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Competition between calcifying and noncalcifying temperate marine macroalgae under elevated CO_2 levels

Laurie C. Hofmann*, Sandra Straub, Kai Bischof

Marine Botany, Bremen Marine Ecology Center for Research and Education, University of Bremen, Leobener Straße NW2, 28359 Bremen, Germany

ABSTRACT: Since pre-industrial times, uptake of anthropogenic CO_2 by surface ocean waters has caused a documented change of 0.1 pH units. Calcifying organisms are sensitive to elevated CO_2 concentrations due to their calcium carbonate skeletons. In temperate rocky intertidal environments, calcifying and noncalcifying macroalgae make up diverse benthic photoautotrophic communities. These communities may change as calcifiers and noncalcifiers respond differently to rising CO_2 concentrations. In order to test this hypothesis, we conducted an 86 d mesocosm experiment to investigate the physiological and competitive responses of calcifying and noncalcifying temperate marine macroalgae to 385, 665, and 1486 µatm CO_2. We focused on comparing 2 abundant red algae in the Northeast Atlantic: *Corallina officinalis* (calcifying) and *Chondrus crispus* (noncalcifying). We found an interactive effect of CO_2 concentration and exposure time on growth rates of *C. officinalis*, and total protein and carbohydrate concentrations in both species. Photosynthetic rates did not show a strong response. Calcification in *C. officinalis* showed a parabolic response, while skeletal inorganic carbon decreased with increasing CO_2. Community structure changed, as *Chondrus crispus* cover increased in all treatments, while *C. officinalis* cover decreased in both elevated-CO_2 treatments. Photochemical parameters of other species are also presented. Our results suggest that CO_2 will alter the competitive strengths of calcifying and noncalcifying temperate benthic macroalgae, resulting in different community structures, unless these species are able to adapt at a rate similar to or faster than the current rate of increasing sea-surface CO_2 concentrations.

KEY WORDS: Competition · *Corallina officinalis* · *Chondrus crispus* · Calcification · Ocean acidification · Mesocosm

INTRODUCTION

Anthropogenic CO_2 production is changing the chemistry of surface ocean waters, and since pre-industrial times, uptake of CO_2 by surface waters has caused a documented change of 0.1 pH units (Caldeira & Wickett 2003). The atmospheric CO_2 concentration is expected to climb to 800–1000 ppm by the year 2100 (Bindoff et al. 2007), and model simulations indicate they could even reach 1900 ppm by 2300 (Caldeira & Wickett 2003, Orr et al. 2005). The atmospheric CO_2 levels expected in 2100 would result in a decrease of surface seawater pH of 0.3 to 0.5 units (Caldeira & Wickett 2005, Orr et al. 2005). Recent research on the consequences of these changes on marine organisms has shown varied responses across multiple taxonomic levels (Fabry 2008, Ries 2009, Kroeker et al. 2010, Fabricius et al. 2011, Rodolfo-Metalpa et al. 2011). In general, calcifying organisms seem to be the most sensitive (Kroeker et al. 2010), but even among calcifiers, the response to elevated CO_2 is not consistent (Fabry 2008, Ries 2009, Fabricius et al. 2011).

*Email: l.hofmann@uni-bremen.de

Calcifying benthic photoautotrophs may be particularly susceptible to the elevated surface-seawater CO_2 concentrations as they are sessile (as adults) and rely on CO_2 as the substrate for photosynthesis. Benthic marine macroalgae are ecologically important as they provide food, refugia, and substrata for diverse marine communities (Paine & Vadas 1969, Lubchenco 1978, Littler & Littler 1984, Gibbons & Griffiths 1986, Eriksson et al. 2006). Therefore, they are essential organisms to study within the context of future climate change. Several authors have investigated the responses of noncalcifying macroalgae to elevated CO_2 concentrations (e.g. Gao et al. 1991, 1993, Mercado et al. 1999, Gordillo et al. 2001, Zou 2005, Cornwall et al. 2012), and have found varied responses. However, calcifying macroalgae, particularly high-magnesium calcite-depositing coralline algae, show pronounced sensitivity to elevated CO_2 concentrations with respect to calcification rates, necrosis, mortality, and recruitment (Jokiel et al. 2008, Kuffner et al. 2008, Martin et al. 2008, Büdenbender et al. 2011, Hofmann et al. 2012, Porzio et al. 2011). Due to the variable reactions of noncalcifying and calcifying macroalgae to elevated CO_2, it is likely that macroalgal communities will show considerable changes in structure and diversity in future oceans. Indeed, tropical community studies have shown that crustose calcifying algae decrease growth rate, cover, and recruitment, while noncalcifying algae show a subsequent increase in cover under elevated CO_2 conditions (Jokiel et al. 2008, Kuffner et al. 2008). Furthermore, Porzio et al. (2011) recently reported that macroalgal species diversity, abundance, and reproduction changes along a natural CO_2 gradient in the Mediterranean. They found that calcitic algae decreased in cover and species richness with decreasing pH. Such a change in macroalgal community structure could have profound effects on the marine fauna, to which coralline algae provide structural support, substrata, and refugia (Bak 1976, Stewart 1982, Coull & Wells 1983, Akioka et al. 1999).

Several studies have shown significant effects of CO_2 on warm-water communities (Jokiel et al. 2008, Kuffner et al. 2008, Porzio et al. 2011). However, little attention has been given to temperate macroalgal communities outside of the Mediterranean, despite the fact that both crustose and articulated coralline algae co-exist with noncalcifying species in diverse macroalgal communities (Hall-Spencer et al. 2008, Martin et al. 2008, Hepburn et al. 2011, Porzio et al. 2011, Russell et al. 2011). Therefore, diverse macroalgal communities provide an ideal platform for investigating the physiological ecology of macroalgal responses to elevated CO_2 concentrations, particularly with respect to their competitive interactions. Demonstrating changes in community structure in response to an external stress is often the main goal of large-scale mesocosm and field-manipulation experiments. However, it is also important to understand the physiology behind the species-specific responses of co-existing organisms to external stress, and how those responses affect competitive interactions that are reflected in community structure and function. To date, no studies have directly linked elevated CO_2-related physiological responses of temperate macroalgal communities to competition between calcifiers and noncalcifiers and the ecological consequences of such competition. Therefore, we conducted a mesocosm experiment over 87 d to determine how the physiology of calcifying and noncalcifying benthic temperate macroalgae is affected by elevated CO_2 levels, how these physiological responses affect their competition, and finally, if changes in competition strengths are reflected at the community level.

MATERIALS AND METHODS

Experimental design and seawater chemistry

On 16 March 2011, macroalgal communities were collected from the coast of Helgoland, Germany where they grow attached to red sandstone rocks in the intertidal zone. We chipped away the rocks using hammers and chisels. As a result, the experimental communities remained intact and attached to their natural substratum. All communities contained the calcifier *Corallina officinalis* and its associated counterparts, which at the time consisted of mostly the noncalcifying red algae *Chondrus crispus*, *Dumontia incrassata*, *Polysiphonia fucoides*, and red calcifying crustose algae. The communities were kept in running seawater overnight, and transported to the Wadden Sea Station of the Alfred Wegener Institute on the North Sea island of Sylt, where the experiment was conducted.

The algae were acclimated to the ambient Wadden Sea seawater in a large outdoor tank with filtered running seawater for 1 wk before initial measurements were taken. The seawater was double-filtered, first with a protein skimmer (Model III P with 2000 l h^{-1} flow rate, Sander Elektroapparatebau) and then with a UV filter (Model 4000/75 Watt, Wiegandt). During the acclimation week, the mesocosms were prepared and the seawater chemistry was monitored.

The mesocosms were cylindrical plexiglass tanks 60 cm tall and 40 cm in diameter. They were surrounded by 2000 l of continuously running seawater for temperature control. The mesocosms also received continuously running seawater and were rigorously bubbled with 1 of 3 CO_2 concentrations: 385 µatm (ambient), 665 µatm, and 1486 µatm CO_2. The CO_2 concentrations were achieved using an HTK 5-channel gas mixing system. Each CO_2 treatment contained 4 tanks (n = 4) and 1 control tank (not furnished with algae).

Once the water chemistry was stable and the algae were acclimated to ambient light and seawater conditions, initial photochemical and photosynthesis measurements were taken, tissue samples were frozen in liquid nitrogen and stored at −80°C for later analysis, and photographs of each community were taken before the communities were sorted randomly into the 12 treatment tanks. Each tank contained 1 rock with exclusively *Corallina officinalis* for growth measurements, 1 rock with only *C. officinalis* and *Chondrus crispus*, and 1 rock containing *C. officinalis* in a more diverse community (> 3 species).

The pH, temperature, and salinity in each tank were monitored twice daily. Water samples for total alkalinity and nutrient analysis were taken weekly. Total alkalinity was measured using a TitroLine alpha 05 plus titrator with an automated sample changer and IoLine IL-Micro pH electrode (SI Analytics). Salinity and temperature were measured using a Portamess 910 Cond conductivity meter, and pH was measured using a WTW SenTix 41 pH electrode connected to a WTW pH 3310 portable pH-meter. The physiological response variables and analysis of community structure were measured monthly. The experiment lasted for 86 d, from March 28 to June 24, 2011.

Growth and calcification of calcifying rhodophyte *Corallina officinalis*

Growth of *Corallina officinalis* was measured by staining the algae thalli. Each treatment tank contained a rock on which only *C. officinalis* was growing. The algae growing on these rocks were stained for 12 to 24 h in alizarin red stain (Rivera et al. 2004) for growth measurements based on length increase. Multiple tips of multiple thalli were measured and averaged for each tank, and this value was counted as 1 replicate.

Calcification rates were determined by measuring the total alkalinity (A_T) of seawater before and after 2 h incubations in small incubation chambers (10 cm diameter × 30 cm height). One rock carrying only *Corallina officinalis* was placed into each chamber during the incubations, which were continuously bubbled with the respective premixed CO_2 treatment levels. Net calcification rates were calculated according to the equation:

$$G_{net} = -0.5\rho_w\,(V/S) \times (\Delta A_T/\Delta t) \quad (1)$$

where G_{net} is net calcification rate (µmol $CaCO_3$ m^{-2} h^{-1}), ρ_w is seawater density (kg l^{-1}), V is seawater volume (l), S is surface area of the algal assemblage (m^2), ΔA_T is change in alkalinity during the incubation, and Δt is incubation period (h). The percentage of the dry weight (DW) of *C. officinalis* thalli consisting of inorganic carbon was measured by determining the ash-free dry weight (AFDW) of the tissue. Thalli fragments were dried for 48 h at 50°C, weighed, placed into pre-burned and pre-weighed crucibles, and incinerated at 400°C for 24 h. The relative percentage of the DW consisting of inorganic carbon was calculated as (AFDW/DW) × 100 %.

Physiological responses of *Corallina officinalis* vs. *Chondrus crispus*

Photosynthesis

Respiration and oxygen production in *Corallina officinalis* and *Chondrus crispus* were measured as outlined in Hofmann et al. (2012), with the modification of 3 min light intervals during photosynthesis-irradiance (*P-E*) curve measurements. Respiration was measured in the dark for 15 min prior to the light steps, which consisted of light intensities ranging from 0 to 1000 µmol photons m^{-2} s^{-1}. Oxygen consumption/production was measured using a Hansatech Chlorolab 3 System (Hansatech Instruments). Maximum photosynthesis rate (P_{max}), photosynthetic efficiency (α), and light saturation point (E_k) were calculated from nonlinear regression analyses based on the model from Eilers & Peeters (1988).

Concentration of phycobilins, soluble proteins, and carbohydrates

The method for measuring phycobilin, protein, and carbohydrate concentrations in a single algal extract was done according to Andria et al. (1999). Algal thalli (100 to 200 mg) previously frozen in liquid

nitrogen and stored at −80°C were ground to a fine powder in pre-chilled shaking flasks using a Mikro-Dismembrator (B. Braun Biotech International). The algal powder was suspended in 5 to 10 ml of cold 0.1 M phosphate buffer (pH 6.8) and kept in the dark at 4°C overnight. The resulting algal extract was split into 3 supernatant fractions that were used for subsequent pigment, protein, and carbohydrate analysis. The pellet was used to determine insoluble carbohydrate content. Methods for measuring phycoerythrin and phycocyanin were taken from Beer & Eshel (1985), protein precipitation was conducted according to Barbarino & Lourenço (2005), and protein concentration was determined by the Bradford method (Bradford 1976). The phenol sulfuric acid method was used to determine soluble and insoluble carbohydrate concentrations (Kochert 1978).

Community analysis

Photochemistry of macroalgal communities

Photochemical parameters of the entire macroalgal communities were measured using a Maxi-Imaging-PAM (pulse amplitude modulated) chlorophyll fluorometer (Walz) equipped with a blue LED-Array illumination unit and a CCD Camera with 1392 × 1040 pixels (Pike, Allied Vision Technologies). The chlorophyll fluorescence measured by the PAM was digitized by the camera and transferred to a PC, which allows the user to obtain an image of the chlorophyll fluorescence for a large area, including mixed communities. The communities were immersed in a beaker containing their treatment water and dark-adapted for 5 min prior to photochemical analysis. Following dark adaptation, the chlorophyll fluorometer measured the dark fluorescence yield (F_o) and maximum fluorescence (F_m) for the entire community. From these parameters, the maximal photosystem II (PS II) quantum yield F_v/F_m was calculated according to the equation:

$$F_v/F_m = (F_m - F_o)/F_m \quad (2)$$

where F_v is variable fluorescence, or the difference between F_m and F_o. Then the communities were exposed to a series of pre-defined increasing photosynthesis-saturating light pulses at 20 s intervals, and the effective PS II quantum yield (Y) was measured after each pulse. The relative electron transport rates (rETR) were then calculated using the equation:

$$r\mathrm{ETR} = 0.5 \times Y \times \mathrm{PAR} \quad (3)$$

where PAR is light intensity of each saturation pulse (µmol photons m^{-2} s^{-1}). The factor 0.5 accounted for the 2 quanta that must be absorbed for every electron transported due to the presence of 2 photosystems. The resulting values were used to produce rETR versus irradiance curves, from which we calculated the maximum rETR ($r\mathrm{ETR}_{max}$), E_k, and the electron transport rate efficiency (α) using the nonlinear model from Eilers & Peeters (1988).

Percent cover, diversity, and dominance

Digital photographs of each macroalgal community were taken monthly and analyzed for percent cover of individual species and community diversity and dominance indexes using the Coral Point Count with Excel extensions (CPCe) software (Kohler & Gill 2006). A 10 × 10 point grid was overlaid on each photograph, and the species that occurred at each point in the community was recorded for the analysis. The percent change in cover was calculated by relating the percent cover of each species over time to the initial percent cover of that species according to the equation $[(\%\ \mathrm{cover}_t - \%\ \mathrm{cover}_i)/\%\ \mathrm{cover}_i] \times 100$, where t is Day 36 or 86, and i is initial.

Statistical analysis

Statistical analyses were applied to test for significance at the 95% ($p < 0.05$) confidence level. When a single response variable for one species was analyzed, a 1-way ANOVA was conducted followed by pairwise comparisons using a Tukey HSD test. When a single response variable was measured over time, a repeated-measures ANOVA was conducted using time as a within-subject factor and CO_2 as the between-subject factor. For analysis of multiple response variables from multiple species over time, a mixed factorial multivariate analysis of variance (MANOVA) was conducted including time as a within-subject factor and CO_2 and species as between-subject factors. A Tukey HSD test was used for pairwise comparisons. For the analysis of proteins, carbohydrates, and phycobiliproteins, the MANOVA was followed by a discriminant analysis. If the data were not normally distributed, they were transformed to fit the assumptions of an ANOVA. When the data did not meet Mauchly's test of sphericity, the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity. Separate MANOVAs were conducted for P-E curve

parameters, chlorophyll *a* fluorescence parameters, and tissue content, because the sampling technique and time of these analyses were slightly different. Correlation analysis was conducted by calculating Pearson's correlation coefficient (Pearson's r) of 2 variables using a 1-tailed test for significance.

RESULTS

Seawater chemistry

Seawater chemistry parameters are outlined in Table 1. The mean CO_2 concentrations in the tanks containing algal communities were slightly lower than the means measured in control tanks, indicating that the algal communities took up ~1% of the dissolved CO_2. The seawater in the highest-CO_2 treatment tanks was saturated with calcite ($\Omega_{calcite} > 1$), and undersaturated with aragonite ($\Omega_{aragonite} < 1$). Weekly measurements of inorganic nitrogen and phosphorus concentrations (nitrate and phosphate) showed seasonal fluctuations. Nitrate concentrations ranged from 7 to 40 µM, and were highest at the beginning of the experiment and lowest after 40 d. Phosphate concentrations started at 0.19 µM and steadily increased during the experiment, with a maximum concentration of 0.94 µM. The increase in phosphate concentration over time was most likely due to the decomposition of spring phytoplankton blooms.

Growth and calcification of *Corallina officinalis*

The growth rate of *Corallina officinalis* after 74 d was influenced by a main effect of CO_2 concentration (1-way ANOVA: df = 2, $F = 9.439$, p = 0.008). It was highest at 385 µatm CO_2 and lowest at 1485 µatm CO_2 (Fig. 1). Calcification rates showed a parabolic relationship to seawater aragonite saturation states ($\Omega_{aragonite}$), with the highest rate measured at $\Omega_{aragonite}$ = 1.65, which corresponded to the 665 µatm CO_2 treatment (Fig. 2a). There was no significant difference between calcification rates at the highest and lowest CO_2 level. However, after 30 d, the skeletal inorganic carbon of *C. officinalis* was significantly positively correlated to aragonite saturation state (linear regression analysis: $r^2 = 0.535$, p = 0.007), and this relationship remained consistent throughout the remainder of the experimental period (Fig. 2b; time series data not shown). Furthermore, there was an inverse relationship between calcification rate and skeletal inorganic carbon when inorganic carbon was above 77.1% of the dry weight; below that point, there was no clear relationship between skeletal inorganic carbon and

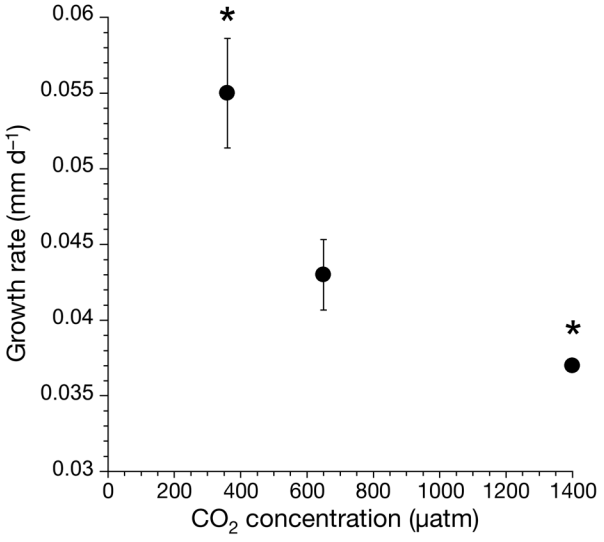

Fig. 1. *Corallina officinalis*. Growth rates (means ± SE) after 74 d exposure to CO_2 treatments, based on length measurements of new material that appeared after staining with alizarin red. *Significant differences in growth rates between CO_2 concentrations ($F = 9.439$, p = 0.008)

Table 1. Seawater (SW) parameters for CO_2-treated tanks without algae (regular font) and with algae (**bold**) calculated from daily measurements throughout the experiment. Algae in the tanks produced slightly lower CO_2 concentrations compared to the tanks without algae due to metabolic uptake of dissolved CO_2. $\Omega_{calcite}$: calcite saturation state, $\Omega_{aragonite}$: aragonite saturation state

CO_2 treatment	pCO_2 (µatm)	pH	$[CO_2]$ (µmol kg^{-1} SW)	$[HCO_3^-]$ (µmol kg^{-1} SW)	$[CO_3^{2-}]$ (µmol kg^{-1} SW)	$\Omega_{calcite}$	$\Omega_{aragonite}$
Ambient	385 ± 20	8.22 ± 0.02	16.1 ± 0.94	1980 ± 24	150 ± 8	3.71 ± 0.2	2.33 ± 0.1
	371 ± 11	**8.25 ± 0.01**	**16.5 ± 0.58**	**2010 ± 28**	**161 ± 4**	**4.00 ± 0.1**	**2.52 ± 0.1**
Medium	665 ± 36	8.01 ± 0.02	28.1 ± 1.7	2103 ± 19	98 ± 6	2.44 ± 0.2	1.53 ± 0.1
	602 ± 15	**8.05 ± 0.01**	**25.4 ± 0.72**	**2088 ± 10**	**106 ± 3**	**2.62 ± 0.1**	**1.65 ± 0.1**
High	1486 ± 73	7.69 ± 0.02	62.4 ± 3.4	2230 ± 13	49 ± 3	1.22 ± 0.1	0.77 ± 0.1
	1380 ± 43	**7.73 ± 0.01**	**58.3 ± 2.1**	**2242 ± 30**	**55 ± 2**	**1.36 ± 0.1**	**0.85 ± 0.03**

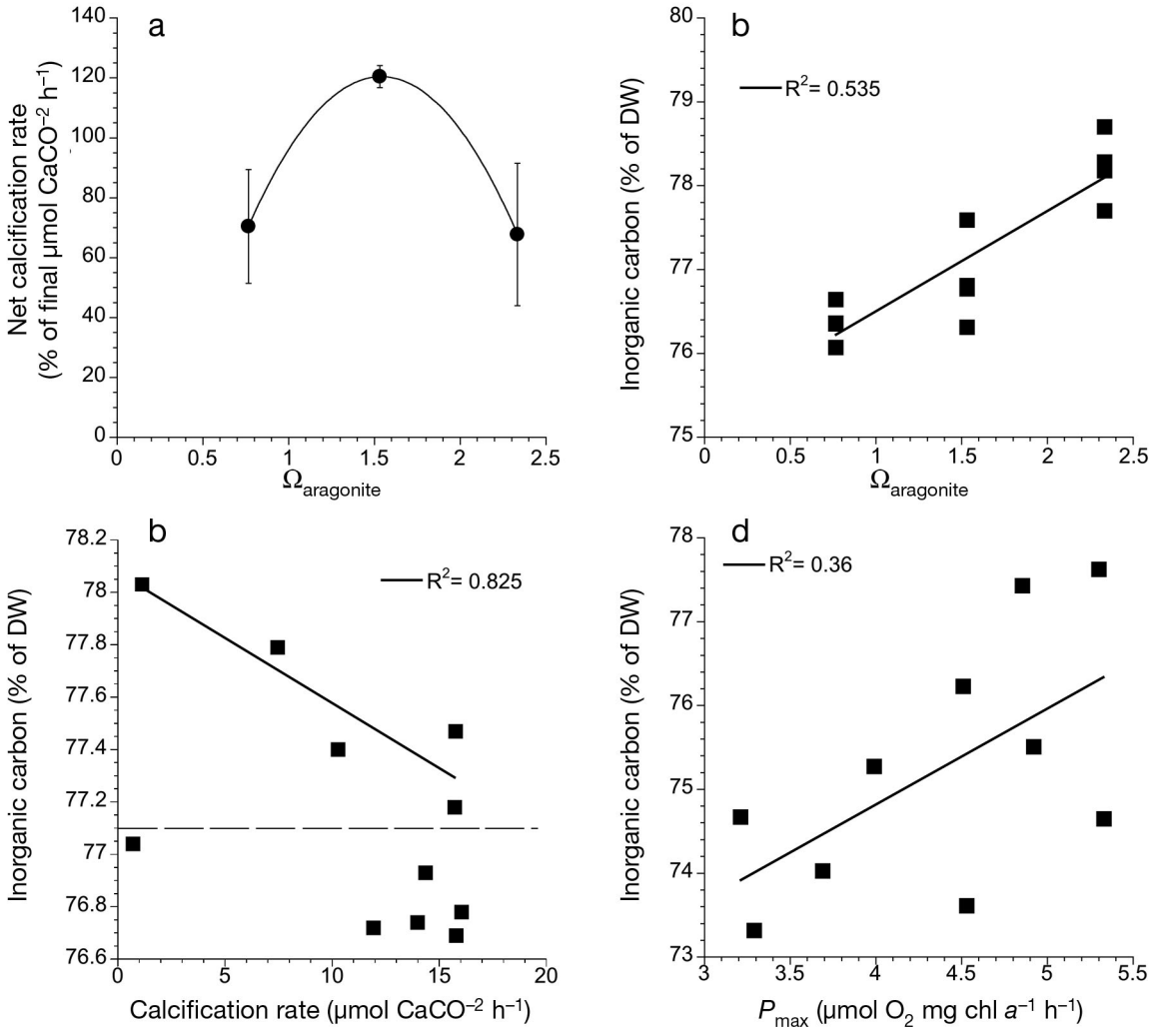

Fig. 2. *Corallina officinalis*. (a) Calcification rates after 38 d exposure to CO_2 treatments as a function of aragonite saturation state ($\Omega_{aragonite}$, means ± SE). (b) Percent inorganic carbon of thalli dry weight (DW) as a function of $\Omega_{aragonite}$. (c) Relationship between inorganic carbon and calcification rate, showing a linear relationship above a cut-off point at 77.1%. (d) Inorganic carbon versus maximum photosynthesis rate (P_{max})

calcification rate (Fig. 2c). In contrast, there was a positive correlation between inorganic carbon and maximum photosynthetic rate (Fig. 2d).

Physiological responses of *Corallina officinalis* vs. *Chondrus crispus*

Photosynthesis

Dark respiration rates E_k, P_{max}, and α of *Corallina officinalis* and *Chondrus crispus* were not significantly affected by CO_2 concentration, but there was a significant main effect of time on α and respiration rate, a significant interaction between time and species with respect to E_k and α, and a significant effect of species on α (Tables 2 & 3, Fig. 3). The interaction between time and species was due to the fact that E_k and photosynthetic efficiency of *C. crispus* were much higher and lower, respectively, after 88 d of exposure compared to the rates after 38 d, which was likely a seasonal effect in response to higher temperatures. Photosynthetic efficiency of *C. officinalis* at the end of the experiment was higher than at the beginning, and was also higher than the photosynthetic efficiency of *C. crispus* after 88 d. The E_k values of *C. officinalis* after 88 d tended to decrease with increasing CO_2 concentration, but the trend was not significant (Pearson's r = –0.567, p = 0.056). In contrast, the E_k values of *C. crispus* were higher at the end of the experiment than at the beginning, regardless of CO_2 concentration.

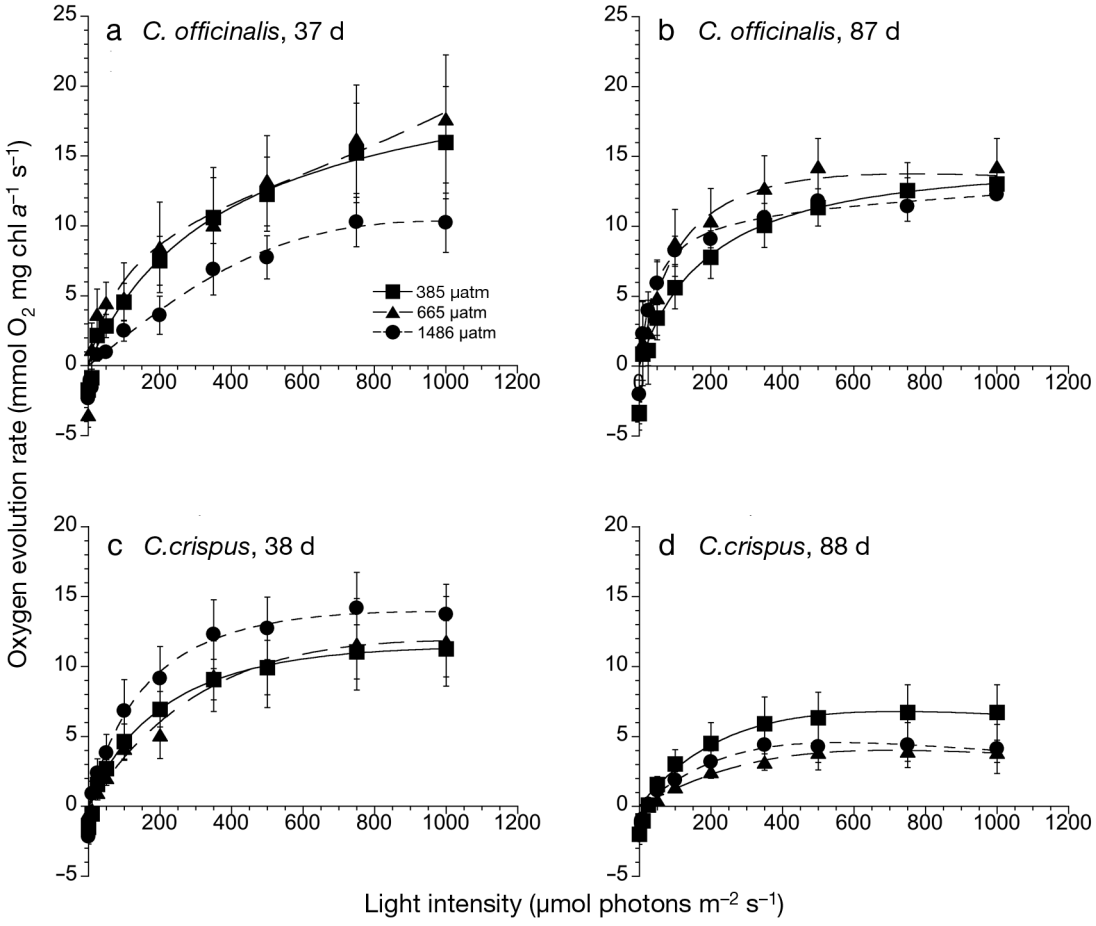

Fig. 3. *Corallina officinalis* and *Chondrus crispus*. Oxygen production as a function of light intensity in *Corallina officinalis* after (a) 37 d and (b) 87 d, and in *Chondrus crispus* after (c) 38 d and (d) 88 d of exposure to CO_2 treatments. Means ± SE are shown

Table 2. *Corallina officinalis* and *Chondrus crispus*. Photosynthetic parameters (means ± SE) measured after 37/38 and 87/88 d exposure to CO_2 treatments. Parameters are based on oxygen evolution measurements at multiple light intensities ranging from 0 to 1000 µmol photons m^{-2} s^{-1}. P_{max}: maximum photosynthetic rate, E_k: light saturation point, α: photosynthetic efficiency. nd: no data

Species	CO_2 level (µatm)	Exposure (d)	P_{max} (mmol O_2 mg chl a^{-1} s^{-1})	E_k (µmol photons m^{-2} s^{-1})	α [mmol O_2 (mg chl *a*)$^{-1}$ (µmol photons m^{-2})]
Corallina officinalis	385	37	16.4 ± 4.2	408 ± 81	0.046 ± 0.015
	665	37	17.1 ± 3.7	nd	0.050 ± 0.024
	1486	37	10.8 ± 2.1	396 ± 23	0.023 ± 0.009
	385	87	13.1 ± 1.5	385 ± 145	0.107 ± 0.071
	665	87	13.8 ± 1.7	165 ± 46	0.200 ± 0.109
	1486	87	11.7 ± 0.3	93 ± 70	0.487 ± 0.251
Chondrus crispus	385	38	11.3 ± 2.0	205 ± 46	0.066 ± 0.018
	665	38	11.9 ± 3.3	280 ± 112	0.050 ± 0.009
	1486	38	14.0 ± 2.3	203 ± 62	0.115 ± 0.054
	385	88	6.9 ± 2.0	279 ± 63	0.033 ± 0.012
	665	88	4.0 ± 0.8	321 ± 97	0.016 ± 0.004
	1486	88	4.6 ± 1.7	208 ± 31	0.021 ± 0.005

Table 3. Mixed factorial MANOVA tests using time as a within-subject variable (WS) and CO_2 and species Sp. as between-subject variables (BS). The lines separating groups of response variables indicate separate tests conducted due to differences in sampling dates or methods. A separate mixed factorial ANOVA was conducted for the percent change in cover because only data for Days 36 and 86 could be analyzed. P_{max}: maximum photosynthetic rate, E_k: light saturation point, α: photosynthetic efficiency, $rETR_{max}$: relative maximum electron transport rate, F_v/F_m: maximum quantum yield of photosystem II. Sol C: soluble carbohydrates, Ins C: insoluble carbohydrates. SWDI: Shannon-Wiener diversity index. D: Simpson's dominance index

Response variable	Factor or interaction																			
	Time (WS)			CO_2 (BS)			Species			Time × CO_2			CO_2 × Species			Time × Species			Time × CO_2 × Sp.		
	F	df	p	*F*	df	p	*F*	df	p	*F*	df	p	*F*	df	p	*F*	df	p	*F*	df	p
P_{max}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
E_k	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6.4	1,11	0.028	–	–	–
α	233.8	1,11	9.3×10^{-9}	–	–	–	5.5	1,11	0.038	–	–	–	–	–	–	6.5	1,11	0.027	–	–	–
Respiration rate	110.9	1,11	4.4×10^{-7}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sol C	99.4	1,15	5.2×10^{-8}	–	–	–	6.1	1,15	0.026	–	–	–	–	–	–	–	–	–	–	–	–
Ins C	119.5	1,15	1.7×10^{-8}	4.9	2,15	0.023	9.0	1,15	0.009	5.6	2,15	0.016	9.9	2,15	0.002	6.7	1,15	0.021	12.2	2,15	0.001
Total proteins	4.6	1,15	0.05	–	–	–	–	–	–	–	–	–	–	–	–	22.1	1,15	2.8×10^{-4}	–	–	–
Phycoerythrin	57.3	1,15	1.7×10^{-6}	4.4	2,15	0.030	17.3	1,15	0.001	7.3	2,15	0.006	–	–	–	25.0	1,15	1.6×10^{-4}	–	–	–
Phycocyanin	–	–	–	–	–	–	13.1	1,15	0.003	5.1	2,15	0.021	–	–	–	–	–	–	3.7	2,15	0.050
$rETR_{max}$	50.1	2,16	1.3×10^{-7}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
E_k	21.4	2,16	3.0×10^{-5}	6.4	2,8	0.022	–	–	–	11.3	4,16	1.6×10^{-4}	–	–	–	–	–	–	–	–	–
α	86.5	2,16	2.6×10^{-9}	6.8	2,8	0.019	–	–	–	6.6	2.01,8.04	0.020	–	–	–	–	–	–	–	–	–
F_v/F_m	25.7	2,16	1.0×10^{-5}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cover change (%)	183.6	1,61	4.7×10^{-20}	24.6	2,61	1.5×10^{-8}	–	–	–	–	–	–	2.7	4,61	0.037	–	–	–	2.7	4,61	0.040
SWDI	7.6	2,18	0.004	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D	5.7	2,18	0.012	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Concentration of phycobilins, soluble proteins, and carbohydrates

A mixed factorial MANOVA test using species and CO_2 as between-subject factors, time as a within-subject factor, and soluble carbohydrates, insoluble carbohydrates, total protein, and phycobiliproteins (phycoerythrin and phycocyanin) as dependent variables showed a significant main effect of time on all variables except phycocyanin. There was a significant interactive effect between all factors on insoluble carbohydrates and phycocyanin, while total proteins were only significantly affected by an interaction between time and species. Both phycobilisomes were affected by an interaction between CO_2 and time, but only phycoerythrin was affected by an interaction between time and species (Table 3).

The phycobiliprotein content in *Corallina officinalis* and *Chondrus crispus* was particularly affected by both time of exposure and CO_2 treatment (Fig. 4a,b). While 35 d of exposure did not have a strong effect on phycobiliprotein content, the concentrations of both phycobiliproteins in *C. crispus* decreased with increasing CO_2 level after 85 d of exposure to the treatments. For *C. officinalis*, the response was not as strong, but phycoerythrin increased in the 665 µatm CO_2 treatment from Day 35 to 85, while the phycocyanin concentration was generally low in this treatment for both time measurements.

During the 3 mo experiment, the concentrations of total protein and soluble and insoluble carbohydrates in both species changed over time and depended on species or CO_2 concentration (Fig. 5). In general, *Chondrus crispus* had higher levels of proteins and carbohydrates compared to *Corallina officinalis* after 35 d, and the total amount of insoluble carbohydrates was elevated in

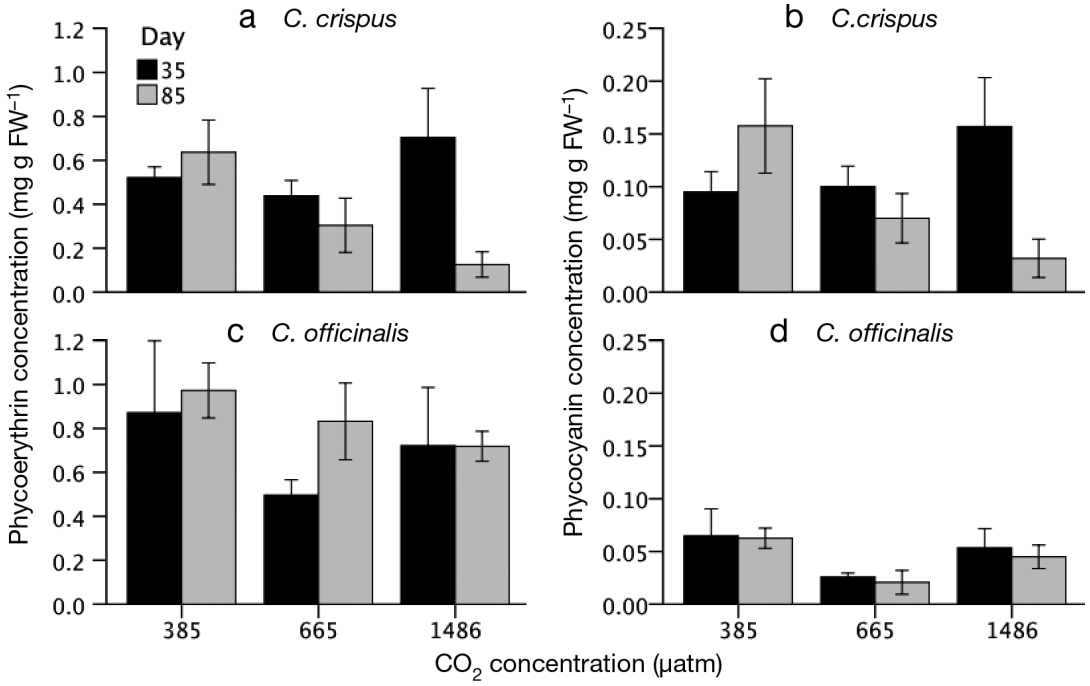

Fig. 4. *Chondrus crispus* and *Corallina officinalis*. Concentration of phycoerythrin and phycocyanin in *C. crispus* (a,b) and *C. officinalis* (c,d) after 35 and 85 d of exposure to CO_2 treatments. FW: fresh weight. Means ± SE are shown

the 2 CO_2 treatments compared to the ambient treatment. However, after longer exposure, there was a sharp decline in protein and carbohydrates (both soluble and insoluble) content in the 2 high-CO_2 treatments. This response may have been related to the combined stress of elevated temperature and CO_2 during the warmest part of the summer (July). *C. officinalis* responded early to the CO_2 treatments after 35 d of exposure by decreasing protein levels. After 85 d, the protein concentrations in *C. officinalis* tissue increased in all treatments, but they did not differ significantly among CO_2 treatments.

A discriminant analysis of the dependent variables was conducted for better interpretation of the mixed factorial multivariate MANOVA. The analysis after 35 d revealed only 1 factor that significantly discriminated the treatment groups ($\Lambda = 0.067$, χ^2 (25) = 47.2, p = 0.005) and explained 93.6% of the variance. However, the analysis after 85 d revealed 2 discriminant functions that explained 65.3 and 30.6% of the variance, respectively (canonical $R^2 = 0.90$ and 0.81 respectively). These discriminant functions in combination significantly discriminated the treatment groups ($\Lambda = 0.013$, χ^2 (25) = 63.5, p < 0.001), and the second function further discriminated the treatment groups alone ($\Lambda = 0.123$, χ^2 (16) = 30.4, p = 0.016). The correlations between the discriminating functions

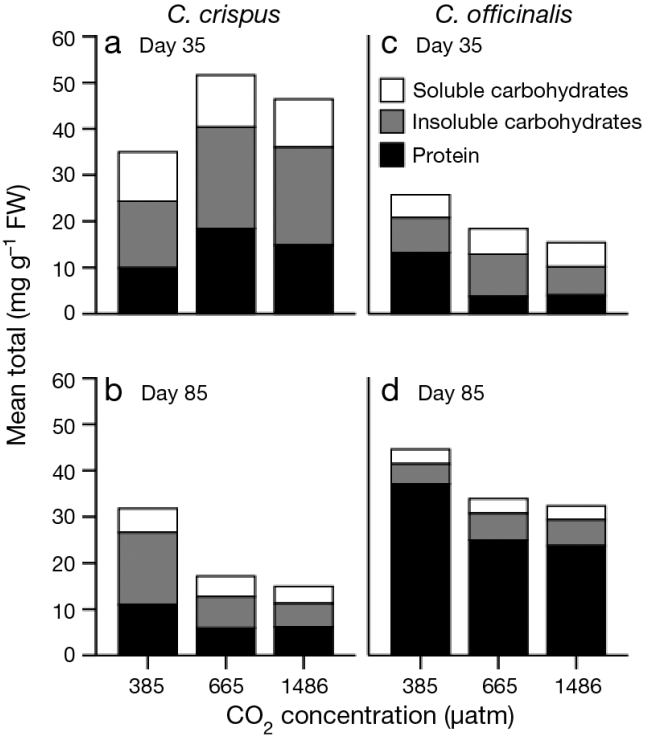

Fig. 5. *Chondrus crispus* and *Corallina officinalis*. Total carbohydrate (soluble and insoluble) and protein concentrations in *C. crispus* (a,b) and *C. officinalis* (c,d) thalli after 35 d (a,c) and 85 d (b,d) of exposure to 3 experimental CO_2 treatments. FW: fresh weight

and the variables showed that after 35 d, all variables except phycoerythrin (r = −0.05) loaded equally low (r = 0.29 to 0.32) on Function 1. After 85 d, insoluble carbohydrates had the highest loading on Function 1 (r = 0.51), while phycoerythrin and proteins had the highest correlations with Function 2 (r = 0.67, r = 0.53, respectively). The combined groups plots for both time periods are shown in Fig. 6. The plot shows that after 35 d, the first function discriminated between the control treatment and the elevated-CO_2 treatments for both species, while after 85 d, both functions strongly discriminated between the ambient- and elevated-CO_2 groups for the 2 species. Overall, Fig. 6 demonstrates that time, CO_2 concentration, and species had an effect on the protein, carbohydrate, and phycobiliprotein concentrations of the algae investigated.

Community responses

Photochemistry

A mixed factorial MANOVA with species (*Chondrus crispus* and *Corallina officinalis*) and CO_2 level as between-subject factors, time of exposure as a within-subject factor, and $rETR_{max}$, electron transport rate efficiency (α), E_k, and F_v/F_m as dependent response variables indicated that there was a significant effect of time on all 4 response variables, and a significant interactive effect between time and CO_2 on $rETR_{max}$ and α (Table 3). After 36 d of exposure to the treatments, all algae investigated (with the exception of *Ulva* spp., which only appeared at the end of the experiment) had higher $rETR_{max}$ values in the ambient treatment than the 2 elevated-CO_2 treatments (Fig. 7). The lower $rETR_{max}$ rates exhibited by algae grown in the elevated-CO_2 treatments resulted from higher nonphotochemical quenching and therefore lower chlorophyll fluorescence yields (Fig. 8). However, by the end of the experiment, the CO_2 effect on chlorophyll fluorescence was no longer visible. The E_k values did not show a strong response to CO_2 after 36 d of exposure, but after 86 d, the values were highest in the 665 µatm CO_2 treatment for crustose coralline algae, *C. crispus*, and *C. officinalis*.

The response of F_v/F_m was not significantly affected by CO_2 (Table 3, Fig. 7). The mean F_v/F_m of *Chondrus crispus*, *Corallina officinalis*, and *Dumontia incrassata* showed a negative trend with increasing CO_2 after 36 d, but the F_v/F_m values recovered after 86 d in the 1486 µatm CO_2 treatment for *C. crispus* and *C. officinalis*. The crustose coralline algae did now show a strong response after 36 d. *Ulva linza*, which appeared later during the course of the experiment, had the lowest mean maximum quantum yield in the 665 µatm CO_2 treatment.

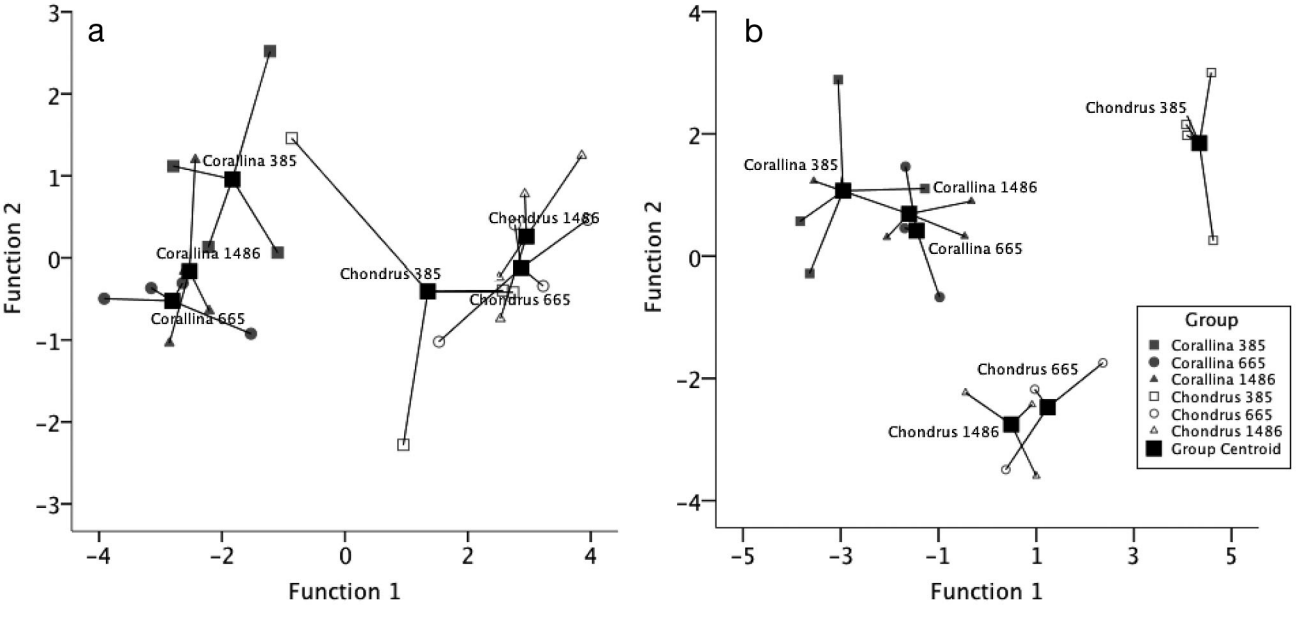

Fig. 6. *Corallina officinalis* and *Chondrus crispus*. Combined groups plots generated by discriminant analysis of carbohydrate (soluble and insoluble), protein, and phycobiliprotein concentrations in *C. officinalis* and *C. crispus* after (a) 35 d and (b) 85 d of exposure to CO_2 treatments. The response variables were grouped based on species and CO_2 treatment and the functions are canonical discriminant functions

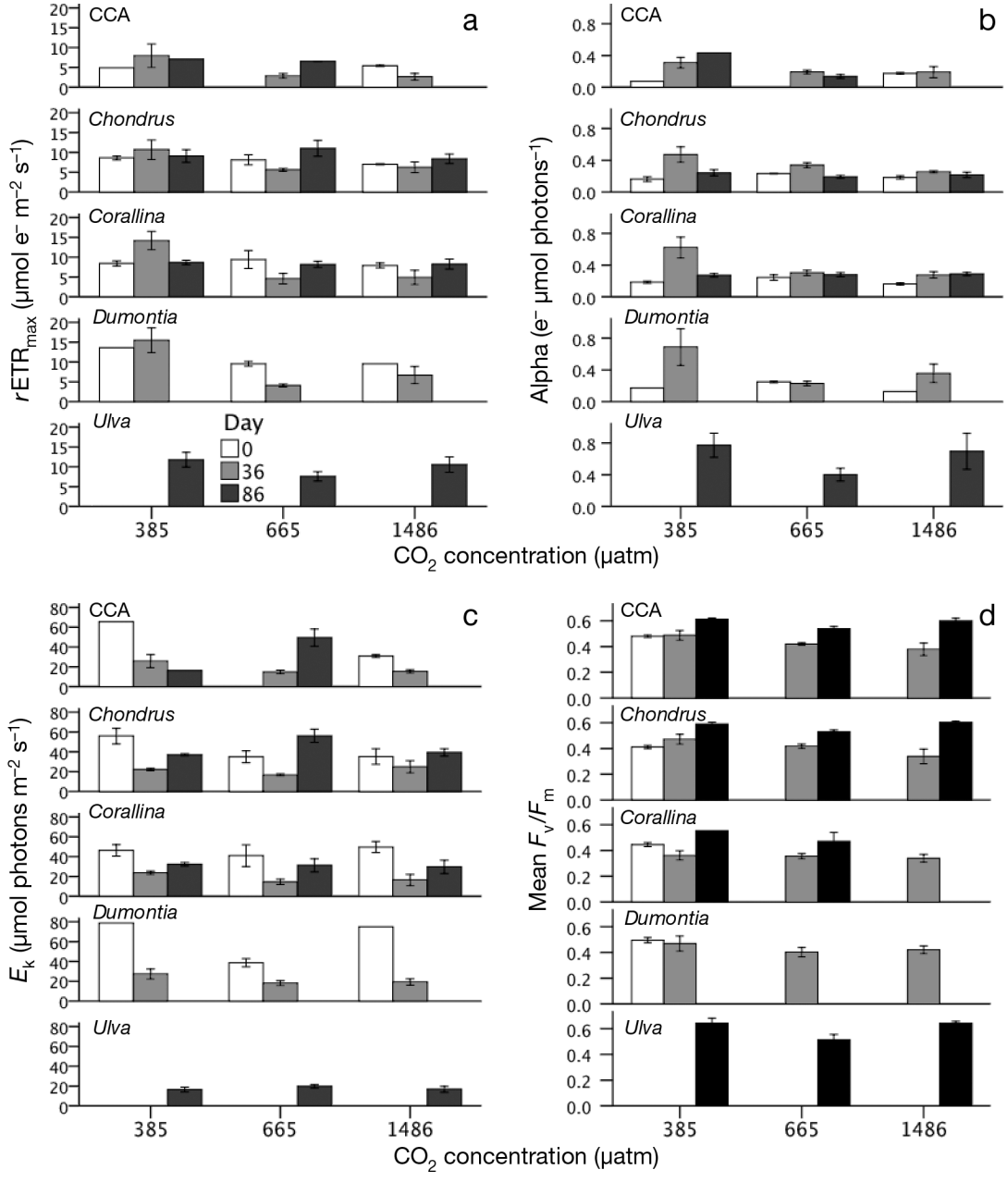

Fig. 7. Photochemical parameters for the 4 genera of macroalgae and the group of crustose coralline algae (CCA) present in the experimental communities based on chlorophyll fluorescence measurements at multiple light intensities ranging from 0 to 600 µmol photons m^{-2} s^{-1}. (a) Relative maximum electron (e^-) transport rates (rETR$_{max}$), (b) electron transport efficiency (alpha), (c) light saturation points (E_k), and (d) maximum quantum yield of photosystem II (F_v/F_m) are shown for each species or algal group at Day 0, 36, and 86 of the experiment. Means ± SE are shown

Percent cover, diversity and dominance

Due to the relatively large size of our mesocosms and long experimental period, we were able to detect changes in macroalgal community structure in response to elevated CO_2. Fig. 9 shows the mean percent cover of all algal species present in the experimental communities. The total macroalgal cover increased over time in all treatments. Community diversity increased and dominance decreased over time (Tables 3 & 4), but neither was significantly affected by CO_2 treatment. When considering individual species, the percent cover of *Ulva linza*, which appeared in all treatments at the end of the experi-

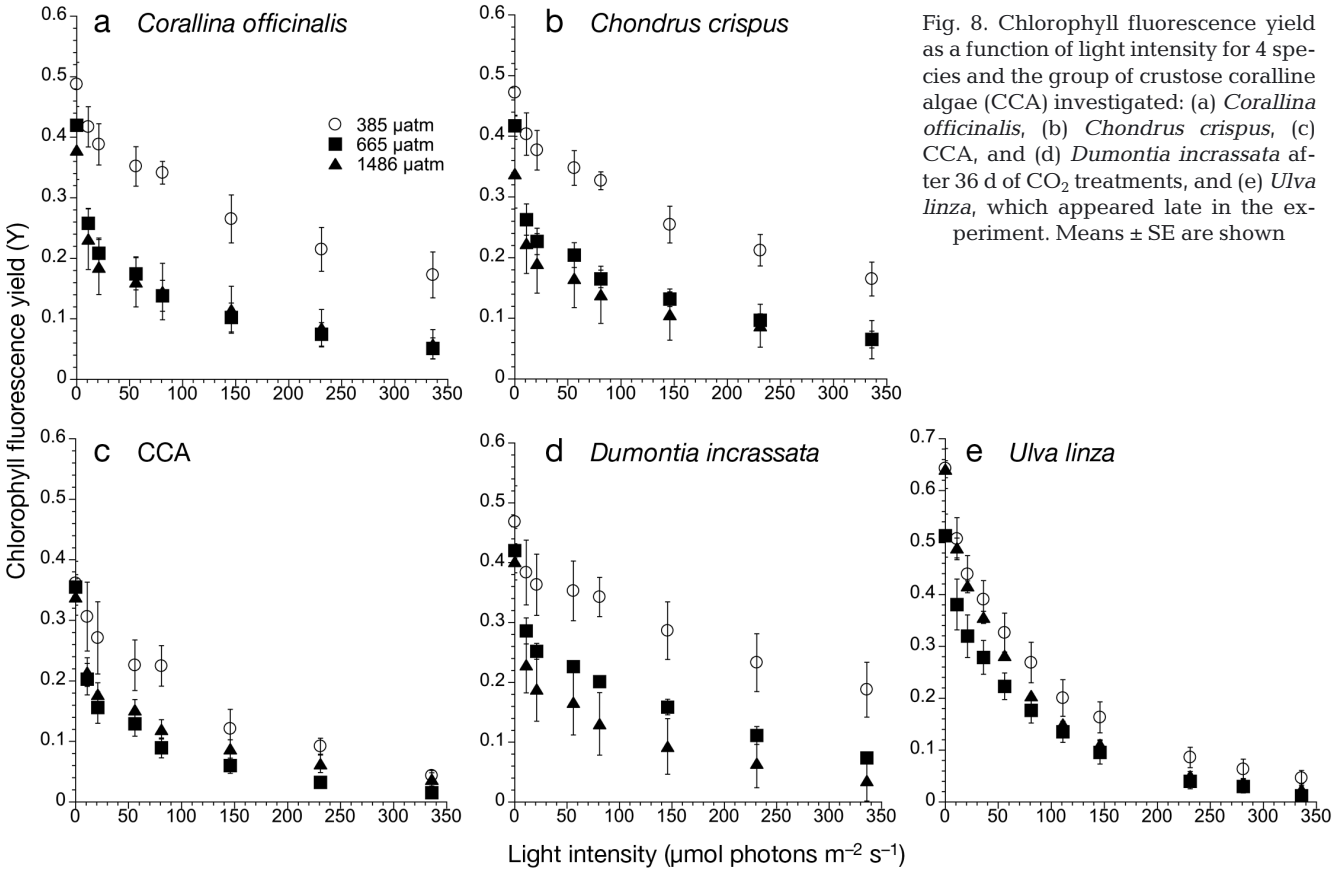

Fig. 8. Chlorophyll fluorescence yield as a function of light intensity for 4 species and the group of crustose coralline algae (CCA) investigated: (a) *Corallina officinalis*, (b) *Chondrus crispus*, (c) CCA, and (d) *Dumontia incrassata* after 36 d of CO_2 treatments, and (e) *Ulva linza*, which appeared late in the experiment. Means ± SE are shown

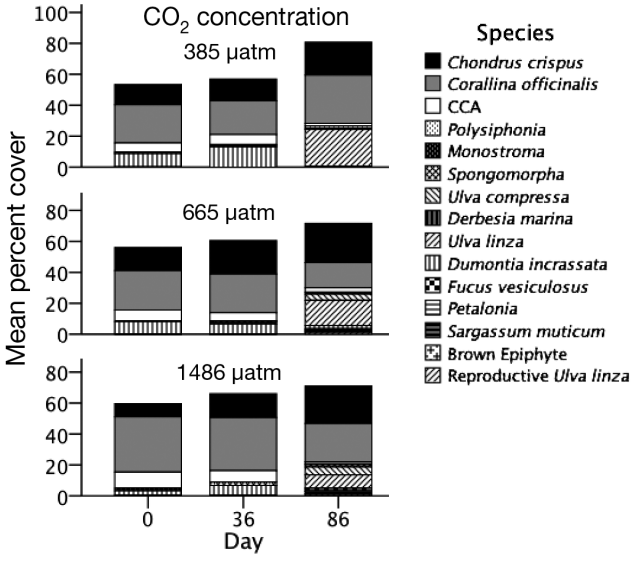

Fig. 9. Mean percent cover of all species of algae present in the experimental communities at Day 0, 36, and 86 for each CO_2 concentration. CCA: crustose coralline algae

Table 4. Shannon-Wiener diversity index and Simpson's reciprocal dominance index (1/D) for the macroalgal communities exposed to CO_2 treatments at Days 0, 36, and 86. Mean ± SE

Day	CO_2 conc. (µatm)	Shannon-Wiener diversity index	1/D
0	385	0.89 ± 0.07	0.88 ± 0.02
	665	0.82 ± 0.04	0.84 ± 0.03
	1486	0.85 ± 0.08	0.81 ± 0.03
36	385	0.87 ± 0.09	0.85 ± 0.03
	665	0.93 ± 0.07	0.81 ± 0.04
	1486	0.91 ± 0.06	0.78 ± 0.03
86	385	1.03 ± 0.07	0.73 ± 0.03
	665	1.09 ± 0.06	0.78 ± 0.03
	1486	1.09 ± 0.08	0.79 ± 0.04

ment, was significantly negatively correlated to CO_2 concentration (Pearson's r = −0.381, p = 0.033; Fig. 9). When the relative percent cover with respect to the calcifiers (*Corallina officinalis* and crustose coralline algae) and the prevalent noncalcifying red alga *Chondrus crispus* are considered, a CO_2 effect is also present. Fig. 10 shows, quantitatively and qualitatively, the percent change in cover over time for these 3 taxa. A mixed factorial ANOVA test indicated that there were significant main effects of time and CO_2 level as well as an interaction between all 3 independent factors (Table 3). *C. crispus* increased in

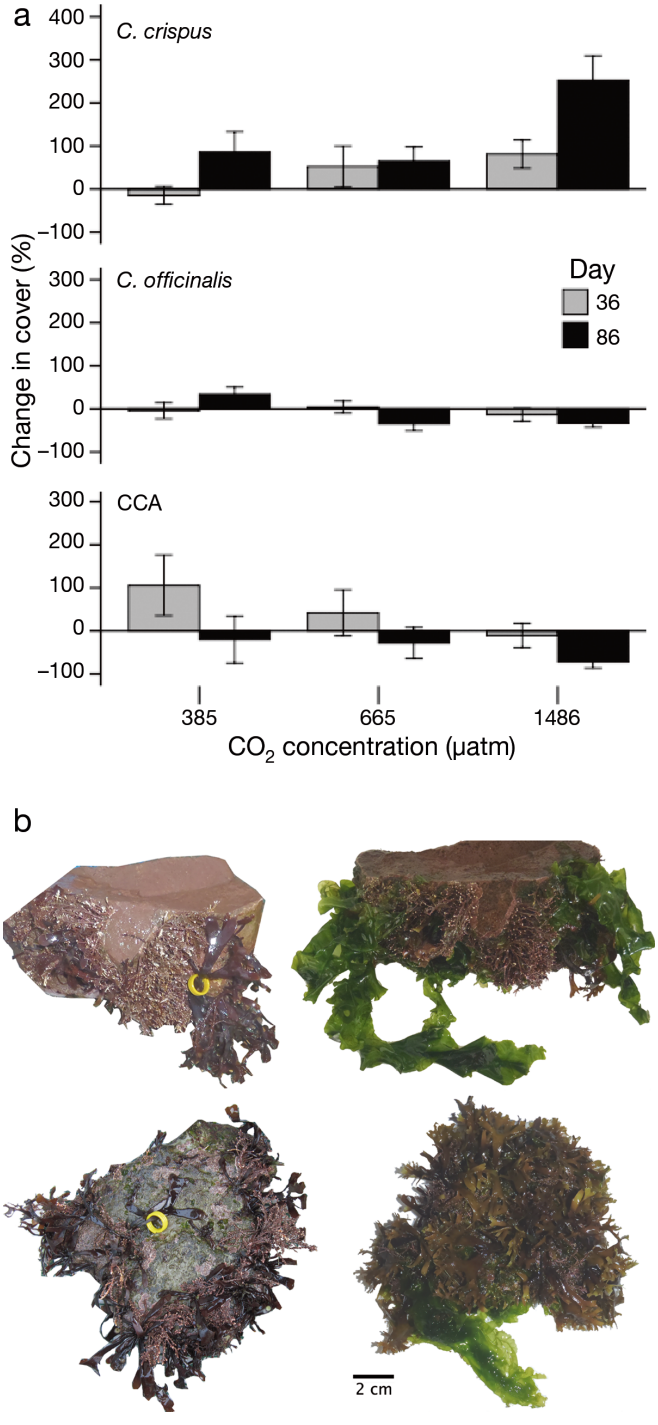

Fig. 10. (a) Change in cover (means ± SE) with respect to initial value after 36 and 86 d exposure to CO_2 treatments for *Chondrus crispus*, *Corallina officinalis*, and crustose coralline algae (CCA). (b) Examples of communities from the ambient-CO_2 (above) and high-CO_2 (below) treatments at Days 0 (left) and 86 (right). In both communities, *Ulva linza* appeared after 86 d, but it is clear in the ambient-CO_2 treatment that *C. officinalis* and *C. crispus* maintain their respective cover, while in the high-CO_2 treatment, *C. crispus* encroaches on the space occupied by *C. officinalis* after 86 d

all treatments over time, but the change in cover was greatest in the 1486 µatm CO_2 treatment after 86 d. In contrast, the calcifying *C. officinalis* decreased in cover in the 1486 µatm CO_2 treatment after just 36 d and in both elevated-CO_2 treatments after 86 d, while the percent cover increased in the ambient treatment. The crustose coralline algae showed the highest decrease in cover at the highest-CO_2 treatment after 86 d due to overgrowth by the noncalcifying species.

DISCUSSION

The physiological responses of the coexisting *Corallina officinalis* and *Chondrus crispus* to elevated CO_2 are complex and shed some light on how competitive interactions may shift between calcifying and noncalcifying macroalgae under future CO_2 conditions. The most striking responses were the decreased growth rates and inorganic carbon in the *C. officinalis* skeleton. Such responses in *C. officinalis* have been previously shown (Hofmann et al. 2012), but we also present net calcification rates, which showed a parabolic relationship to CO_2 concentration (the highest rate was at 665 µatm CO_2). Such a calcification response has been reported for another coralline algae, *Neogoniolithon* sp. (Ries et al. 2009). Those authors attributed the higher calcification rates at pCO_2 concentrations between 600 and 1100 µatm to higher rates of photosynthesis providing more energy for pH regulation. In fact, evidence of CO_2 fertilization of photosynthesis in calcifying macroalgae is weak. Gao & Zheng (2010) actually found a decrease in photosynthetic rates of *Corallina sessilis* at 1000 ppmv CO_2 compared to 380 ppmv, and Hofmann et al. (2012) and Cornwall et al. (2012) showed no significant differences in maximum photosynthetic rates of *C. officinalis* at CO_2 concentrations >1300 µatm.

We measured the highest maximum photosynthesis rate and calcification rate in algae grown in the 665 µatm CO_2, suggesting that moderately high CO_2 concentrations can stimulate photosynthesis and calcification to a limited extent. However, this stimulation of calcification rates does not necessarily result in a higher net deposition of $CaCO_3$, as skeletal inorganic carbon of *Corallina officinalis* decreased with increasing CO_2 concentration and increasing net calcification rate (above a threshold of 77% inorganic carbon). These results are consistent with Hofmann et al. (2012), who found that the area of deposited $CaCO_3$ between *C. officinalis* cells decreased under

elevated CO_2. The reason for the discrepancy between calcification rates and skeletal inorganic carbon content could be higher dissolution rates in the dark. Martin & Gattuso (2009) found significantly higher dissolution rates of dead *Lithothamnion cabiochae* thalli under elevated CO_2 (700 ppm) compared to ambient concentrations (400 ppm), but found no significant difference in net calcification rates after 1 mo of exposure to the experimental CO_2 treatments. Similar discrepancies between net calcification and dissolution rates have been found for some corals and mollusks (Rodolfo-Metalpa et al. 2011). In the present study, the stimulation of calcification at moderate CO_2 concentrations allowed *C. officinalis* to maintain its inorganic skeleton despite (most likely) higher dissolution rates in the dark. However, there is a threshold CO_2 level beyond which calcification and photosynthesis are no longer able to maintain ambient deposition rates of $CaCO_3$, resulting in a less calcified skeleton. This threshold must lie somewhere between 1000 and 1400 µatm CO_2 for temperate coralline macroalgae, based on the following: previously reported calcification rates of coralline algae under elevated CO_2 concentrations (Anthony et al. 2008, Martin & Gattuso 2009, Ries et al. 2009, Gao & Zheng 2010), the results of the present study, and the evidence that photosynthetic rates and efficiency in *Corallina* spp. are decreased under CO_2 concentrations beyond 1000 µatm (Gao & Zheng 2010, Hofmann et al. 2012).

While *Corallina officinalis* is able to maintain a heavily calcified skeleton under moderately elevated CO_2, the energy cost of elevating calcification rates may still have an effect on the competitive success of this species. For example, growth rates and protein levels were both lower after 35 d of exposure to 665 µatm CO_2, while the calcification rate was elevated. In contrast, the noncalcifying *Chondrus crispus* elevated its cellular protein and carbohydrate content after 35 d of exposure. In benthic calcifying animals, such an energy trade-off between net calcification rates and other physiological processes has been postulated (Findlay et al. 2011). Those authors found that net calcification rates can be maintained or even elevated under high CO_2 conditions, but at costs such as increased metabolism or lower predation-avoidance response. The presence of protective organic or tissue layers is also a significant factor affecting the responses of different calcifying organisms (Rodolfo-Metalpa et al. 2011). Therefore, an organism's ability to cope with the changes associated with ocean acidification will depend on its ability to obtain additional resources needed to supply the high energy demands of maintaining calcification. These changes in energy allocation are likely to have contributed to the community shift observed in our study where the noncalcifying *C. crispus* increased while the calcifying *C. officinalis* decreased in cover at both elevated CO_2 levels. The observed community shift cannot be explained simply by changes in photosynthetic rates, as *C. crispus* showed only a marginal increase in oxygen production (nonsignificant at the 95% CI level) at the highest-CO_2 treatment after 35 d, and no change relative to the ambient level after 85 d. The photosynthetic response of *C. crispus* is not surprising, as many authors have already shown that noncalcifying algae do not always respond to elevated CO_2 by increasing photosynthesis, and in fact some even decrease their photosynthetic rates (García-Sánchez et al. 1994, Mercado et al. 1999, Gordillo et al. 2001, Zou 2005). Therefore, nutrient availability is important for when interpreting the responses of algae to elevated CO_2.

Several of the responses measured showed sensitivity to the interaction between exposure time and CO_2 concentration, suggesting that seasonal fluctuations in inorganic nutrient supply also influence the effects of CO_2 on marine calcifying algae. At the beginning of our experiment, nitrate concentrations were high but decreased steadily during the first 40 d of the experiment. Then they slowly began to increase again but only to 40% of the initial level (data not shown). Furthermore, water temperature constantly increased during the summer season and reached maximum levels at the end of the experiment. The combination of relatively low nitrate availability and high temperature could explain the generally positive physiological responses to elevated CO_2 concentrations in *C. crispus* at the end of the experiment. Gordillo et al. (2001) reported the significance of nitrogen availability in the response of the noncalcifying green alga *Ulva rigida* to elevated CO_2 concentrations. Nitrogen-limited algae exposed to elevated CO_2 concentrations showed increased growth rates but decreased net photosynthesis and soluble protein concentration. We saw a similar response in *C. crispus* after 85 d of exposure to elevated CO_2 concentrations when nitrate levels were much lower than during the first 35 d. The evidence from Gordillo et al. (2001) and the changes in physiological responses of *C. crispus* over time in our study support the notion that noncalcifying algae differ in their response to CO_2 depending on external energy availability. Therefore, in areas exposed to the combination of elevated CO_2 and eutrophication, changes in community structure between the coverage of calci-

fiers and noncalcifiers could be amplified. Such a response has already been shown in a kelp understory, where turf algae expanded at the expense of calcifiers under elevated CO_2 and nutrient conditions (Russell et al. 2009).

Our results indicate that elevated surface-seawater CO_2 concentrations reachable within the next 100 to 200 yr could change the structure of temperate intertidal macroalgae communities containing dominant calcifying macroalgae such as *Corallina* spp. The observed changes in community structure would have important ecological implications. *C. officinalis* often grows in areas with strong currents and wave action, and serves as a habitat and buffer for meiofauna and substrate for other algae (Dommasnes 1968). With the decrease in abundance or even disappearance of this species, many other algae would lose their habitats and substrata for growth, while changes in carbohydrate content of the different macroalgal species would change the nutritional content and palatability of the algae for grazers.

The rapid increase in CO_2 concentrations applied to our treatments and in almost all ocean acidification studies is often considered unrealistic. However, the observed changes in community structure are consistent with the observations of other authors who have investigated competition between calcifiers and noncalcifiers that have been exposed to naturally different CO_2 concentrations for a long period (at least decades). For example, Porzio et al. (2011) report a significant reduction of calcitic macroalgae cover and the dominance of a few noncalcifying species, and Martin et al. (2008) report a lower abundance of calcifying seagrass epiphytes in areas with naturally elevated CO_2 concentrations resulting from submerged volcanic vents. These observations are similar to our results, in that both high-magnesium calcite-depositing taxa (*Corallina officinalis* and crustose coralline algae) decreased in cover in both elevated-CO_2 treatments, while *Chondrus crispus* cover increased the most in the highest-CO_2 treatment. Our study adds to the evidence that crustose coralline algae are particularly susceptible to ocean acidification (Anthony et al. 2008, Jokiel et al. 2008, Kuffner et al. 2008, Martin et al. 2008, Büdenbender et al. 2011).

In conclusion, our study has added to the limited pool of long-term mesocosm experiments and contributes to increasing evidence that elevated surface-seawater CO_2 concentration will affect community composition of temperate rocky-shore macroalgae communities by altering the competitive relationship between calcifiers and noncalcifiers.

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