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Species interactions can shift the response of a maerl bed community to ocean acidification and warming

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Abstract

Predicted ocean acidification and warming are likely to have major implications for marine organisms, especially marine calcifiers. However, little information is available on the response of marine communities as a whole to predicted changes. Here, we experimentally examined the combined effects of temperature and partial pressure of carbon dioxide (pCO_2) increases on the response of maerl bed assemblages, composed of living and dead thalli of the free-living coralline alga *Lithothamnion corallioides*, epiphytic fleshy algae, and grazer species. Two three-month experiments were performed in the winter and summer seasons in mesocosms with four different combinations of pCO_2 (ambient and high pCO_2) and temperature (ambient and + 3°C). The response of maerl assemblages was assessed using metabolic measurements at the species and assemblage scales. Gross primary production and respiration of assemblages were enhanced by high pCO_2 conditions in the summer. This positive effect was attributed to the increase in epiphyte biomass, which benefited from higher CO_2 concentrations for growth and primary production. Conversely, high pCO_2 drastically decreased the calcification rates in assemblages. This response can be attributed to the decline in calcification rates of living *L. corallioides* due to acidification as well as increased dissolution of dead *L. corallioides*. Future changes in pCO_2 and temperature are likely to promote the development of non-calcifying algae to the detriment of the engineer species *L. corallioides*. The development of fleshy algae may be modulated by the ability of grazers to regulate epiphyte growth. However, our results suggest that predicted changes will negatively affect the metabolism of grazers and potentially their ability to control epiphyte abundance. Here, we demonstrate that the response of marine communities to climate change will depend on the direct effects on species physiology and the indirect effects due to shifts in species interactions. This double, interdependent

response underlines the importance of examining community-level processes, which integrate species interactions, to better understand the impact of global change on marine ecosystems.

1. Introduction

A growing body of literature predicts that ocean acidification and warming will be the main anthropogenic drivers affecting marine species by the end of the century (Kroeker et al., 2013). Due to the increase in atmospheric CO_2, seawater surface temperatures have been predicted to increase by 0.71-2.73°C and pH to decline by 0.07-0.33 units in the surface ocean by the end of the 21st century (Bopp et al., 2013).

Species interactions are a key element in ecosystem functioning and are likely to play an important role in species responses to climate change (O'Connor et al., 2011; Hansson et al., 2012; Kroeker et al., 2012). To date, most research has focused on the impact of ocean acidification and warming on the response of single species (Yang et al., 2016); studies examining the effects of climate change on marine communities are scarce in the literature (Alsterberg et al., 2013). Understanding the mechanisms and interactions that occur among marine communities that face the predicted changes is necessary for a better overview of marine ecosystem response. Climate change is likely to strongly alter interactions between macroalgae (e.g. calcifying and non-calcifying macroalgae; Olabarria et al., 2013; Short et al., 2014; Short et al., 2015), interactions between grazers and macroalgae (Poore et al., 2016; Sampaio et al., 2017) as well as prey-predator dynamics (Asnaghi et al., 2013; Jellison et al., 2016), inducing drastic consequences on the structure and functioning of marine ecosystems (Widdicombe and Spicer, 2008; Hale et al., 2011).

Maerl beds feature high structural and functional diversity arising primarily from the numerous species interactions that occur in this environment — in particular, interactions between fleshy and calcareous macroalgae and grazers and macroalgae (Hily et al., 1992; Guillou et al., 2002; Grall et al., 2006). The accumulation of living and dead thalli of free-living coralline algae (Corallinaceae, Rhodophyta) creates a complex three-dimensional structure that provides habitat for many faunal and floral species (Foster et al., 2007; Amado-Filho et al., 2010; Peña et al., 2014), some of which have high commercial value (Grall and Hall-Spencer, 2003). In some locations, dead maerl can reach high proportions compared with living maerl (Hily et al., 1992), thereby contributing substantially to the local carbonate dynamics (Martin et al., 2007).

The main species inhabiting maerl beds may respond differently to ocean acidification and warming. Coralline algae are known to be among the most vulnerable species facing ocean acidification (McCoy and Kamenos, 2015; Martin and Hall-Spencer, 2016), due to their highly soluble Mg-calcite skeleton (Morse et al., 2006). The deleterious consequences of ocean acidification have also been demonstrated for other calcareous marine taxa, such as mollusks (Gazeau et al., 2013; Parker et al., 2013) and echinoderms (Dupont et al., 2010), with reductions in survival, growth, development, and abundance (Kroeker et al., 2013). Conversely, some species can benefit from the increase in CO_2 concentration and temperature. Positive responses, such as increases in primary production and growth, have been found mostly among non-calcifying organisms, such as fleshy algae and seagrasses (Koch et al., 2013; Pajusalu et al., 2013).

Here, we experimentally investigated the impact of ocean acidification and warming on the metabolism and the interactions of the main maerl-forming species in Brittany *Lithothamnion coralloides* and the epiphytic fleshy macroalgae and main grazer (gastropods and sea urchins) associated with it. Because the response of species and communities to climate change is also likely to vary depending on seasonal changes in environmental factors (Godbold and Solan, 2013; Martin et al., 2013; Baggini et al., 2014), experiments were performed in both winter and summer conditions. The response of marine communities to climate change is likely to be influenced by the direct effects of environmental stressors on individual organisms, and by the indirect effects induced by shifts in interspecific interactions (Harley et al., 2012; Auster et al., 2013). In the present study, we therefore performed metabolic measurements at the species and at the community scale. At the species scale, studying species physiology is useful for understanding how organisms cope with changing climatic conditions and for analyzing the community metabolic response. Community-scale measurements provide information on the potential shifts in species interactions induced by climate change. In particular, we tested the hypothesis that climate change will increase epiphytic fleshy algal growth, exacerbating the deleterious consequences of predicted changes on *L. corallioides* metabolism. We also investigated whether the predicted changes can modify interactions between grazers and macroalgae, and their ability to regulate epiphytic biomass.

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2. Materials and methods

2.1. Species collection and assemblages

Organisms were collected from a maerl bed in the Bay of Brest, France (48°18'N 4°23'W) using a naturalist's dredge (width: 1 m, height: 0.2 m, net: 1.5 m long) deployed from the research vessel *Albert Lucas*. In the Bay of Brest, maerl beds are located at depths of between 0.7 and 6.8 m, according to the tide (Dutertre et al., 2015). We deliberately selected thalli of the maerl species *L. corallioides* Crouan and Crouan, 1867 that were devoid of any apparent epiphytes; nonetheless, they were not cleaned so as to retain any epiphyte spores that may have been present on their surface. Medium-sized individuals of the three main species of grazers living in maerl beds were also sampled: two gastropod species (sea snails) *Gibbula magus* Linnaeus, 1758 and *Jujubinus exasperatus* Pennant, 1777 and an urchin species *Psammechinus miliaris* Müller, 1771 (Grall et al., 2006). Samples were collected on 24 January 2015 (winter conditions) and 15 September 2015 (summer conditions). In each season, 1 kg of living thalli of *L. corallioides*, 500 g of dead thalli of *L. corallioides*, 40 individuals of *G. magus* (shell length range 17-29 mm; Table S1), 40 individuals of *P. miliaris* (test diameter range 11-23 mm), and 80 individuals of *J. exasperatus* (shell height range 5-11 mm) were randomly selected and transported in seawater tanks to the Roscoff Marine Station. To mitigate the stress experienced by the species during sampling and transport, they were kept in open-flow aquaria at ambient pH and *in situ* temperature conditions at the time of collection for at least one week before starting the experiments. No mortality was recorded during this period.

2.2. Experimental design

Two three-month long experiments were conducted for both winter (March to June 2015) and summer (September to December 2015) conditions.

For each season, 20 artificial assemblages were created and randomly assigned to 20 15 L aquaria. Each assemblage was composed of 45 g of living *L. corallioides* thalli, 20 g dead *L. corallioides* thalli, two *G. magus* individuals, two *P. miliaris* individuals and four *J. exasperatus* individuals, according to the proportions observed on maerl beds.

Algae and grazers were acclimated to laboratory conditions for 7 days. Then, the pH was gradually decreased by 0.05 units per day over 7 days and temperature increased by 0.5°C per day. The pH was controlled by modifying pCO_2 through CO_2 bubbling.

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At each season, two pCO_2 conditions were tested, each with two temperature conditions to examine the interaction between pCO_2 and temperature. There were therefore four conditions:

1) ambient pCO_2 and ambient temperature (control, A-pCO_2; T)

2) high pCO_2 and ambient temperature (H-pCO_2; T)

3) ambient pCO_2 and high temperature (A-pCO_2; T + 3°C)

4) high pCO_2 and high temperature (H-pCO_2; T + 3°C).

Ambient pCO_2 conditions (A-pCO_2) were determined according to *in situ* winter (7.98) and summer (8.06) mean pH_T (pH on the total scale) monitored above maerl beds in the Bay of Brest (data from Martin, unpublished data). High pCO_2 (H-pCO_2) corresponded to the "business-as-usual" scenario predicted for the end of the century, with a pH decrease of -0.33 units (RCP8.5; Bopp et al., 2013). Ambient temperature (T) corresponded to *in situ* winter (10.0°C) and summer (17.1°C) conditions in the Bay of Brest recorded by SOMLIT (from 2003 to 2014), and high temperature (T + 3°C) was determined according to the business-as-usual scenario predicted for 2100 (Bopp et al., 2013).

The pH and the temperature were controlled in four 100 L tanks, continuously supplied with filtered (5 µm) natural seawater, with a high water flow rate of 150 L h^{-1} per tank. They were maintained by an off-line feedback system (IKS Aquastar, Karlsbad, Germany) that activated or stopped heaters and solenoid valves, controlling temperature and CO_2 (Air Liquide, France) bubbling in the tanks, respectively. Each 100 L tank provided seawater to five 15 L aquaria for each of the four conditions using pumps. The water flow rate was 15 L h^{-1} in each aquarium. Temperature was maintained constant in aquaria with water baths. Seawater pH (pH_T, expressed on the total hydrogen ion concentration scale, Dickson et al., 2007) and temperature were monitored every two days in the 20 aquaria, at different times of the day. Seawater pH_T and temperature measurements were carried out using a pH probe associated with a temperature sensor (PHC101, Hach Lange, IntelliCAL). The pH probe was calibrated using Tris/HCl and 2-aminopyridine/HCl buffers (Dickson et al., 2007). The pH values of the off-line feedback system were adjusted from measurements of pH_T carried out every two days in each aquarium. Total alkalinity (A_T) was also monitored during the experiment in each aquarium at different times of the day (n = 28). For A_T analyses, seawater samples (60 mL) were filtered through 0.7 µm Whatman GF/F filters and immediately poisoned with a mercuric chloride solution to prevent further biological activity (Dickson et al., 2007). A_T was determined using open-cell

titration on an automatic titrator (Titroline alpha, Schott SI Analytics, Mainz, Germany) according to the method developed by Dickson et al. (2007). A_T was calculated using a Gran function applied to pH values ranging from 3.5 to 3.0 (Dickson et al., 2007) and corrected using standard reference material provided by the Andrew G. Dickson laboratory (CRM Batch 111, accuracy of ± 6 µmol kg^{-1}). Salinity was measured every 2 weeks with a conductivity probe (CDC401, Hach Lange, IntelliCAL, accuracy of 0.1) and remained constant during experiments (35.2 ± 0.2). From A_T and pH_T measurements, dissolved inorganic carbon (DIC), saturation state of seawater with respect to aragonite (Ω_{Ar}) and saturation state of seawater with respect to calcite (Ω_{Ca}) were calculated with CO2SYS software. Mean temperature and parameters of the carbonate chemistry are given in Table 1.

Irradiance was set to the mean *in situ* daily irradiance at 5 m depth in the Bay of Brest according to Martin et al. (2006): 30-40 µmol photons m^{-2} s^{-1} in winter and 90-100 µmol photons m^{-2} s^{-1} in summer. The light was provided by two or four 80 W fluorescent tubes (JBL Solar Ultra Marin Day, JBL Aquaria, Nelson, New Zealand) above the aquaria under a 10/14 h or 14/10 h light/dark photoperiod, for winter or summer conditions, respectively.

2.3. Metabolic measurements

After three months in experimental conditions, metabolic measurements were performed at the species and assemblage level using incubations in acrylic respirometry chambers (Engineering and Design Plastics Ltd, Cambridge, UK). For species-scale measurements, each species was incubated separately. Community-scale measurements were performed on assemblages, incubating all individuals from all species present in each aquarium. The chamber volume was adapted to species size. It was of 80 mL for *J. exasperatus* and epiphytes, 185 mL for *P. miliaris, G. magus* and living and dead *L. corallioides*, and 600 mL for the assemblages. Before incubation, epiphytic algae that spontaneously grew on *L. corallioides* during the experiments were carefully removed and incubated separately. Metabolic measurements (net photosynthetic and respiration rates) for the main epiphytic algae *Rhodymenia ardissonei* and *Solieria chordalis* were only examined in the summer, when their biomass was sufficient for measurements. Species were placed on a plastic grid above a stir bar in the chambers to ensure the seawater was well mixed. For *G. magus* and *P. miliaris*, net calcification, respiration and excretion (ammonia release) rates were measured. For *J. exasperatus*, only respiration rates were measured due to its limited size and metabolic rates. For grazers, physiological rates were measured under ambient irradiance. For each grazer species,

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individuals present in each aquarium were incubated together. For living and dead *L. corallioides* and assemblages, net photosynthetic and light calcification rates were measured under ambient irradiance, and respiration and dark calcification rates were measured in the dark. For light incubations, chambers were placed inside aquaria to control temperature. For dark incubations, chambers were placed in a plastic crate filled with aquaria seawater in an open circuit to keep the temperature constant. Incubation duration was adjusted to keep oxygen saturation above 80%. Incubations lasted approximately from 1 h for *G. magus* to 2.5 h for dead maerl. For assemblages, the metabolism was measured from the incubations of all species together.

Oxygen concentrations were measured at the beginning and at the end of each incubation, using an optical fiber system (FIBOX 3, PreSens, Regensburg, Germany). Reactive spots were calibrated with 0% and 100% buffer solutions. Net primary production (NPP, µmol O_2 g DW^{-1} h^{-1}) or respiration (R, µmol O_2 g DW^{-1} h^{-1}) rates were calculated following Eq. (1):

$$\text{NPP or R} = \frac{\Delta O_2 \times V}{\Delta t \times DW} \quad (1)$$

where ΔO_2 is the difference between the initial and final oxygen concentrations (µmol O_2 L^{-1}), V the volume of the chamber (L), Δt the incubation time (h), and DW the dry weight of the species incubated (g). The dry weight was obtained after 48 h at 60°C. For gastropods, the body was separated from the shell to consider the dry weight of the body only.

For algae and the assemblages, gross primary production (GPP) was calculated following Eq. (2):

$$GPP = NPP - R \quad (2)$$

Control incubations containing only seawater were carried out to correct for oxygen fluxes due to any additional biological activity in seawater. Oxygen fluxes calculated in control chambers were subtracted from oxygen fluxes of chambers containing algae.

Seawater samples were taken in the aquaria at the beginning of the incubation and in the chambers at the end of the incubations (except for fleshy algae and *J. exasperatus*) to measure ammonium (NH_4^+) concentration and total alkalinity (A_T). To do so, 45 mL seawater samples for NH_4^+ analyses were fixed with reagent solutions and stored in the dark. NH_4^+ concentrations were determined according to the Solorzano method (Solorzano, 1969). Absorbance was measured by spectrophotometry at a wavelength of 630 nm (spectrophotometer UV-1201V, Shimadzu Corp, Kyoto, Japan). For grazers, ammonia excretion rates (E, µmol NH_4^+ g DW^{-1} h^{-1}) were calculated following Eq. (3):

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$$E = \frac{\Delta NH_4^+ \times V}{\Delta t \times DW} \quad (3)$$

where ΔNH_4^+ is the difference between the initial and final ammonium concentrations (µmol NH_4^+ g DW^{-1} h^{-1}).

For A_T analyses, 60 mL seawater samples were filtered through 0.7 µm Whatman GF/F filters and were immediately poisoned with a mercuric chloride solution. Total alkalinity was determined according to the method described above. Net calcification rates at light and in the dark (G_l and G_d, respectively; in µmol $CaCO_3$ g DW^{-1} h^{-1}) were calculated according to the alkalinity anomaly technique (Smith and Key, 1975) and corrected for NH_4^+ fluxes (Gazeau et al., 2015). This correction was applied to calcareous species and assemblage incubations following Eq. (4):

$$G_l \text{ or } G_d = \frac{(-\Delta A_T + \Delta NH_4^+) \times V}{2 \times \Delta t \times DW} \quad (4)$$

where G_l is the net calcification in the light, G_d is the net calcification in the dark, ΔA_T is the difference between the initial and final A_T (µeq L^{-1}).

After the three-month experiments, epiphytic algae that spontaneously grew on *L. corallioides* during experiments were picked off and dried at 60°C for 48 h to determine their dry weight.

2.4. Chlorophyll *a* analysis

At the end of the experiments, thalli of living and dead *L. corallioides* were collected in each aquarium and immediately frozen at -20°C pending analyses. Then samples were freeze-dried and crushed into a powder using a mortar, in the dark. An aliquot of 0.15 g of powder was precisely weighed and suspended in 10 mL of 90% acetone and stored in the dark at 4°C for 12 h. Samples were then centrifuged at 4000 rpm. The supernatant was collected and absorbance was measured at 630 (A_{630}), 647 (A_{647}), 664 (A_{664}), and 691 (A_{691}) nm. Chlorophyll *a* (Chl *a*) concentrations (µg g DW^{-1}) were calculated from Ritchie (2008) following Eq. (5):

$$\text{Chl } a = \frac{(-0.3319\, A_{630} - 1.7485\, A_{647} + 11.9442\, A_{664} - 1.4306\, A_{691}) \times V}{mp} \quad (5)$$

where V is the volume of acetone (mL) and *mp* the mass of powder (g).

2.5. Data analysis

The influence of season, temperature and pCO_2 was tested on metabolic rates of grazers (*P. miliaris*, *G. magus* and *J. exasperatus*), living and dead maerl, epiphytic biomass and assemblages. Even after transformations, the data were non-normally distributed. Therefore, analyses were conducted using a three-way permutational multivariate analysis of variance (PERMANOVA), based on Euclidian distance (Anderson, 2001). PERMANOVAs were run with 4999 permutations (Anderson, 2001), using season (two levels: winter and summer), temperature (two levels: ambient and elevated temperature) and pCO_2 (two levels: ambient and elevated pCO_2) as fixed orthogonal factors (n = 5). These statistical analyses were performed with the PRIMER 7 & PERMANOVA+ software package.

The effects of pCO_2 and temperature on the physiological rates of the epiphytic algae *R. ardissonei* and *S. chodalis* were only tested in the summer. Because assumptions of normality (Shapiro test) and homogeneity of variances (Bartlett test) were not met, two-way non-parametric Scheirer-Ray-Hare tests were performed. These statistical analyses were carried out using the statistical package R, version 3.2.2.

3. Results

3.1. Metabolic responses of grazers to acidification and warming

In the urchin *P. miliaris*, high temperature (+3°C) reduced *P. miliaris* R in the summer, while pCO_2 had no significant effect on *P. miliaris* R (Fig. 1a; Table 2). *P. miliaris* G_l was significantly affected by the triple interaction between season, temperature and pCO_2 (Fig. 1b), with a negative impact due to the combined effect of temperature and pCO_2 increase in the summer, but no interaction effects in the winter. The combined increase of temperature and pCO_2 significantly affected *P. miliaris* E (Table 2; Fig. 1c).

Neither temperature nor pCO_2 increases significantly affected *G. magus* R, G_l and E (Table 2; Fig. 1d-f). In *J. exasperatus*, R was positively affected by the temperature increase, but in winter conditions only (Table 2; Fig. 1g). *J. exasperatus* R was negatively influenced by the pCO_2 increase in the winter, but positively in the summer.

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3.2. Metabolic responses of living *L. corallioides* to acidification and warming

Living maerl GPP did not differ among temperature and pCO_2 conditions regardless of the season (Table 3; Fig. 2b,c). R was significantly reduced by the high temperature condition in the winter, whereas an increase in R was observed in the summer. Chlorophyll *a* content was negatively affected by the high temperature condition in the winter only (Tables 3,4). Temperature had a positive effect on the G_l of living maerl. Conversely, G_l was significantly reduced under high pCO_2 (Table 3; Fig. 2d). G_d was significantly affected by an interaction between season and temperature (Fig. 2e). G_d was positively affected by temperature in winter, but no effect was detected in the summer. A significant decline in G_d occurred under high pCO_2 regardless of the season. Net dissolution, because G_d was negative, was recorded in the winter under high pCO_2 conditions.

3.3. Metabolic responses of dead *L. corallioides* to acidification and warming

The high temperature condition (+3°C) did not affect dead maerl GPP or R (Table 3; Fig. 2g,h). The pCO_2 increase did not affect dead maerl GPP in either season. However, there was an interaction between season and pCO_2, with a decrease in R under high pCO_2 in the summer. Chlorophyll *a* content was significantly affected by the temperature and pCO_2 interaction (Tables 3,4). Dead maerl G_l significantly increased under high temperature (Fig. 2i). Conversely, a negative impact of high pCO_2 was on G_l in the winter and summer. In the dark, net dissolution was observed on dead maerl regardless of the temperature and pCO_2 conditions (Fig. 2j). No temperature effect was observed on dark dissolution. However, dark dissolution rates were significantly higher under high pCO_2 treatments, regardless of the season.

3.4. Growth and metabolic responses of epiphytic algae to acidification and warming

Mean GPP and R for the two epiphytic algae *R. ardissonei* and *S. chordalis* measured in the summer are presented in Figure 3. *R. ardissonei* GPP was not affected by high temperature or pCO_2 conditions, and R was reduced under high pCO_2 (Table 5; Fig. 3b,c). In *S. chordalis*, GPP was significantly affected by the interaction between temperature and pCO_2 (Table 5; Fig. 3e). R was enhanced by the high temperature and pCO_2 conditions (Fig. 3f).

The mean biomass of epiphytic fleshy algae at the end of the experiment was significantly higher in the summer than in the winter (+81%, 3-way PERMANOVA, df = 1, F = 5.3, p=0.027, Fig. 4). Epiphyte biomass was significantly affected by the

triple interaction between season, temperature and pCO_2 (3-way PERMANOVA, df = 1, F = 4.9, p=0.035), with high pCO_2 having a positive effect on epiphyte biomass in the winter. In the summer, this positive effect was only detected under high temperature.

3.5. Metabolic responses of assemblages to acidification and warming

No temperature effect was observed on NPP, GPP and R in either season (Table 6; Fig. 5a-c). The high pCO_2 condition enhanced NPP in both seasons. The combined effect of season and pCO_2 affected GPP, with a positive effect of pCO_2 increase in the summer only. Similarly, R significantly increased under high pCO_2 in summer conditions. An interactive effect of season and temperature was detected for G_l, which increased under high temperature in the summer only (Fig. 5d). Conversely, high pCO_2 reduced G_l regardless of the season. In the dark, net dissolution was observed in the winter, but net precipitation occurred in summer conditions at high temperature (Fig. 5e). G_d was significantly affected by the triple interaction between season, temperature and pCO_2. In the winter, high pCO_2 increased net dissolutions rates, and high temperature reduced them. In the summer, the interactive effect of temperature and pCO_2 increase was more complex, with a decrease in G_d detected under high temperature conditions only.

4. Discussion

Our study demonstrates that the response of maerl bed communities to increased temperature and pCO_2 conditions is a complex function of direct effects of climate variables on species physiology and shifts in species interactions. Results show that predicted changes may alter interactions among calcifying and fleshy macroalgae via overgrowth of epiphytic algae and an increase in competition with underlying maerl. Interactions between grazers and macroalgae were also affected because the grazer physiology was adversely affected by acidification and warming with potential consequences on epiphyte biomass regulation. Our results underscore the importance of examining community-level processes to integrate species interactions in the study of the impact of global change on marine ecosystems.

Assemblage GPP and R were not affected by the high temperature and pCO_2 conditions in the winter. Conversely, in the summer, GPP and R increased under high pCO_2 conditions. The response of assemblage GPP and R appeared closely related to changes in epiphyte biomass and productivity. For instance, the high biomass of epiphytic algae in the summer led to high

contribution to oxygen fluxes. Under high pCO_2 conditions, the higher availability of CO_2 as substrate for photosynthesis may stimulate epiphyte productivity and growth (Koch et al., 2013). The two main epiphytic algae that grew during the experiments, *R. ardissonei* and *S. chordalis*, are naturally found in maerl beds in Brittany (Peña et al., 2014). The response of the alga *S. chordalis* to increased temperature and pCO_2 differed from that of *R. ardissonei*. This difference suggests that the response is species-specific, even among fleshy algae, as demonstrated by Kram et al. (2016). *R. ardissonei* GPP was not affected by increased temperature and pCO_2, but its R was significantly lower under high pCO_2. Within the same genus, Cook et al. (1986) showed that *Rhodymenia palmate* can potentially use HCO_3^- as source of inorganic carbon for photosynthesis. The same process may occur in *R. ardissonei*, suggesting that this alga is not carbon-limited at current oceanic pCO_2 levels. In contrast to *R. ardissonei*, increased pCO_2 stimulated *S. chordalis* GPP under ambient conditions of temperature. In their study, Short et al. (2014) indicate that the overgrowth of filamentous algae occurs synergistically with high pCO_2 levels and decreased photosynthesis in coralline algae. Here, the stimulation of epiphyte productivity and growth under high pCO_2 is likely to increase the competition with underlying maerl, especially through reduction in incident light.

Although assemblages were mainly composed of living and dead maerl, the response of GPP and R of *L. corallioides* to increased temperature and pCO_2 differed from that observed in assemblages. For example, the temperature increase of +3°C reduced living *L. corallioides* R in the winter, but increased R in the summer. Under high pCO_2 conditions, although CO_2 availability for photosynthesis was higher, no difference was observed in *L. corallioides*, probably due to the ability of this species to employ inorganic carbon acquisition mechanisms (Kübler and Dudgeon, 2015). Interestingly, GPP, R and chlorophyll *a* content of dead maerl were of the same magnitude as for living maerl. Although live algae prevent bio-fouling by shedding their surface layers (Keats et al., 1997; Villas Bôas and Figueiredo, 2004), post-mortem colonization by photosynthetic endolithic assemblages may occur within dead crusts (Diaz-Pulido et al., 2012). Moreover, dead thalli may represent a substrate for the settlement of crustose coralline algae that cover small parts of some thalli. Crustose coralline algae colonization may also contribute to the observed GPP and R values. In dead maerl, only R decreased under high pCO_2, while no effect was detected for GPP.

These findings also suggest the importance of dead maerl to assemblage carbonate fluxes during the experiments. For example, endolithic algae appear to play an important role in the dissolution of a crustose coralline alga (CCA) species,

285 *Porolithon onkodes* (Reyes-Nivia et al., 2014). Through their photosynthesis, endolithic algae may elevate interstitial pH within the *P. onkodes* skeleton (Reyes-Nivia et al., 2013), increasing carbonate cement precipitation (Diaz-Pulido et al., 2014). Within dead *L. corallioides*, the presence of endolithic algae combined with the presence of small patches of CCA on the surface of thalli may explain the calcification rates observed in light and dissolution in dark. Considering the high Mg content in the skeleton of *L. corallioides*, increased pCO_2 likely promotes the dissolution of dead thalli. Alternatively, the 290 increase in dissolution observed in the present study may be associated with a reduction of CCA recruitment over the surface of dead thalli under acidified conditions (Jokiel et al., 2008). These results are consistent with the negative response to increased pCO_2 observed here in assemblage G_l and G_d values, which appeared strongly related to the response of living maerl calcification rates. The high sensitivity of coralline algae to ocean acidification has already been attributed to their high Mg-calcite content (Morse et al., 2006; Hofmann and Bischof, 2014). In the present study, the pCO_2 increase had 295 adverse consequences on assemblage G_d, both in the winter and summer. In the dark, assemblage R reduced seawater pH by releasing CO_2, and hindered the precipitation of $CaCO_3$ (Cornwall et al., 2013). Under high pCO_2 conditions, the combined effect of acidification and assemblage R in the dark is likely to increase the sensitivity of living and dead *L. corallioides* to dissolution (Andersson et al., 2009). Moreover, as discussed above, the overgrowth of epiphytic algae under high pCO_2 increased assemblage R in the dark. Therefore, the negative effect of ocean acidification on *L. corallioides* G_d would be 300 exacerbated by the presence of epiphytic algae, which promote a decline in pH in the dark. In light, several studies have suggested that moderate growth of fleshy macroalgal communities may reduce the impact of ocean acidification on coralline calcification by reducing the CO_2 concentration of seawater through photosynthesis (Semesi et al., 2009; Short et al., 2014). However, the present findings do not support this idea, because a decline in G_l was observed under high pCO_2 despite high epiphyte biomass. Under high pCO_2, the overgrowth of epiphytic fleshy algae induced by ocean acidification in the summer 305 may reduce light, oxygen and nutrient availability for underlying maerl, affecting its primary production and calcification (D'Antonio, 1985; Short et al., 2014). Thus, overgrown maerl would be negatively affected by the direct effect of ocean acidification on calcification rates and indirect effects due to shifts in competition dynamics with fleshy epiphytic algae (Kuffner et al., 2008).

In regard to the present results, the regulation of epiphyte biomass by grazers appears essential to maintain the proper functioning of maerl bed communities (Guillou et al., 2002). In mollusks and urchins, several studies have demonstrated a link between feeding rates and other metabolic processes, such as respiration, calcification and excretion (Carr and Bruno, 2013; Navarro et al., 2013; Noisette et al., 2016). In mollusks, a wide range of responses to ocean acidification and warming have been revealed (Gazeau et al., 2013; Parker et al., 2013). The differences in sensitivity of mollusks to ocean acidification depend on several parameters, such as the form of $CaCO_3$ they precipitate during calcification (Ries et al., 2009), as well as their ability to regulate the acid-base balance (Gutowska et al., 2010). Our results corroborate these studies, given that *G. magus* and *J. exasperatus* responded differently to acidification and warming. Increased temperature and pCO_2 had no effect on *G. magus* with regard to the metabolic functions tested. However, despite the apparent resistance of *G. magus* to the applied changes, other physiological parameters that we did not test here may have been affected, such as feeding rates, somatic growth, enzyme activity or immune response (Parker et al., 2013). The respiration rates of *J. exasperatus* showed a decline under high pCO_2 in the winter. The lower growth of epiphytes and biofilm in winter may reduce the energy available to maintain the metabolism under stressful conditions (Thomsen et al., 2013; Pansch et al., 2014). This reduced energy availability may induce changes in energy partitioning and decrease R under high pCO_2. In the summer, the increased R under high pCO_2 can be attributed to higher food supply, which is likely to increase the resistance of *J. exasperatus* to climate change, as reported for several marine taxa (Ramajo et al., 2016).

Given the relatively high resistance of *G. magus* and *J. exasperatus* to predicted changes, the metabolic response of *P. miliaris* appears to have stronger implications on assemblage functioning. For example, *P. miliaris* is considered as one of the main macro-epiphytic grazers on maerl beds in the Bay of Brest (Guillou et al., 2002). During the experiments, *P. miliaris* likely played an important role in the regulation of epiphytic biomass. The response of G_1 to temperature and pCO_2 changes was complex. The interaction between temperature and pCO_2 observed in the summer may cause changes in energy partitioning, thereby inducing a trade-off between metabolic processes at the expense of respiration and excretion (Garilli et al., 2015). However, the effect of temperature and pCO_2 on the calcification of *P. miliaris* must be considered carefully. For instance, urchins defecated carbonate pellets following consumption of maerl thalli. These feces are likely to dissolve during incubation, introducing a bias in the measurement of calcification (Gazeau et al., 2015). In the summer, temperature increase

by 3°C reduced *P. miliaris* respiration rates. Moreover, the decrease in excretion under high temperature and pCO_2 conditions was modulated by the interaction between these two factors. Temperature is a major factor affecting physiological processes in ectotherms such as metabolic rates and growth (Kordas et al., 2011). In *P. miliaris*, summer temperatures are likely to exceed the physiological thresholds of organisms, inducing a metabolic decline when maintained at 20°C. Although this decline has only been measured for respiration and excretion, the increase in temperature is also likely to affect sea urchin feeding efficiency (Thomas et al., 2000; Carr and Bruno, 2013). Therefore, the ability of *P. miliaris* to regulate epiphyte biomass may be significantly altered under predicted acidification and warming conditions.

In addition to the impact of climate change on grazer-fleshy macroalgae interactions, predicted changes may also considerably alter the interaction between grazers and coralline algae. Asnaghi et al. (2013) demonstrated that the grazing activity by urchins may exacerbate pCO_2 effects on coralline algae. Ocean acidification may alter the structural integrity of coralline algae, increasing its sensitivity to grazing (Johnson and Carpenter, 2012; Ragazzola et al., 2012). Coralline algae may thus be more susceptible to grazing by urchins, which also benefit from a higher carbonate uptake from their diet to modulate their response to ocean acidification (Asnaghi et al., 2013). In *L. corallioides*, the decrease in calcification rates may alter its structural integrity and increase its susceptibility to grazing, especially by urchins, which are considered as important bioeroders of coralline algae in marine ecosystems (Ballesteros, 2006; O'Leary and McClanahan, 2010), particularly in maerl beds (Lawrence, 2013).

In conclusion, the community response to climate change does not appear to be only the result of individual species' metabolic responses, but also strongly depends on shifts in species interactions. Our results suggest that ocean acidification and warming will strongly destabilize communities through both direct effects on species physiology and changes in the interaction strengths between coralline algae, fleshy algae and grazers. Under the predicted business-as-usual conditions, epiphyte overgrowth may exacerbate the negative impact of climate change on underlying coralline algae. Here, we also demonstrated that climate change may affect grazer physiology, with major consequences on their ability to regulate epiphyte biomass. Climate change may also affect other components that we did not assess in the present study, such as algal palatability and potential changes in grazer trophic behavior (Campbell et al., 2014; Duarte et al., 2015; Poore et al., 2013;

Poore et al., 2016). In line with this study, further work should focus on the impact of climate change on marine communities and species interactions to better understand the consequences on ecosystem functioning.

Authors' Contributions

EL SM PR JG JC designed the experiments; EL SM JC collected the data; EL ML analyzed the data; EL SM PR prepared the manuscript with contributions from all co-authors.

Competing interests

The authors declare that they have no conflict of interest.

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Table 1. Physicochemical parameters (mean ± SE) of seawater in each experimental condition (A-pCO_2 = ambient pCO_2; H-pCO_2 = high pCO_2; T = ambient temperature; T+3°C = high temperature) in the winter and the summer. pH_T and temperature were monitored every two days in each aquarium (n = 35). Total alkalinity values (A_T) are means (± SE) of 28 samples measured in each aquarium. The CO_2 partial pressure (pCO_2), dissolved inorganic carbon (DIC), and saturation states of seawater with respect to aragonite (Ω_{Ar}) and calcite (Ω_{Ca}) were calculated from pH_T, temperature, salinity, and A_T using CO2SYS.

	Experimental condition	pCO_2 (µatm)	pH_T	Temperature (°C)	A_T (µmol kg^{-1})	DIC (µmol kg^{-1})	Ω_{Ar}	Ω_{Ca}
WINTER	A-pCO_2; T	490 (± 5)	7.97 (± 0.04)	10.1 (± 0.3)	2348 (± 6)	2189 (± 6)	1.84 (± 0.02)	2.89 (± 0.02)
	H-pCO_2; T	1183 (± 10)	7.63 (± 0.03)	10.1 (± 0.3)	2342 (± 7)	2306 (± 7)	0.89 (± 0.01)	1.40 (± 0.01)
	A-pCO_2; T+3°C	513 (± 5)	7.97 (± 0.03)	13.7 (± 0.1)	2341 (± 5)	2166 (± 5)	2.01 (± 0.01)	3.14 (± 0.02)
	H-pCO_2; T+3°C	1087 (± 18)	7.64 (± 0.03)	13.6 (± 0.2)	2329 (± 2)	2266 (± 4)	1.09 (± 0.01)	1.70 (± 0.02)
SUMMER	A-pCO_2; T	426 (± 4)	8.03 (± 0.04)	17.1 (± 0.2)	2359 (± 3)	2127 (± 3)	2.60 (± 0.02)	4.03 (± 0.03)
	H-pCO_2; T	948 (± 9)	7.72 (± 0.03)	17.1 (± 0.2)	2382 (± 4)	2279 (± 4)	1.45 (± 0.01)	2.24 (± 0.02)
	A-pCO_2; T+3°C	432 (± 4)	8.01 (± 0.04)	20.0 (± 0.5)	2364 (± 3)	2109 (± 3)	2.88 (± 0.02)	4.43 (± 0.03)
	H-pCO_2; T+3°C	879 (± 7)	7.74 (± 0.02)	20.2 (± 0.3)	2369 (± 2)	2238 (± 2)	1.71 (± 0.01)	2.64 (± 0.02)

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Table 2. PERMANOVA results for the effects of season, temperature (T) and pCO_2 on respiration, net calcification and excretion rates in the urchin *Psammechinus miliaris* and the two gastropods *Gibbula magus* and *Jujubinus exasperatus* (n = 5). Significant p-values are shown in bold ($\alpha = 0.05$). Degrees of freedom = 1; F: pseudo F-statistic

		Respiration µmol O_2 g DW^{-1} h^{-1}		**Net Calcification µmol $CaCO_3$ g DW^{-1} h^{-1}**		**Excretion µmol NH_4^+ g DW^{-1} h^{-1}**	
		F	p-value	F	p-value	F	p-value
Psammechinus miliaris	Season	257.7	**<0.001**	2.8	0.11	15.2	**<0.001**
	T	17.1	**<0.001**	3.1	0.088	3.4	0.072
	pCO_2	2.2	0.15	0.7	0.41	0.1	0.071
	Season x T	14.7	**<0.001**	3.5	0.078	4.2	0.052
	Season x pCO_2	3.9	0.055	0.0	0.89	3.8	0.063
	pCO_2 x T	2.3	0.14	5.6	**0.025**	4.6	**0.037**
	Season x T x pCO_2	0.3	0.59	5.1	**0.028**	0.1	0.74
		F	p-value	F	p-value	F	p-value
Gibbula magus	Season	383.6	**<0.001**	26.4	**<0.001**	206.7	**<0.001**
	T	0.1	0.72	0.1	0.72	2.7	0.11
	pCO_2	0.2	0.64	0.6	0.43	0.6	0.43
	Season x T	0.3	0.59	0.1	0.77	1.1	0.31
	Season x pCO_2	1.5	0.23	0.8	0.37	2.4	0.13
	pCO_2 x T	0.1	0.79	0.0	0.99	0.1	0.79
	Season x T x pCO_2	0.2	0.66	0.3	0.62	0.7	0.41
		F	p-value				
Jujubinus exasperatus	Season	0.9	0.35				
	T	6.5	**0.017**				
	pCO_2	0.0	0.92				
	Season x T	8.7	**0.005**				
	Season x pCO_2	14.0	**<0.001**				
	pCO_2 x T	0.8	0.38				
	Season x T x pCO_2	0.4	0.54				

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585 Table 3. Summary of PERMANOVA for the effects of season, temperature (T) and pCO_2 on net and gross primary production, respiration, chlorophyll *a* content and light and dark calcification rates of living and dead *Lithothamnion corallioides* (n = 5). Significant p-values are shown in bold (α = 0.05). Degrees of freedom = 1; F: pseudo F-statistic

		Net production µmol O_2 g DW^{-1} h^{-1}		**Gross production µmol O_2 g DW^{-1} h^{-1}**		**Respiration µmol O_2 g DW^{-1} h^{-1}**		**Chlorophyll a µg chl a g DW^{-1}**		**Light calcification µmol $CaCO_3$ g DW^{-1} h^{-1}**		**Dark calcification µmol $CaCO_3$ g DW^{-1} h^{-1}**	
		F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value
LIVING *L. corallioides*	Season	2.3	0.14	34.3	**<0.001**	309.0	**<0.001**	0.2	0.66	85.7	**<0.001**	214.8	**<0.001**
	T	4.1	**0.049**	2.7	0.1	0.9	0.34	0.0	0.84	6.4	**0.015**	4.5	**0.041**
	pCO_2	0.2	0.71	0.1	0.78	0.1	0.79	1.4	0.26	6.1	**0.015**	140.8	**<0.001**
	Season x T	0.1	0.78	1.5	0.22	35.7	**<0.001**	6.2	**0.018**	0.3	0.59	4.8	**0.033**
	Season x pCO_2	1.1	0.31	0.8	0.39	0.1	0.76	0.0	0.88	1.7	0.22	0.2	0.63
	pCO_2 x T	5.6	**0.021**	3.0	0.089	3.4	0.071	0.0	0.89	1.1	0.32	0.4	0.54
	Season x T x pCO_2	0.1	0.74	0.0	0.91	0.7	0.42	0.1	0.75	0.0	0.98	0.5	0.48
		F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value
DEAD *L. corallioides*	Season	60.8	**<0.001**	78.2	**<0.001**	115.6	**<0.001**	1.8	0.18	34.4	**<0.001**	0.1	0.71
	T	3.1	0.086	3.3	0.075	2.5	0.13	0.3	0.58	4.0	**0.049**	0.6	0.45
	pCO_2	1.2	0.30	1.9	0.18	4.9	**0.043**	3.5	0.074	29.8	**<0.001**	44.8	**<0.001**
	Season x T	2.8	0.099	2.8	0.11	1.3	0.26	0.1	0.81	3.4	0.073	0.0	0.88
	Season x pCO_2	0.6	0.46	1.2	0.29	4.0	0.053	1.4	0.26	0.2	0.69	2.0	0.16
	pCO_2 x T	0.4	0.53	0.6	0.46	0.8	0.37	24.0	**<0.001**	2.6	0.12	0.6	0.46
	Season x T x pCO_2	1.1	0.31	0.7	0.40	0.0	0.94	0.2	0.68	2.9	0.11	0.6	0.45

590 Table 4. Chlorophyll *a* content (mean ± SE) of living and dead *L. corallioides* in the different pCO_2 (A-pCO_2 = ambient pCO_2; H-pCO_2 = high pCO_2) and temperature (T = ambient temperature; T+3°C = high temperature) treatments, after being maintained three months in winter and summer conditions, n = 5

	Chlorophyll *a* (µg chlorophyll g DW^{-1})			
	A-pCO_2/T	H-pCO_2/T	A-pCO_2/T+3°C	H-pCO_2/T+3°C
Living *L. corallioides*				
Winter	59.84 (± 1.97)	61.66 (± 3.83)	52.93 (± 3.44)	56.85 (± 2.52)
Summer	55.03 (± 2.95)	57.63 (± 3.99)	60.35 (± 0.70)	62.19 (± 3.75)
Dead *L. corallioides*				
Winter	47.09 (± 2.72)	39.39 (± 5.65)	39.15 (± 2.20)	46.36 (± 2.19)
Summer	52.21 (± 1.92)	36.30 (± 1.83)	43.63 (± 0.90)	47.96 (± 2.54)

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Table 5. Summary of the effects of pCO_2 and temperature (T) and their combined effect on gross production and respiration of the two epiphytic algae *R. ardissonei* and *S. chordalis* in the summer (n = 5). Statistical analyses were performed using a two-way crossed Scheirer-Ray-Hare test. Significant p-values are presented in bold (α = 0.05). Degrees of freedom = 1; F: pseudo F-statistic

		Net production µmol O_2 g DW^{-1} h^{-1}		**Gross production µmol O_2 g DW^{-1} h^{-1}**		**Respiration µmol O_2 g DW^{-1} h^{-1}**	
Rhodymenia ardissonei		F	p-value	F	p-value	F	p-value
	T	0.8	0.37	0.2	0.68	1.3	0.25
	pCO_2	0.0	0.96	0.8	0.38	8.6	**0.003**
	pCO_2 x T	1.0	0.31	1.0	0.31	0.7	0.42
Solieria chordalis		F	p-value	F	p-value	F	p-value
	T	0.1	0.76	0.1	0.80	5.5	**0.019**
	pCO_2	3.0	0.08	8.3	**0.011**	3.9	**0.049**
	pCO_2 x T	5.8	**0.016**	7.5	**0.014**	0.0	0.48

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Table 6. Summary of PERMANOVA for the effects of season, temperature (T) and pCO_2 on net and gross primary production, respiration and light and dark calcification rates, measured on assemblages (n = 5). Significant p-values are presented in bold (α = 0.05). Degrees of freedom = 1; F: pseudo F-statistic

		Net production µmol O_2 g DW^{-1} h^{-1}		**Gross production µmol O_2 g DW^{-1} h^{-1}**		**Respiration µmol O_2 g DW^{-1} h^{-1}**		**Light calcification µmol $CaCO_3$ g DW^{-1} h^{-1}**		**Dark calcification µmol $CaCO_3$ g DW^{-1} h^{-1}**	
		F	p-value	F	p-value	F	p-value	F	p-value	F	p-value
Assemblages	Season	30.2	**<0.001**	50.6	**<0.001**	219.7	**<0.001**	290.5	**<0.001**	19.3	**<0.001**
	T	1.2	0.28	2.2	0.14	1.7	0.19	38.1	**<0.001**	10.0	**0.003**
	pCO_2	15.7	**<0.001**	9.6	**0.004**	2.1	0.16	46.0	**<0.001**	14.9	**<0.001**
	Season x T	1.7	0.21	0.2	0.66	0.1	0.73	15.7	**<0.001**	3.8	0.054
	Season x pCO_2	0.6	0.43	7.1	**0.013**	10.9	**0.001**	0.1	0.78	0.4	0.54
	pCO_2 x T	2.7	0.11	0.4	0.53	0.1	0.72	0.2	0.71	2.6	0.12
	Season x T x pCO_2	0.4	0.55	1.2	0.29	1.3	0.27	3.3	0.080	4.7	**0.039**

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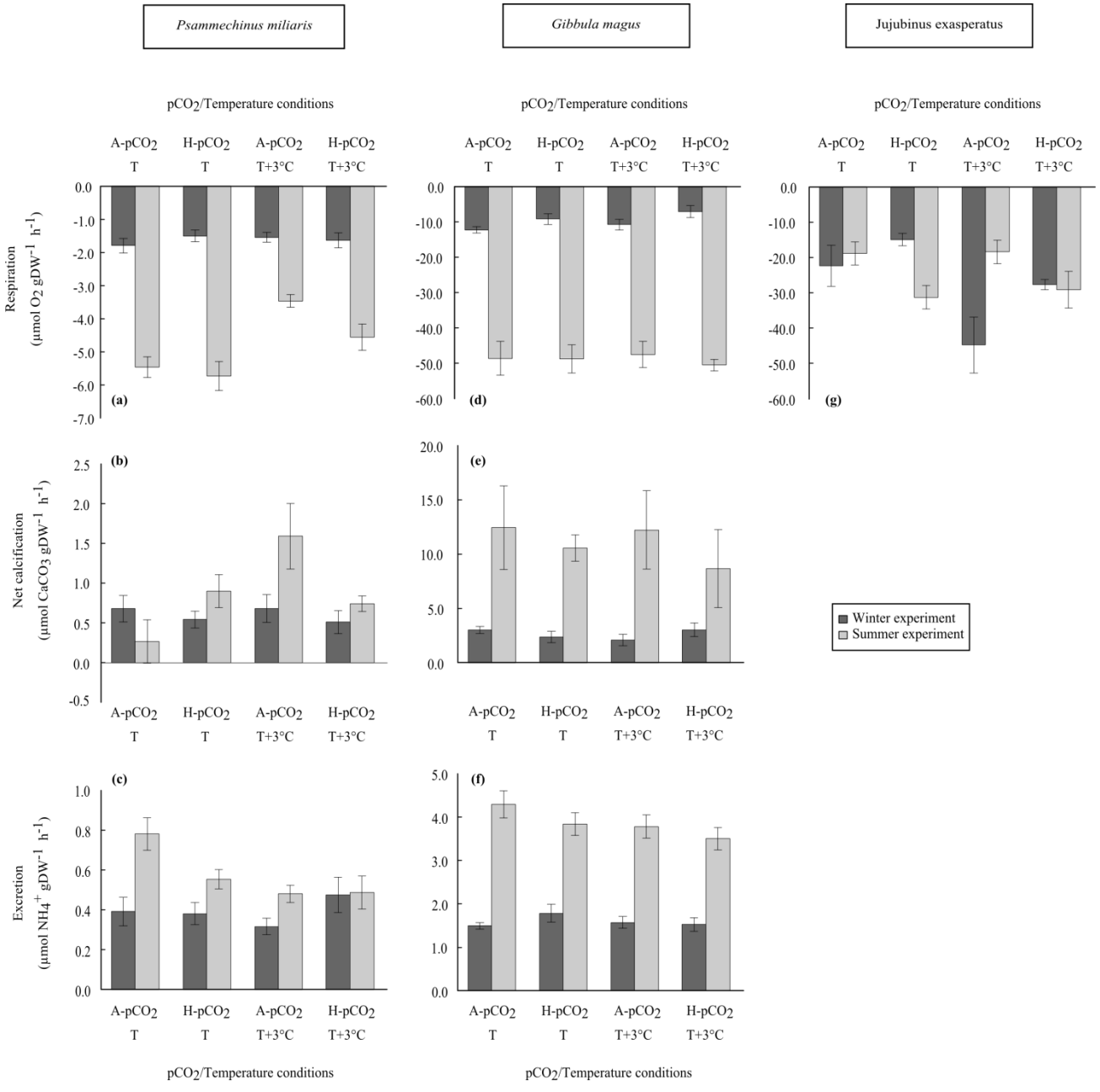

Fig. 1. Respiration, net calcification and excretion rates (mean ± SE) of the grazers *P. miliaris* (a to c), *G. magus* (d to f) and respiration of *J. exasperatus* (g) in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High-pCO_2) and temperature (T = Ambient temperature; T+3°C = High temperature) conditions. The species were maintained in assemblages for three months in winter (dark gray) and summer conditions (light gray). n = 5

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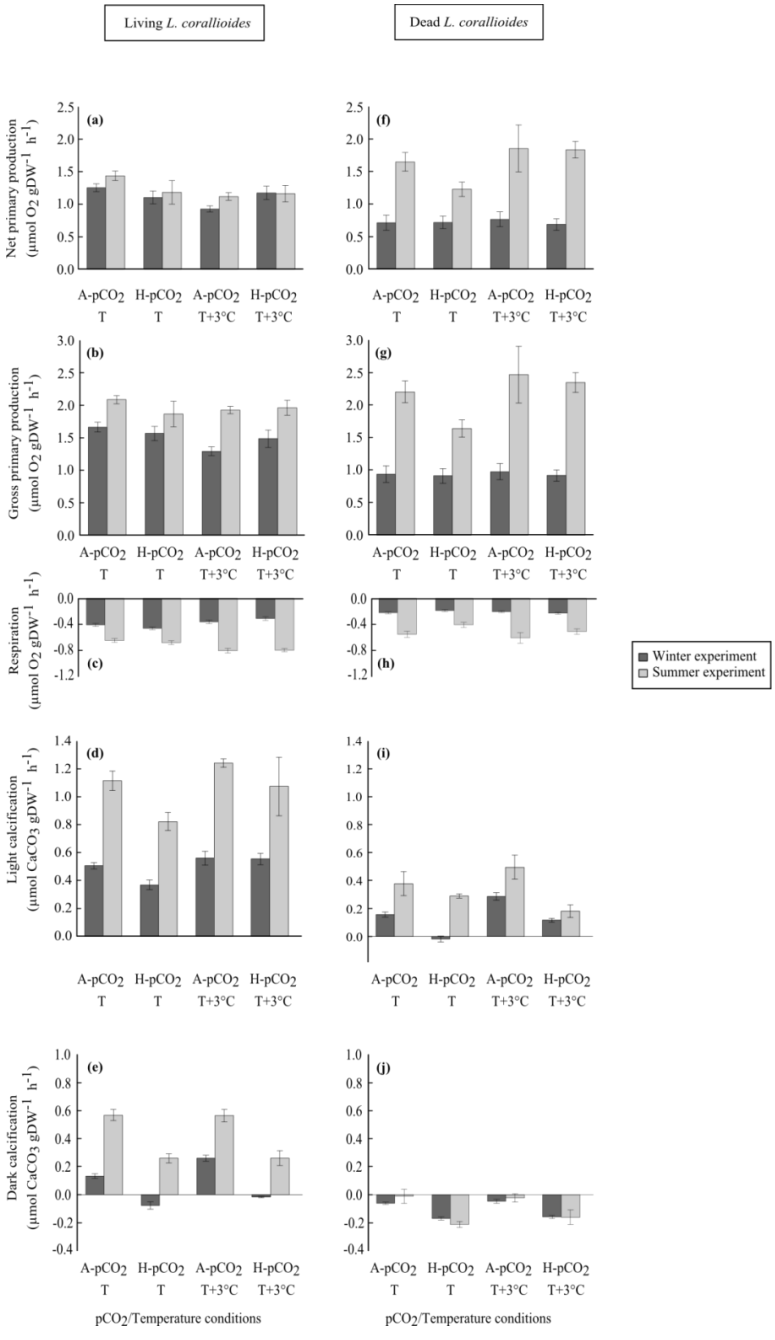

Fig. 2. Net and gross primary production, respiration, light and dark calcification rates (mean ± SE) of living (a to e) and dead thalli (f to j) of *L. corallioides* in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High-pCO_2) and temperature (T = Ambient temperature; T+3°C = High temperature) treatments, after three months in winter (dark gray) and summer conditions (light gray). n = 5

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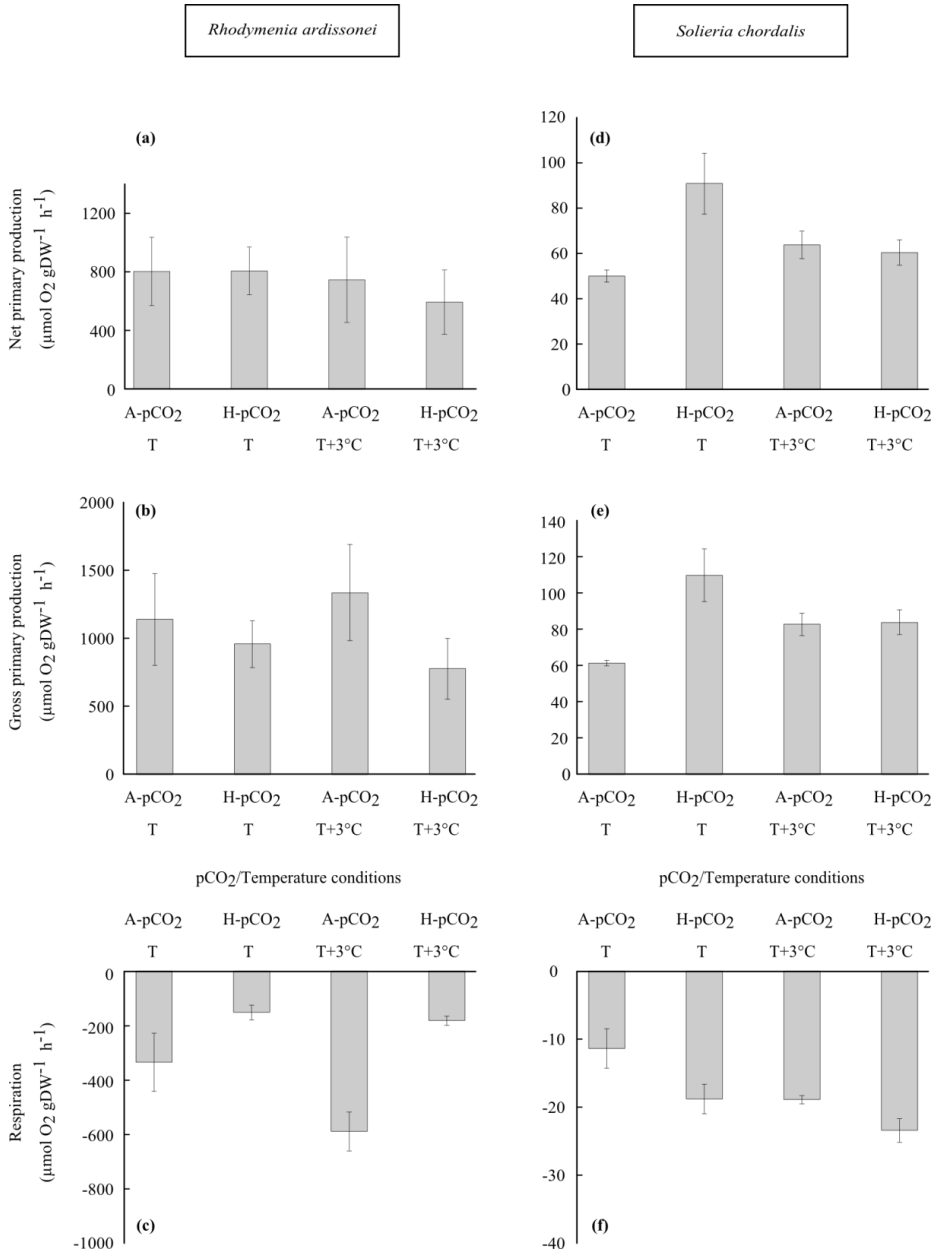

610 Fig. 3. Summer net and gross primary production and respiration rates (mean ± SE) of the two main epiphytic fleshy algae *Rhodymenia ardissonei* (a to c) and *Solieria chordalis* (d to f), in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High-pCO_2) and temperature (T = Ambient temperature; T+3°C = High temperature) treatments. n = 5

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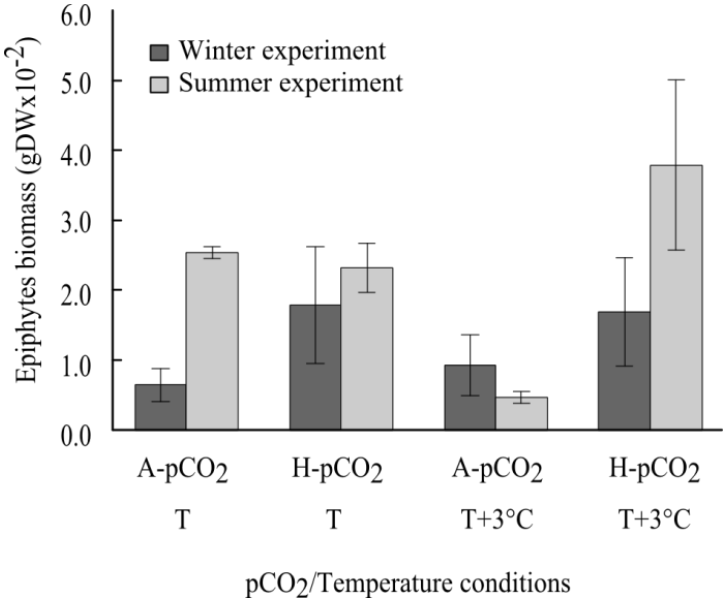

Fig. 4. Biomass of epiphytic fleshy algae obtained in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High-pCO_2) and temperature (T = Ambient temperature; T+3°C = High temperature) treatments, after the three-month experiments in winter (dark gray) and summer (light gray) experiments. n = 5

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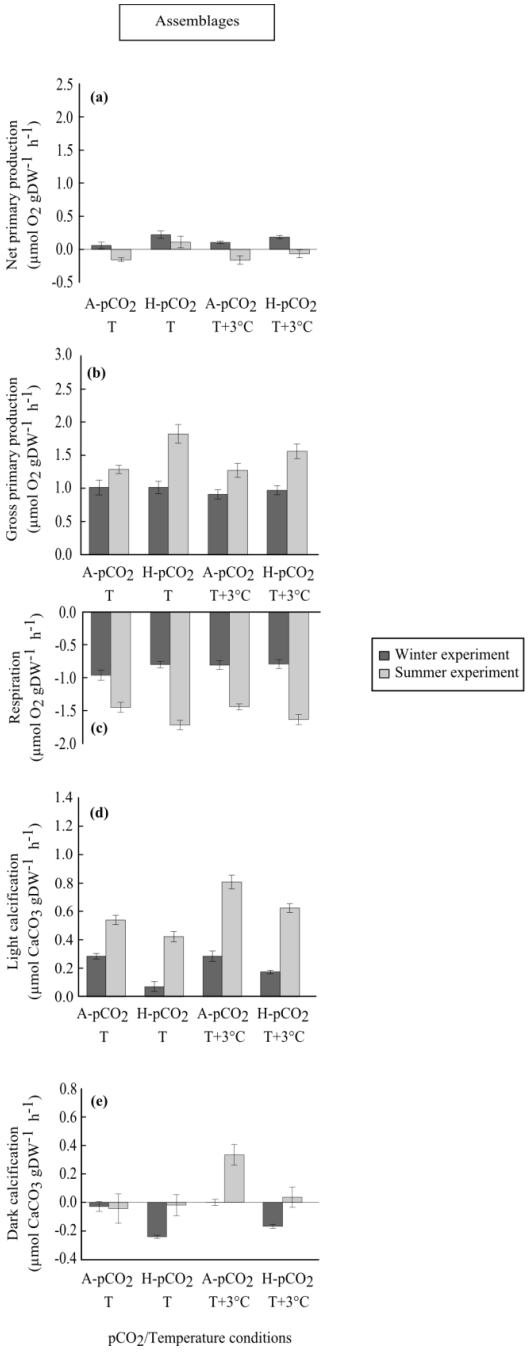

Fig. 5. Net and gross primary production (a and b, respectively), respiration (c) and light and dark calcification rates (d and e, respectively) rates (mean ± SE) of assemblages in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High-pCO_2) and temperature (T = Ambient temperature; T+3°C = High temperature) treatments. The assemblages were maintained during three months in winter (dark gray) and summer conditions (light gray). n = 5