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ORIGINAL PAPER

Physiological responses of the calcifying rhodophyte, *Corallina officinalis* (L.), to future CO_2 levels

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Abstract Future atmospheric CO_2 levels will most likely have complex consequences for marine organisms, particulary photosynthetic calcifying organisms. *Corallina officinalis* L. is an erect calcifying macroalga found in the inter- and subtidal regions of temperate rocky coastlines and provides important substrate and refugia for marine meiofauna. The main goal of the current study was to determine the physiological responses of *C. officinalis* to increased CO_2 concentrations expected to occur within the next century and beyond. Our results show that growth and production of inorganic material decreased under high CO_2 levels, while carbonic anhydrase activity was stimulated and negatively correlated to algal inorganic content. Photosynthetic efficiency based on oxygen evolution was also negatively affected by increased CO_2. The results of this study indicate that *C. officinalis* may become less competitive under future CO_2 levels, which could result in structural changes in future temperate intertidal communities.

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Introduction

Ocean acidification, which is described as a pH decrease in ocean surface waters caused by the dissolution of anthropogenically produced atmospheric CO_2, is currently under heavy investigation due to its potential impacts on marine organisms. The future atmospheric CO_2 levels are predicted to reach 800 microatmospheres (μatm) by 2100, based on the "business as usual" scenario or 1,000 μatm based on the most liberal predictions released by the Intergovernmental Panel on Climate Change (IPCC). These atmospheric CO_2 concentrations correspond to a predicted 0.3–0.5 unit drop in pH of the surface ocean waters relative to current conditions (Caldeira and Wickett 2005; Orr et al. 2005). A corresponding shift in the speciation of dissolved inorganic carbon (DIC) occurs as the system attempts to buffer itself, and the result is a lower saturation state for calcium carbonate ($CaCO_3$)—the main skeletal building block for all marine calcifying organisms.

A decreased saturation state of $CaCO_3$ could make calcification more difficult for marine calcifying organisms, and some organisms have already been reported to decrease calcification rates under ocean acidification conditions (Gao et al. 1993a, b; Langdon et al. 2000, 2003; Riebesell et al. 2000; Jokiel et al. 2008; Kuffner et al. 2008). However, despite the large degree of effort that has already been made to investigate the effects of ocean acidification, the biological and ecological consequences of this scenario are not well understood because of the highly variable responses of different organisms (Ries et al. 2009). These variable responses are likely due to physiological processes other than calcification that are also affected by higher CO_2 levels, lower pH, and lower $CaCO_3$ saturation states that accompany ocean acidification.

Calcifying autotrophic organisms are especially interesting to investigate due to the complex physiological processes

of photosynthesis and calcification that play a large role in the fitness of these organisms, and the important ecological niches that they occupy. In tropical environments, corals are heavily studied in ocean acidification research due to the symbiotic relationship between the photosynthesizing zooxanthellae and the heterotrophic coral (Reynaud et al. 2003; Langdon and Atkinson 2005; Krief et al. 2010). In temperate environments, calcifying micro- and macroalgae are crucial organisms to investigate because of their high primary production and contribution to global biogeochemical cycles (Thierstein and Young 2004) and importance in providing structural support, refugia, and substrata for inter- and subtidal marine communities (Coull and Wells 1983; Hicks 1986; Akioka et al. 1999). Calcifying algae are unique organisms that conduct both photosynthesis and calcification—two processes that are intricately linked to DIC (CO_2, HCO_3^-, CO_3^{2-}). Because the speciation of DIC and subsequently the seawater carbonate system will be altered under ocean acidification conditions, photosynthesis and calcification in calcifying algae could be heavily impacted.

Carbon dioxide is the main substrate for photosynthesis. The availability of CO_2 for photosynthesis is lower in seawater than in air due to its slower rate of diffusion in seawater. Therefore, marine photosynthetic organisms have acquired efficient methods for carbon uptake called carbon concentrating mechanisms (CCMs). These mechanisms enhance photosynthetic efficiency by concentrating CO_2 at the reaction site of the carbon fixing enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RubisCO), and thereby decreasing competition by oxygen molecules (photorespiration). These mechanisms also compensate for the fact that bicarbonate ion (HCO_3^-) concentrations are higher than dissolved CO_2 concentrations, and therefore involve the enzyme carbonic anhydrase that increases the interconversion of HCO_3^- and CO_2. Some macroalgae even have the ability to use HCO_3^- directly via anion exchange processes (Drechsler et al. 1993; Axelsson et al. 1995; Larsson et al. 1997). As a result, photosynthesis is very efficient at ambient DIC conditions, but it is not always saturated, as several macroalgal species have shown increased photosynthetic and growth rates at increased CO_2 concentrations (Gao et al. 1991, 1993a, b; Kübler et al. 1999; Zou 2005). On the other hand, some macroalgae have even shown decreased photosynthetic performance and/or growth (García-Sánchez et al. 1994; Israel et al. 1999) or no response at all (Israel and Hophy 2002). Clearly, the photosynthetic responses of macroalgae to high CO_2 concentrations are variable and complex, and for calcifying macroalgae, the responses may be even more complex due to the process of calcification.

Calcifying macroalgae must balance both photosynthesis and calcification. Some studies have indicated that these two processes are linked (Borowitzka and Larkum 1976b) and that both are strongly light dependent (Pearse 1972; LaVelle 1979; Borowitzka and Larkum 1976a; Borowitzka 1981). The mechanism of calcification in *Halimeda* spp. has been heavily studied (Borowitzka and Larkum 1976a, b, c; De Beer and Larkum 2001) and is reported to occur as the deposition of aragonite crystals in the intercellular spaces of the alga. Photosynthesis is thought to stimulate calcification by removing CO_2 from the intercellular spaces and thereby increasing the local pH and carbonate ion concentration (Borowitzka and Larkum 1976b). The coralline red algae (Corallinaceae) have no intercellular spaces like *Halimeda* spp., but their cell walls have an organic matrix that is thought to provide a nucleation site for calcite crystals (Borowitzka 1981; Pueschal et al. 1992), and the mechanism for calcification is also thought to be related to the localized increase in pH at the cell-seawater interface created by photosynthetic uptake of CO_2 (Digby 1977). Coralline algae deposit $CaCO_3$ as high magnesium-calcite, which is the most soluble form of $CaCO_3$. As a result, coralline algae could be some of the first calcifying organisms to be negatively impacted by ocean acidification. Some authors have already reported the negative effects of high CO_2 on coralline algae (Gao et al. 1993a, b; Gao and Zheng 2010; Kuffner et al. 2008; Martin and Gattuso 2009), but there is a definite need for a better understanding of how different species will respond to increased CO_2 concentrations. Therefore, the goal of this study was to increase our understanding of how the coralline alga *Corallina officinalis* will respond to the levels of CO_2 predicted to be in future ocean surfaces.

Corallina officinalis is an upright calcifying alga found in the inter- and subtidal on rocky coastlines, often at exposed locations and in tidal-drainage channels. It is a late successional species with a complex morphological structure (Littler and Littler 1980). *Corallina* spp. often form extensive macroalgal turfs that cover large areas of the intertidal and provides substratum, habitat, and refugia for a number of important marine organisms (Coull and Wells 1983; Hicks 1986; Akioka et al. 1999; Kelaher 2002, 2003). On the rocky coast of the island of Helgoland in the North Sea, *C. officinalis* is abundant and is an important species in the macroalgal community. It can be found in isolated patches and in extensive turfs and is often closely associated with the other red algae *Chondrus crispus* and *Mastocarpus stellatus* and under a dense cover of *Fucus serratus* and *Ascophyllum nodosum*. The main goals of the current study were to determine the physiological responses, including growth, photosynthesis, and calcification, of *C. officinalis* to increased CO_2 concentrations expected to occur within the next century and beyond. Changes in the physiology of this species could have significant impacts on the surrounding communities in rocky tidal environments.

Materials and methods

Experimental set-up

Corallina officinalis specimens were collected from the intertidal zone of the northern coast of Helgoland, Germany, in October 2009 and stored in bottles containing natural seawater until they were transferred into culture at the University of Bremen, Germany. Cultures were maintained at 15°C, 33 psu (Reef Salt, ab Aqua Medic GmBH Bissendorf, Germany), 50 µmol photons m^{-2} s^{-1} light intensity (Osram Luminux Plus Daylight L18 W/11-860), and a 12:12 h light:dark cycle in artificial seawater containing ¼ strength Provasoli enrichment medium (Provasoli 1968). Calcium chloride ($CaCl_2 \cdot 2H_2O$) was added to the enrichment medium to a final concentration of 10 mM to ensure the presence of calcium for the calcifying algae.

Three CO_2 concentrations were used for experimental treatments: 384 µatm (ambient atmospheric CO_2 level), 1,313 µatm, and 1,939 µatm. The three treatments were aimed to achieve ambient CO_2, 1× and 2× the A1F1 scenario for 2100 from the IPCC Special Report on Emissions Scenarios, but the medium CO_2 treatment was higher than expected due to technical restraints. Four replicate tanks containing 400 mg of algae were used for each CO_2 treatment level. One additional tank without algae was monitored at each CO_2 level to detect any potential effects of the algae on the water chemistry, but they were not used in the statistical analysis. The desired CO_2 concentrations were manipulated using the Aquamedic CO_2 Set Professional (Bissendorf, Germany), which consisted of computer-controlled gas valves that were set to open and close when the recorded pH rose above or below, respectively, the desired pH. The CO_2 was bubbled directly into each 20-liter treatment tank until the pH level corresponding to the desired CO_2 concentration was reached. Once the desired pH was reached, the gas valve was automatically closed and the CO_2 bubbling stopped. Manual monitoring of the pH in each tank was conducted using a WTW 3310 pH meter, electrode model SenTix Mic (Weilheim, Germany) calibrated with standard buffer solutions pH 4 and 7 AVS Titrinorm (BDH Prolabo from VWR International, Dublin, Ireland). The pH electrode was calibrated with standard NBS buffers. The pH units were corrected to the total scale using a calibration with Tris buffers at 1°C intervals over a temperature range of 5–22°C. The pH was measured twice a day and before and after every water change. Water samples (25 ml) were taken weekly for total alkalinity (TA) analysis. The seawater carbon chemistry was calculated using the CO2SYS program (version 14) originally designed by Lewis and Wallace (1998). The data input included pH (total scale) and TA, and the constants from Mehrbach et al. (1973) were used. The artificial seawater was filtered through a Whatman polycap capsule filter (0.45 µm pore size) and circulated in each tank using magnetic stir bars. The tanks were covered with clear plastic to limit gas evaporation, and nutrients were added after each water change (every other day) during a total exposure time of 4 weeks.

Growth, inorganic content, and carbonic anhydrase

Growth rates were determined by weighing the fresh weight of one marked algal thallus in each treatment tank over time after blotting the thalli dry with paper towels. Relative growth rates (RGR) expressed as percent of fresh weight per day (%FW day^{-1}) were calculated as $\ln(W_t/W_0)/t \times 100$, where W_t and W_0 are the fresh weights at time t (days) and time 0. Inorganic content was determined by drying the algae at 70°C for 24 h followed by burning for 24 h at 400°C in a muffle furnace. The area of calcified material between cells was calculated from transmission electron microscopy images provided from the University of Hamburg, Germany. Transmission electron microscopy (TEM) was performed according to Quader (1985). Briefly, the algae were fixed with 2% glutaraldehyde in cacodylate buffer (75 mM, pH 7.0) for 1.5 h, postfixed with 1% osmium tetroxide at 4°C overnight. The samples were dehydrated through a series of graded acetone concentrations, 30–100%, and finally embedded in plastic according to Spurr (1969). Ultrathin sections were obtained with a ultramicrotome (Ultracut E, Leica-Reichert-Jung, Nußloch, Germany) and stained with uranyl acetate followed by lead citrate (Reynolds 1963). Sections were viewed with a LEO 906 E TEM (LEO, Oberkochen, Germany) equipped with the MUltiScan CCD Camera (Model 794) of Gatan (Munich, Germany) using the Digital Micrograph 3.3 software from Gatan to acquire, visualize, analyze, and process image data. Images were analyzed with ImageJ (Image Processing and Analysis in Java, National Institutes of Health, Bethesda, Maryland, USA). Carbonic anhydrase activity was measured using the method described by Haglund et al. (1992). Samples of frozen (−80°C) *C. officinalis* (50–100 mg) were ground in extraction buffer (50 mM Tris, pH 8.5, 25 mM dithiothreitol, 25 mM isoascorbic acid, 5 mM EDTA) with a chilled mortar and pestle. The reaction was started by adding 3 ml of algal extract or buffer with no algae (blank) to 2 ml of ice-cold CO_2 saturated water (substrate) in a glass tube. The time it took for the pH to drop 0.4 units within the pH range of 8.1–7.1 was recorded. Enzyme activity was calculated as $(t_b/t_s - 1)$/FW, where t_b and t_s are the time in seconds it took for the pH to drop 0.4 units for the blank and the sample, respectively, and FW is fresh weight of the algal sample. Several aliquots of each algal extract were measured sequentially in order to ensure reproducibility of the assay.

Photosynthesis measurements

Photochemical parameters were measured using Pulse Amplitude Modulated (PAM) fluorescence (PAM 2100, Heinz Walz GmbH, Effeltrich, Germany) at time 0, 3 days, 9 days, 2 weeks, and 4 weeks following the methods by (Bischof et al. 1999). Specimens were dark adapted for 5 min in order to obtain maximum photosynthetic efficiency values (Fv/Fm) and rapid light curves consisting of 30 s light intervals (0–2, 100 µmol photons m^{-2} s^{-1}) were conducted to measure relative electron transport rates (rETR). Oxygen production was measured using a Hansatech Chlorolab 3 System consisting of a S1 clark type polarographic oxygen sensor, DW3 electrode chamber, Oxylab electrode control unit, LH36/2R red LED light (660 nm), Quantitherm PAR/Temperature sensor, and Oxylab32 Windows software (Hansatech Instruments Ltd, Norfolk, England). A range of 50–100 mg fresh weight (FW) of algal tissue was placed in the electrode chamber containing 15 ml of filtered, artificial seawater at the respective treatment levels. Photosynthesis–irradiance curves were produced by measuring oxygen production for 10 min (following 15 min of dark adaptation) at a range of increasing light intensities (0–1,000 µmol photons m^{-2} s^{-1}) produced by the LH36/2R LED light source during a series of automated steps. During the incubation, the culture medium was aerated with a small magnetic stir bar. Oxygen measurements were made before and after the four-week experiment. Total oxygen production was calculated with respect to chlorophyll *a* content, which was extracted in 2 ml dimethyl fluoride (DMF) for 24 h at 4°C in the dark and calculated according to Inskeep and Bloom (1985).

Statistics

For each of the response variables including growth, inorganic content, and carbonic anhydrase activity, a treatment effect of CO_2 was tested using a one-way analysis of variance (ANOVA) in the statistics program PASW Statistics 18.0 (IBM SPSS Statistics Inc.). Normality and homogeneity of variances were tested using Kolmogorov–Smirnov and Levine tests, respectively. The photosynthesis–irradiance curves generated from PAM fluorescence and oxygen evolution were fit to the Eilers and Peeters (1988) model. The following photosynthetic parameters were calculated from the model: maximum photosynthetic rate ($rETR_{max}$ or P_{max}), photosynthetic efficiency (alpha, α), and saturation irradiance (I_k). The curve fit parameters were calculated for each individual replicate and averaged for statistical analysis. Linear regressions and Pearson correlations were conducted to investigate relationships between photosynthetic performance and CO_2/pH.

Results

The seawater chemistry parameters for each of the three CO_2 treatments are shown in Table 1. A one-way ANOVA followed by a Tukey's post hoc test for multiple comparisons showed that there was a significant difference between the pH units of the three treatments during the 4-week experiment ($p < 0.01$).

Growth, inorganic content, and carbonic anhydrase

Relative growth rates (%FW day^{-1}) showed no significant decline with respect to exposure time, so growth rates measured during the duration of the experiment were combined to obtain an overall mean at each pH condition. A multiple comparison test showed that growth rates in the low pH treatments (pH 7.67, pH 7.84) did not differ significantly from each other, but growth rates at both low pH treatments were significantly lower than those in the ambient pH treatment (Table 2, $p = 0.005$, $p = 0.056$, respectively).

A one-way ANOVA indicated that pH had a significant treatment effect on the percent inorganic material in *C. officinalis* (Table 2, $p = 0.017$). The algae exposed to ambient pH conditions had significantly higher inorganic content (81.8% ± 0.54) than the algae exposed to the lowest pH condition (79.3% ± 0.38; Tukey's post hoc test, $p = 0.048$). Furthermore, the amount of calcium carbonate deposited as calcite on the cell walls was significantly lower at pH 7.67 (13.7% ± 1.8) than at pH 8.30 (18.8% ± 1.7), which was clearly visualized in transmission electron microscopy images (Fig. 1a). Despite a trend, carbonic anhydrase activity was not significantly affected

Table 1 Seawater chemistry for the three treatments, including mean (±SE) pH, pCO_2, HCO_3^-, CO_3^{2-}, and $\Omega_{calcite}$

pH	pCO_2 (µmol kg SW^{-1})	HCO_3^- (µmol kg SW^{-1})	CO_3^{2-} (µmol kg SW^{-1})	$\Omega_{calcite}$
8.300 (0.0089) $n = 155^a$	384 (0.82)	3,271 (7.0)	465 (0.99)	11.24 (0.02)
7.843 (0.0117) $n = 124^b$	1,313 (1.46)	3,878 (4.3)	191 (0.21)	4.62 (0.01)
7.668 (0.0125) $n = 124^c$	1,939 (0.21)	3,870 (0.42)	129 (0.01)	3.12 (0.00)

Only the starting conditions (without algae) for pCO_2, HCO_3^-, CO_3^{2-}, and $\Omega_{calcite}$ are shown; they decreased slightly after algal addition, but only by 2–7%. Different letter superscripts represent significant treatment effects of pH ($p < 0.001$)

Table 2 Mean (±SE) relative growth rates, inorganic material, carbonic anhydrase activity, and F_v/F_m ratios for the three CO_2 treatments after 28 days

pCO_2 (μmol kg SW^{-1})	Relative growth rate (% day^{-1})	Inorganic material (% of dry weight)	CA activity (units g FW^{-1})	F_v/F_m
384	1.97 (0.15)[a]	81.8 (0.54)[a]	43.42 (1.82)	0.61 (0.01)
1,313	1.11 (0.15)[b]	80.1 (0.72)[ab]	76.37 (15.57)	0.62 (0.01)
1,939	1.35 (0.24)[b]	79.3 (0.38)[b]	70.89 (5.43)	0.61 (0.02)

Unshared superscript letters signify significant treatment effects

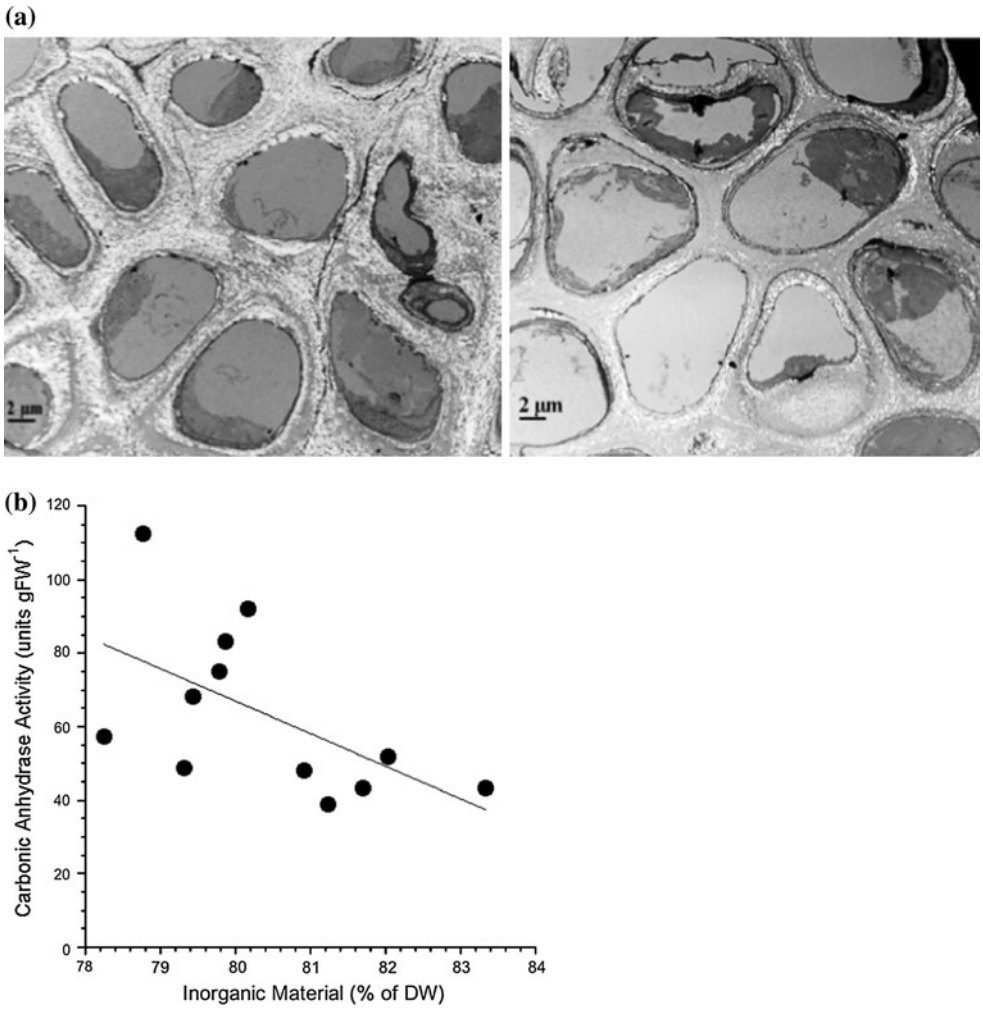

Fig. 1 **a** Transmission electron microscopy images of longitudinal sections through the youngest tip of each *C. officinalis* thalli. Calcite deposition (*white material*) between the cells is higher in algae grown under ambient (*left*) versus high (*right*) CO_2. **b** Significant negative correlation between carbonic anhydrase activity and inorganic material after 28 days of exposure to CO_2 treatments (Pearson correlation = −0.057, $p = 0.027$, $n = 12$)

by pH ($p = 0.08$), but was significantly negatively correlated to inorganic content after 28 days of exposure (Fig. 1b, Pearson correlation = −0.057, $p = 0.027$, $n = 12$).

Photosynthesis

The mean Fv/Fm ratios of *C. officinalis* did not differ significantly with respect to pH condition (Table 2). The F_v/F_m values of *C. officinalis* after 28 days of exposure to high CO_2 levels were not significantly different from initial F_v/F_m values prior to exposure, indicating that there was no culture effect or pH effect on F_v/F_m. As a result, we concluded that the algae were not photosynthetically stressed under the higher CO_2 conditions.

Photosynthesis–irradiance (P–I) curves generated from PAM fluorescence measurements and photosynthetic O_2 evolution measurements show mean relative electron transport rates (rETR) and mean O_2 evolution, respectively, as functions of light intensity for *C. officinalis* 28 days after exposure to different pH conditions (Fig. 2). At ambient pH, relative electron transport rate and oxygen evolution showed similar nonlinear trends with respect to light

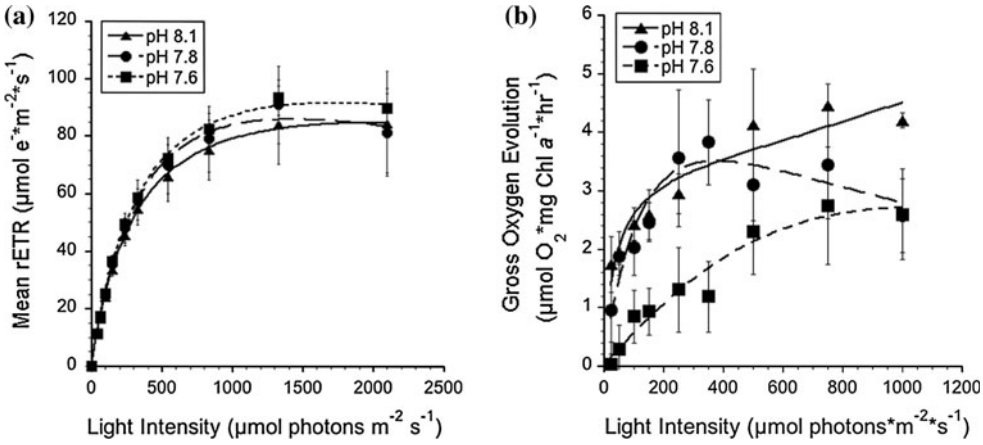

Fig. 2 **a** Mean (±SE, $n = 4$) relative electron transport rates and **b** mean (±SE, $n = 3$) gross O_2 evolution as a function of light intensity for *C. officinalis* 28 days after exposure to the pH treatments

Table 3 Mean (±SE) maximum photosynthetic rates (P_{max}), light compensation points, and respiration rates from oxygen evolution measurements and the maximum relative electron transport rates ($rETR_{max}$), photosynthetic efficiencies (α), and light saturation points (I_k) for PAM fluorescence measurements of *C. officinalis* exposed to different CO_2 levels

CO_2 treatment	O_2 evolution			PAM fluorescence		
pCO_2 (µmol kg SW^{-1})	P_{max} (µmol O_2 mg Chl a^{-1} h^{-1})	Light compensation point (µmol photons m^{-2} s^{-1})	Respiration rate (µmol O_2 mg Chl a^{-1} h^{-1})	$rETR_{max}$ (µmol e^- m^{-2} s^{-1})	α (µmol e^- µmol photons)	I_k
384	2.66 (0.17)	17.4 (6.3)	-0.66 (0.07)^{ab}	68.6 (1.9)	0.315 (0.009)	217.9 (3.0)
1,313	3.89 (0.70)	31.4 (8.1)	-1.24 (0.26)^{a}	86.2 (13.5)	0.321 (0.019)	265.7 (32.8)
1,939	1.92 (0.73)	27.5 (5.2)	-0.34 (0.08)^{b}	92.2 (6.5)	0.332 (0.009)	279.2 (25.0)

Unshared subscript letters for respiration rates represent significant differences at the 95% confidence level

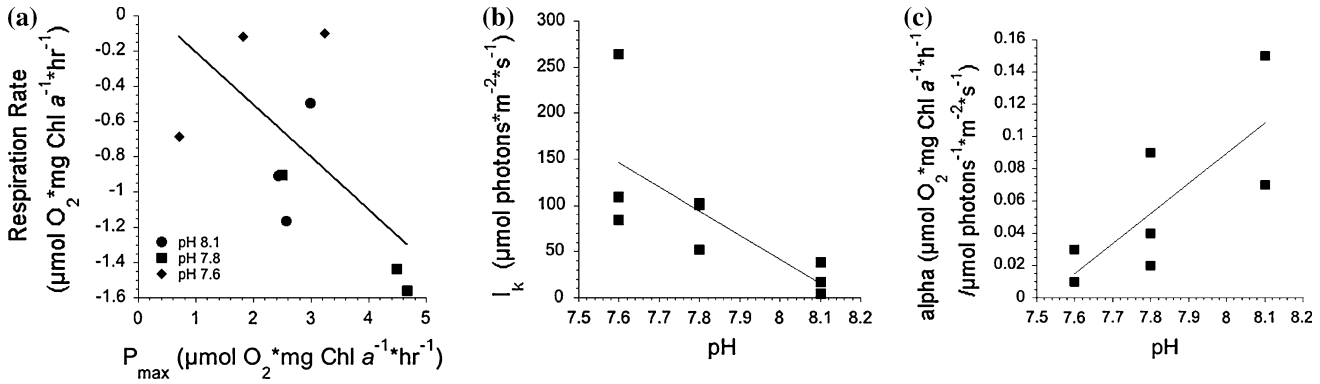

Fig. 3 **a** Respiration rates as a function of maximum photosynthetic rates; **b** light saturation point and **c** photosynthetic efficiency as a function of pH for *C. officinalis* after 28 days of exposure to the 3 pH levels. All data are from oxygen evolution measurements

intensity. However, the two P–I curves (rETR and O_2 evolution) deviate from each other with decreasing pH. The nonlinear curve fit parameters for rETR were not significantly affected by pH and are shown in Table 3. In terms of oxygen evolution, maximum photosynthetic rate (P_{max}) and light compensation point were not significantly affected by pH, but there was a significant treatment effect of pH on respiration rates (Table 3, $p = 0.021$). Respiration rates were highest at pH 7.8 and lowest at pH 7.6, and overall they increased (became more negative) significantly with increasing maximum photosynthetic rates (Fig. 3a, $R^2 = 0.622$, $p = 0.012$). Furthermore, a significant linear regression indicated that I_k decreased with increasing pH (Fig. 3b, $R^2 = 0.547$, $p = 0.023$), and there was a significant linear correlation between α and pH (Fig. 3c, Pearson correlation = 0.586; $p = 0.049$).

Discussion

The results of this study indicate that several aspects of *C. officinalis* physiology could be significantly affected by

increasing surface seawater CO_2 concentrations. Growth of *C. officinalis* was affected by increased CO_2 concentrations, as both increased CO_2 treatments caused significantly slower growth rates than in the algae grown at ambient CO_2 concentrations. Our growth rates and the negative effect of CO_2 on growth complement those of (Gao and Zheng 2010) who found that growth of *C. sessilis* was lower at 1,000 ppmv CO_2 (0.9% day^{-1}) than at 380 ppmv CO_2 (2.1% day^{-1}). Because *C. officinalis* is a slow growing macroalga relative to non-calcifying species (Colthart and Johansen 1973; Andrake and Johansen 1980), significant reductions in growth could have large implications for reproduction, recruitment, and overall community structure in intertidal communities dominated by the calcifying alga.

In contrast to growth, which showed a clear negative response to increased CO_2 concentrations, photosynthetic parameters showed inconsistent responses to elevated CO_2 levels. The F_v/F_m ratios remained the same across all treatments, but the negative correlation between pH and $rETR_{max}$ indicated that at the light intensity used in this study, *C. officinalis* was not photosynthetically saturated with respect to inorganic carbon because maximum electron transport rates increased with increasing CO_2 concentration (decreasing pH). On the other hand, the O_2 evolution data suggested that *C. officinalis* photosynthesis was negatively affected in the low pH treatment, due to the low P_{max} and photosynthetic efficiency relative to the other treatments. The discrepancy between the photosynthetic responses detected in the PAM fluorometry measurements and the O_2 evolution measurements indicate that elevated CO_2 may increase non-assimilatory electron flow in Photosystem I, which is not detectable by PAM fluorescence. In general, the rETR and O_2 evolution data did not complement each other at high irradiances, which has been reported earlier to be a result of critically low yield values and photoinhibition at high irradiances (Hanelt and Nultsch 1995; Beer and Axelsson 2004). However, even at low irradiances, the rETR and O_2 evolution data for the two low pH treatments did not complement each other—they were only similar at the ambient pH treatment (8.3). Russell et al. (2009) found that elevated CO_2 had a negative effect on the effective quantum yield of coralline crusts, while Russell et al. 2011 found no CO_2 effect on their relative electron transport rate. The relative electron transport rates may therefore not be a good indication of macroalgal health under elevated CO_2 conditions. In contrast, the O_2 evolution measurements directly measure a product of photosynthesis and are therefore more likely to represent the actual physiological response of the algae, compared to the relative electron transport rate. Furthermore, Gao and Zheng 2010 previously reported the inhibitory effect of elevated CO_2 (1,000 ppmv) on photosynthesis and calcification in *C. sessilis*. Therefore, we conclude that the photosynthetic efficiency and maximum photosynthetic rate were negatively impacted at the lowest pH (7.6)/highest CO_2 concentration based on oxygen evolution. This decrease in photosynthetic efficiency may have been linked to the decreased physiological health of the algae as evidenced by decreased growth rates and lower calcite deposition under elevated CO_2 concentrations.

While growth and inorganic content were expected to decrease under high CO_2 concentrations, photosynthesis and carbonic anhydrase showed unexpected responses. We expected that photosynthetic efficiency would increase with increasing CO_2, as the algae would have more substrate for RubisCO. In parallel, we expected the carbonic anhydrase activity to be downregulated when more CO_2 was available, which has been found in previous algal studies under elevated CO_2 levels (Nelson et al. 1969; García-Sánchez et al. 1994; Rost et al. 2003). While no significant treatment effect of pH was detected for carbonic anhydrase activity due to high variability within the assay measurements, the algae exposed to the two high CO_2 concentrations had roughly 40% higher CA activity than algae grown under ambient CO_2 conditions. We thus hypothesize that this stimulation of carbonic anhydrase activity may have been an attempt by the algae to compensate for decreased calcification/increased dissolution under high CO_2 concentrations. Carbonic anhydrase has been shown to play a role in the calcification process of many organisms, particularly corals, by regulating the internal and external cellular speciation of dissolved inorganic carbon (Kingsley and Watabe 1987; Nimer et al. 1994; Al-Horani et al. 2003; Rahman et al. 2007; Tambutté et al. 2007). Under high CO_2 concentrations, the following mechanism of external calcification in *C. officinalis* could be possible (originally proposed by Tambutté et al. 2007 for an azooxanthellate coral):

1. $CO_2 + H_2O \rightarrow 1\,H^+ + HCO_3^-$
2. $HCO_3^- \rightarrow CO_3^{2-} + H^+$
3. $Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3$

In the first reaction, carbonic anhydrase catalyzes the conversion of CO_2 into HCO_3^- and then CO_3^{2-}, thereby producing two protons and decreasing the pH outside of the cell. These protons could be removed from the site of calcification by a Ca^{2+}-ATPase, which catalyzes the exchange of $2H^+$ for Ca^{2+} in some algae, such as *Chara corallina* (McConnaughey and Falk 1991). It is also possible that carbonic anhydrase catalyzes the conversion of HCO_3^- to CO_3^{2-} in reaction 2, as suggested by Digby (1977). However, the stimulated CA activity could not completely compensate under the elevated CO_2 conditions, as inorganic content was low even when CA was stimulated. Furthermore, the question still remains why oxygen evolution and photosynthetic efficiency decreased under high CO_2 conditions compared to ambient, especially when CA activity was increased and CO_2 was abundant. Gao and Zheng

(2010) found the same negative response of photosynthesis under elevated CO_2 concentrations in *C. sessilis*, and suggested increased non-photochemical quenching and higher energy requirements were likely under CO_2 stress, resulting in lower growth and photosynthesis. In the current study, perhaps increased cyclic electron flow in Photosystem I created more ATP for the upregulation of CA activity, which therefore contributed to the decrease in photosynthetic efficiency and growth in *C. officinalis*.

In future studies with *C. officinalis* it will be necessary to separately measure both external and internal carbonic anhydrase activity in order to determine if both enzymes increase their activity in response to increased CO_2, or if only external CA is affected. Giordano and Maberly (1989) reported that *C. officinalis* only contained internal carbonic anhydrase. However, Mercado et al. (1997) reported the presence of CA in the related alga *Corallina elongata* after developing a more sensitive method for detecting the enzyme. Furthermore, Ragazzola (2009) found evidence for the presence of external CA in *C. officinalis* when photosynthesis was inhibited by the impermeable sulfonamide inhibitor acetazolamide (AZ), and the activity of external carbonic anhydrase in *C. officinalis* has been detected in our lab since this study was conducted. Therefore, future work will clarify how this enzyme is responding to increased CO_2 levels both inside and outside of the cells.

The clear response of decreased inorganic material production and lower growth rates in *C. officinalis* under elevated CO_2 conditions indicate that there are physiological impacts of ocean acidification on this alga that may impact the ecology of rocky intertidal macroalgal communities. Slower growth rates and weaker (i.e., less calcium carbonate) skeletons may lower the ability of *C. officinalis* to compete with other macroalgae, particularly non-calcifying species, in its natural environment. Gao and Zheng (2010) suggested that the inorganic skeleton of *C. sessilis* provides protection from UV exposure, due to the exacerbated inhibition of photosynthesis they observed under the combined stress of elevated CO_2 concentrations and UV exposure in this species. Moreover, as UV radiation as a single stress factor was shown to strongly affect competition between non-calcifying rhodophytes (Bischof et al. 2000, 2006), the combination of these two stressors might have strong influences on community structure. Furthermore, several studies have shown that biotic and abiotic stressors such as increased grazing pressure and high CO_2 levels lower recruitment of crustose calcifying algae (Belliveau and Paul 2002, Jokiel et al. 2008, Kuffner et al. 2008, Martin et al. 2008), while turf macroalgal cover increases (Russell et al. 2009; Connell and Russell 2010; Russell et al. 2011). If increased CO_2 levels weaken the skeletal structure of *C. officinalis*, the potential combination of increased grazing pressure, slower growth, higher UV stress, and less recruitment will likely cause a phase shift in *C. officinalis* communities toward more fleshy, non-calcifying macroalgae, and could even amplify changes in competition between non-calcifying algae. While both calcifying and non-calcifying algae provide important habitat and shelter for many marine organisms, erect calcifying algae such as *C. officinalis* contribute to the strength of the intertidal community structure and provide refugia for organisms in environments with high wave action (Stewart 1982; Coull and Wells 1983; Kelaher 2002, 2003)—all important ecological roles that could be interrupted under high CO_2 conditions.

In conclusion, during our relatively short-term experiment conducted at CO_2 concentrations 1 and 2 times those that could be reached by 2100 (AIFI scenario, IPCC Special Report on Emissions Scenarios), *C. officinalis* growth was negatively affected by these conditions. Furthermore, inorganic carbon production and photosynthetic efficiency (based on O_2 evolution) were significantly negatively affected at the highest CO_2 concentration investigated in this study. The results indicate that *C. officinalis* will be physiologically disadvantaged if CO_2 concentrations in surface oceans reach above 1,000 µatm, and could be negatively affected at even lower concentrations that are expected by the end of the century. The ecological implications of these physiological disadvantages are that this calcifying species could be less competitive under future CO_2 scenarios compared to fleshy macroalgae unless it is able to adapt. However, the exact ecological impacts on *C. officinalis* and the surrounding macroalgal community are still unknown, especially when considering adaptation strategies. Therefore, future work should focus on identifying how the physiological impacts observed in this study may induce changes in ecological relationships among macroalgae in temperate macroalgal environments.

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