca* abundances differed between the tidal heights considered (Gastaldi et al. 2016, this study), evidencing differences in the conditions for growth at each tidal height. Although in our study area *U. lactuca* was, by far, more abundant than *H. perlevis*,

species with low cover may have major effects on the benthic communities (Bulleri et al. 2017). Increases in the mean cover of *H. perlevis* on the order of 200 to 1000% as the environment becomes milder (as found in this study) may have strong ecological relevance since sponges can be functionally important members of temperate shallow benthic environments (e.g. by providing food and refuge to other organisms, nutrient cycling, clearing of the water column, benthic–pelagic coupling; see Bell 2008a), even when they are not usually the dominant species. Predation also seems to have a large effect on sponge abundance (Pawlik et al. 2002, 2013, Wulff 2006, 2012); however, no evident marks were observed, suggesting that predatory pressure is not a determinant factor for sponge abundance at BSA. Moreover, predation on *H. perlevis* has not been reported along its wide distribution area (Bell 2008b).

Our experiments showed a shift from a positive to a negative interaction between both species in the intertidal and subtidal areas, respectively. Macroalgae may positively affect sponges through the provision of structural support and protection against desiccation (Palumbi 1985, Carballo et al. 2006, Ávila et al. 2013). Moreover, canopies promote water retention on the substratum surface and can buffer high temperatures during low tides (Beermann et al. 2013). In our case, the provision of hard structures seems unlikely to be the mechanism by which *U. lactuca* benefits *H. perlevis*, since *U. lactuca* is a thin laminar alga with no erect structure (Fig. 1B). Protection from desiccation was observed between the coralline alga *C. vancouveriensis* and the intertidal sponge *H. panicea* (Palumbi 1985); however, it is also unlikely that this is the mechanism involved here, since the highest desiccation occurred under artificial shading in the intertidal height during our shading experiment, where sponge growth was also high. *U. lactuca* canopy and artificial shading nevertheless effectively reduced the substrate temperature and direct solar radiation, coinciding with the highest growth of *H. perlevis*. Although we are unaware of the causal effect of substrate temperature on sponge growth, direct solar radiation does affect several sponge species (Jokiel 1980); however, both mechanisms remain to be tested in *H. perlevis*.

Macroalgae may also negatively affect sponges through other mechanisms such as releasing allelopathic compounds at physical contact; increasing sedimentation; and, in the case of sponges harbouring photosynthetic symbionts, through shading (Maldonado et al. 2008, Freeman & Thacker 2011, González-Rivero et al. 2012). Moreover, macroalgae

and kelp forests are known to negatively affect their understory communities by physical abrasion, by anoxia events (D'Avanzo & Kremer 1994, Connell 2003) or even by smothering or reducing flow rates (Eckman & Duggins 1991, Madsen et al. 2001, Peterson et al. 2004), thus affecting sponge pumping behavior by promoting refiltration (Duckworth et al. 2004, Yahel et al. 2005, Hadas et al. 2008). *Ulva* spp. produce chemical compounds that inhibit recruitment and development of invertebrates and other macroalgae (Hellio et al. 2004). In the subtidal, enhanced sedimentation seems unlikely to be the mechanism by which *U. lactuca* affects *H. perlevis*, since the sponge can grow on muddy gravel and soft mud (Stone 1970, Gastaldi et al. 2016). Shading also seems unlikely, since *H. perlevis* grew more under artificial shading and in procedural controls in the subtidal (see Fig. 4C,D). Although this may be interpreted as a cage artifact, this is rather because *U. lactuca* thalli were not present at both treatments. In addition, results from the intertidal did not show this pattern, suggesting a procedural artifact. Also, we did not observe a consistent response between response variables (change in surface area and dry biomass), probably related to the growth pattern of transplants, growing first laterally as a thin layer of tissue, as observed for encrusting sponges (Ayling 1983), and thus greatly increasing surface area with a minimal change in biomass.

The SGH states that negative interactions are more important under benign environmental conditions, while positive ones are more important when environmental conditions become harsher (Bertness & Callaway 1994). Although the original formulation of the SGH included competition as the main negative interaction, it can be generalized to include other negative interactions (see Daleo & Iribarne 2009), such as those related to physical or chemical stress, which are the most likely to have occurred in our work, although there may also have been competition for light by symbionts. Thus, as predicted by the SGH, the interaction between *H. perlevis* and *U. lactuca* switched from positive to negative at the extremes of desiccation stress. However, the magnitude of the stress factors may vary temporally and the tolerance of the interacting species may differ depending on the particular stress factors involved (e.g. radiation, temperature, food availability). Hence, it may be expected that the responses of the interacting species also differ temporally according to such stress factors (Kawai & Tokeshi 2007). Changes from competition to facilitation proposed in the original formulation of the SGH do not consider particular

situations where stress levels vary over time (Bulleri et al. 2014). Therefore, it is reasonable to expect that the strength of positive and negative interactions (and the resulting balance) changes over the temporal scale. Considering that coastal environments are under increasing anthropogenic pressure, increases in nitrogen concentrations lead to increased growth and abundance of macroalgae, promoting the occurrence of macroalgal blooms (Teichberg et al. 2010). Therefore, temporal extension or increased frequency of *U. lactuca* blooms might alleviate intertidal stress on *H. perlevis* beyond spring, thus leading to a higher abundance of intertidal sponges.

Physical stress (e.g. desiccation, heat) is one of the main factors affecting species interaction and community structure in intertidal habitats (Bertness et al. 1999, 2006). In temperate environments, however, different physical stress factors vary in their intensity over the year (e.g. variability in temporal harshness or consumer pressure). Therefore, species interactions should be studied taking into account the environmental variability (as well as other stress factors such as eutrophication), since the sign and strength of such interactions are likely to change under these variable conditions (Bulleri et al. 2014). This will allow us to understand the mechanisms that drive species interaction and, thus, to predict how communities will be shaped in a changing world.

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