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Increased irradiance availability mitigates the physiological performance of species of the calcifying green macroalga *Halimeda* in response to ocean acidification

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ABSTRACT

Although negative responses of tropical calcifying organisms to ocean acidification have been widely reported, the modulating potential of irradiance combined with elevated pCO_2 has not been well studied. In this study, the interactive effects of elevated pCO_2 and irradiance availability on the physiology of calcifying macroalgae *Halimeda cylindracea* and *Halimeda lacunalis* were investigated using a fully factorial, 28-day aquaria coupling experiment. The results of the present study demonstrate that elevated pCO_2 negatively influences growth, photosynthesis, calcification and other physiological processes of both *Halimeda* species. However, these negative effects could be mitigated to some extent by increased irradiance availability. Specific growth rate (*SGR*), net calcification rates (G_{net}) and maximum quantum yield (F_v/F_m) decreased significantly by 6.84%–86.70%, 51.78%–62.29% and 2.37%–28.91% in elevated pCO_2 treatments. However, *SGR*, G_{net} and F_v/F_m increased by 3.39%–84.78%, 29.61%–40.68% and 1.68%–6.92% in high irradiance conditions, respectively. Chl-*a* in elevated pCO_2 treatments was 7.75%–61.25% lower than ambient pCO_2 conditions, while the carotenoid content increased by 12.12%–57.45% in low irradiance conditions from day 20–28. Malondialdehyde (MDA) content was higher in elevated pCO_2 treatments. However, there was also a two- to four-fold increase in proline content in elevated pCO_2 treatments. Tissue total organic carbon (TC_{org}) and nitrogen (TN) were positively correlated to CO_2 enrichment. The results of the current study suggested that elevated pCO_2 negatively influenced the physiological responses of *Halimeda*, while increased irradiance availability may enhance the metabolic performance in response to ocean acidification.

1. Introduction

Over the last two centuries, the oceans have become a large CO_2 sink, sequestering about 2×10^9 metric tons of carbon per year [1]. It is predicted that there will be an increase in the rate of anthropogenic CO_2 emissions in the future [1]. The current level of atmospheric CO_2 is 394 ppmv, and this is anticipated to approach nearly 1000 ppmv by the end of this century [2]. It is likely that this will result in pH values in surface seawaters decreasing by about 0.3–0.4 units [2–4]. Koch et al. [5] have reported that at current ocean pH (~8.04) levels, CO_2 will account for the greatest increase (> 250%) in dissolved inorganic carbon (DIC) by the end of 2100. Ocean acidification (OA) is receiving substantial scientific attention, with an increasing number of studies concluding that OA is a major threat to marine calcifying organisms [6–8]. Excessive CO_2 dissolved in seawater can increase the concentrations of bicarbonate (HCO_3^-) and hydrogen ions (H^+), which cause a decline in pH values and the saturation state (Ω) of different crystallization forms of calcium carbonate in the marine environment [3]. Gradual shifts in CO_2, which alter the proportion and concentration of DIC, affects physiological metabolic processes, including growth, photosynthesis and calcification in a range of marine organisms [5]. These changes in seawater chemistry may have significant negative effects on calcifying organisms, as carbonate ions (CO_3^{2-}) in seawater are used to build their calcified skeletons and shells [9]. While OA, as a solitary factor, can induce various physiological responses, it remains difficult to ascertain the ultimate impacts of OA on marine calcifiers, with large

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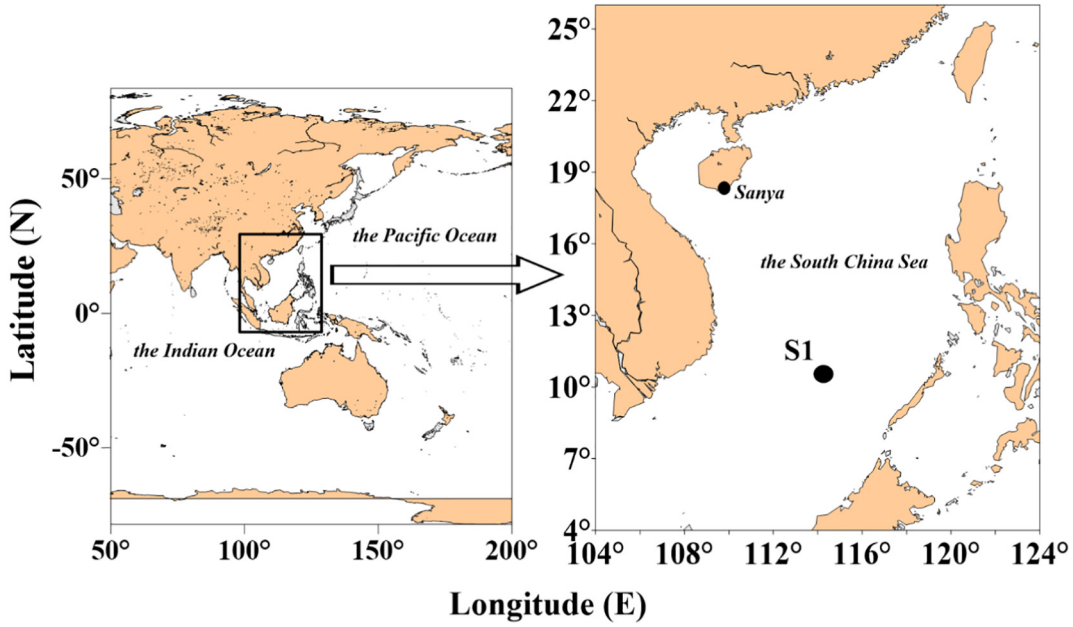

Fig. 1. The geographic location of the sampling site (S1) in the South China Sea and the location of the experimental facility in Sanya, Hainan Island.

differences reported between taxa using different meta-analyses [10,11].

Calcifying macroalgae, which are an essential part of coral reef ecosystems, may be considered particularly sensitive to increases in atmospheric CO_2 (pCO_2), which is cause for concern, as many coral reef inhabitants rely upon these biogenic structures for shelter and habitat [12]. OA (elevated pCO_2) can impact algal physiological performance, such as photosynthesis, respiration and growth, which are all metabolically linked [13]. Meyer et al. [14] have described the differential effect of increases in inorganic carbon (pCO_2) (DIC) and organic carbon (DOC) availability on the physiology of two marine calcifiers, *Halimeda incrassata* and *Udotea flabellum*, and pointed out that photosynthesis and calcification rates of *H. incrassata* are negatively affected by both DIC and DOC enrichment while *U. flabellum* is less affected. With the negative effects on calcifying macroalgae caused by elevated pCO_2 and concomitant lowering of carbonate saturation states (Ω) [15], an understanding of their physiological and ecological responses to OA is urgently required. Irradiance is another important environmental factor for many tropical calcifying species, with photon energy absorbed by light-harvesting complexes via electron transport systems [14,16]. Previous studies have shown that low irradiance may limit the ability of macroalgae to utilize HCO_3^- or to accumulate a carbon concentrating mechanism by increasing their reliance on CO_2 diffusion [17]. Therefore, it is important to design experiments combining elevated pCO_2 concentrations with other factors, especially irradiance intensity, as multiple environmental stressors probably have more complex interactive or/and cumulative effects on the metabolic processes of calcifying organisms than a single stressor [18,19]. Unfortunately, the factors mentioned above have received limited attention in terms of experimental analysis. Vásquez-Elizondo and Enríquez [16] suggested that moderate levels of light stress were maintained under reduced pH (7.90) resulting in no impact on coralline algal photosynthesis, while moderate adverse effects on calcification rates were observed.

Halimeda species are green calcareous macroalgae that are widely distributed in tropical and subtropical lagoons and reefs, which contribute to reef carbonate accretion [20], and contribute to biodiversity by providing a complex benthic topography [21,22]. *Halimeda* segments may be shed intermittently by living thalli or after holocarpic sexual reproduction, with $CaCO_3$ production rates reaching up to 0.8–1.4 kg m^{-1} yr^{-1} in coral reef systems [23]. Due to its specific growth locations and depth range, *Halimeda* experiences varied sunlight irradiance conditions [24,25]. Previous studies have reported that *Halimeda* species that are adapted to low versus high irradiance environments may respond differently to elevated pCO_2, due to irradiance acclimation and differences in seawater carbon chemistry [19,25]. Deeper waters are generally low in irradiance, but are enriched in CO_2 under a relatively stable environment [26]. In contrast, dissolved CO_2 exhibits diel fluctuations in shallow waters and lagoons due to photosynthetic CO_2 uptake during the day and respiratory CO_2 emissions at night [27,28]. The interactive influences of elevated temperature (ocean warming), and nutrients with ocean acidification on calcifying organisms have already been substantially reported, particularly in relation to coral reefs [29,30]. However, few studies have examined the metabolic effects of irradiance availability and CO_2-induced seawater acidification on *Halimeda* [25], despite irradiance being one of most important basic environmental factors which affects growth, calcification, and photosynthesis, pigment accumulation of autotrophic organisms.

The South China Sea is a marginal sea [31], where tropical coral communities occur and develop principally around the offshore islands and archipelagos, which act as multidimensional habitats and shelters for a diversity of marine organisms [31]. Members of the genus *Halimeda* are widely distributed in this area, growing in sandy or/and epilithic substrata anchored by a massive bulbous holdfast or matted siphons [32]. Macroalgal *Halimeda* species may experience different irradiance intensities by altering their distribution depth, which affects algal biomass accumulations [33]. Thus, the aim of this study was to determine how physiological responses of *Halimeda* species to elevated pCO_2 are affected by irradiance availability. It is hypothesized that increases in irradiance availability may mitigate photosynthesis, calcification and other aspects of relative physiological performance, even under high CO_2 concentrations. These physiological descriptions will allow the development of a common physiological method to understand the response of calcifying macroalgae to global environmental changes.

2. Materials and methods

2.1. Sample collection

Two *Halimeda* species (*Halimeda cylindracea* and *Halimeda lacunalis*)

were collected off the archipelago at sampling station S1 in the South China Sea at depths ranging from 3 m to 15 m with distinct irradiance levels (Fig. 1) in July 2018. All samples were carefully uprooted from the substrate keeping the whole thalli intact. Afterwards, specimens were transported to the ship and temporarily cultured in a continuous flow-through seawater system until they were brought back to laboratory in Sanya, China.

In the laboratory, the algae were incubated in a large mesocosm tank that received a constant supply of fresh seawater (~50 L/min) at ~28 °C, 33 ppt, pH 8.12, and 80 μmol photons m^{-2} s^{-1} with a 12:12 h light/dark cycle for two weeks [9]. During the algae acclimation period, all individuals turned a healthy green color and no obvious mortality was observed.

2.2. Experimental design and treatments

After the preliminary incubation period, the two *Halimeda* species were haphazardly subjected to experimental treatments of two irradiance intensities and two elevated pCO_2 levels within aquaria for four weeks (October 10th 2018–November 8th 2018). The experiment was conducted using a 2 × 3 factorial design to test the mixed effects on Halimeda physiology. A total of 18 aquaria (30 L) were assigned to one of six treatments as shown in Table 1. There was a total of 20 individuals of two species (240–280 g FW, fresh weight) in one aquarium. Filtered seawater changes were conducted twice a week. Irradiance levels for high light treatments averaged 180 μmol photons m^{-2} s^{-1} (T4–T6) and were reduced to 30 μmol photons m^{-2} s^{-1} in the low light treatments (T1–T3). While irradiance levels at the sampling site were comparatively higher (300–500 μmol photons m^{-2} s^{-1}), prior studies have demonstrated that saturation irradiance (I_k) for field populations of *Halimeda* species typically varies from 30 to 200 μmol photons m^{-2} s^{-1} [9,34,35], which included the irradiance range selected in the present study. Irradiance intensities were created using a combination of white FMW39TS and blue FMB5AT5 light bulbs (Arcadia, Redhill, UK) and determined by a radiometer (ELDONET Terrestrial Spectroradiometer, Germany) [9]. Carbon dioxide enrichment (elevated pCO_2) was achieved by bubbling different concentrations of mixed CO_2 and ambient air (CE100C, Wuhan Ruihua Instrument and Equipment Ltd., China). The selected elevated pCO_2 treatments comprised the current average seawater pCO_2 level of 400 ppmv (T3, T6), the predicted average pCO_2 level 1000 ppmv (T2, T5) for the year 2100 [36], and the expected pCO_2 level 1600 ppmv (T1, T4) during extreme episodes by the end of year 2100 [37].

2.3. Monitoring experimental conditions

During the experimental period, seawater temperature, pH (NBS scale), salinity and total alkalinity (TA) were monitored daily to ensure consistency between the treatments. Temperature and salinity were measured using a calibrated handheld YSI meter (YSI, Yellow Springs, OH, USA) and pH values were monitored using a Mettler Toledo pH electrode. Prior to carrying out measurements, the pH probe was calibrated using three-point calibration with standard NBS buffer solutions for 4.01, 7.00 and 9.21 (relative accuracy ± 0.01 units). TA was determined using the titration method (Metroham 877 Titrino Plus titrator, Switzerland) with an automated sample changer and pH probe. Measured seawater parameters (temperature, salinity, pH and TA) were used to calculate dissolved inorganic carbon components (CO_2, HCO_3^- and CO_3^{2-}) and the aragonite saturation state (Ω_{Ar}) with the program CO2SYS [38].

Table 1
Factorial design used to test the effects of elevated pCO_2 at two irradiance levels.

Treatments (pCO_2 × irradiance)	Seawater pCO_2 (ppmv)	Irradiance (μmol photons·m^{-2}·s^{-1})
T1	1600	30
T2	1000	30
T3	400	30
T4	1600	180
T5	1000	180
T6	400	180

2.4. Growth characteristics and tissue mineral content

At the beginning and end of the experiment, all individuals of the two *Halimeda* species in each treatment were collected and weighed using an electronic balance (AR224CN, OHAUS, New Jersey, USA; accuracy, ~0.1 mg). The specific calculation process of special growth rate (*SGR*) of two *Halimeda* species was conducted according to the method described by Wei et al. [39].

The production of new segments (%) on tracked thalli was evaluated at the beginning and end of the experiment by calculating the proportion of new segments. Tissue mineral content was assumed to be $CaCO_3$ content and was determined following the method described by Peach et al. [25].

2.5. Measurement of calcification rates

The calcification rates in each treatment were calculated according to the alkalinity anomaly technique [34]. Fragments (5.0 g) of each *Halimeda* species were incubated in 1.0 L transparent glass bottles with filtered seawater under experimental (T1–T6) conditions while being stirred by an electro-magnetic stirrer. Changes in TA from 0 to 8 h after incubation were calculated using the following equation:

$$G_{net} = 0.5p \times (TA_0 - TA_n) \times V/(FW \times t)$$

where G_{net} is the net calcification rate (μmol $CaCO_3$ g FW^{-1} h^{-1}), p is the density of seawater (1.025 kg L^{-1}), TA_0 represents the initial TA of seawater, TA_n represents the TA of seawater after n hours of incubation (8 h in this study), V is the volume of the incubation chamber (L), FW is algal fresh weight (g), and t is the incubation time (h).

2.6. Photosynthetic performance and pigment contents

To determine photosynthetic performance, the maximum quantum yield (F_v/F_m) of *Halimeda* was measured every four days using a DIVING-PAM fitted with an 8.0 mm diameter fiberoptic cable (Heinz Walz GmbH, Effeltrich, Germany). On each sampling date, a random branchlet (~3.0 cm) in each treatment was removed and dark adapted for approximately 20 min before measurement. In addition to photosynthetic performance, pigment content, including chlorophyll (Chl-*a*) and carotenoid, were determined according to the method described by Ritchie [40].

2.7. Physiological responses

To gain further insight into the physiological relevance of elevated pCO_2 and irradiance, proline and malondialdehyde (MDA) were analyzed as biochemical markers under stress conditions. During treatments, segments were collected every four days for physiological assays. Proline accumulation in *Halimeda* exposed to each treatment was measured following the method described by Shan et al. [41].

The occurrence of MDA was also considered as a useful indirect evaluation of general lipid peroxidation under certain environmental stresses whose equivalents were calculated as described by Hodges et al. [42].

2.8. Tissue carbon, nitrogen and phosphorus content

The total organic carbon (TC_{org}), nitrogen (TN) and phosphorus

(TP) content were measured at the end of experiment. Samples of both *Halimeda* species tissue were rinsed with distilled water 3–5 times and then dried in an oven at 60 °C until a constant weight was obtained. Subsequently, all samples were finely ground to powders with an automatic grinding mill (FSTPRP-24, Jingxin, Shanghai, China). Separate tissue samples from same individuals were also analyzed for TC_{org} by acidification of the inorganic fraction with 1 N HCl (100 µl per 3.0 mg dry tissue). Samples for TC_{org} and TN were measured using a CHN elemental analyzer (Flash EA300, Thermo Scientific, Milan, Italy). TP content was determined from the same ground tissues as mentioned above using a calorimetrically modified molybdenum blue method described by Koroleff [43].

2.9. Statistical analysis

All statistical analyses were conducted using Microsoft Excel 2010 and Minitab 16.0 software. The sampling map and figures were created using Surfer 8.0 software and Origin 8.1, respectively. All data were tested for normality and equal variance prior to statistical analysis and were reported as mean ± standard deviation. All response variables measured were analyzed using a multivariate analysis of variance (MANOVA) with elevated pCO_2 and irradiance as the independent factors. Tukey's test was used to identify significant differences between treatment means, with differences considered significant at $P < 0.05$ and extremely significant at $P < 0.01$. P values were significant at the 95% confidence level.

3. Results

3.1. Seawater monitoring

The seawater chemistry conditions for each treatment during the experimental period are shown in Table 2. Seawater temperature and salinity were similar across all treatments with mean values of 28.40 °C and 32.81 ppt, respectively. There was no significant difference ($P > 0.05$) between these values. Treatments without CO_2 enrichment had high pH values and lower alkalinity levels than those with CO_2 enrichment ($P < 0.01$). The corresponding daily ranges for calculated values of CO_2 concentrations of ambient seawater ranged from 12.2 to 13.2 µmol kg^{-1}, and was much lower than the range of 30.7 to 31.8 µmol kg^{-1} in T2 and T5 (1000 ppmv) and 64.0 to 65.9 µmol kg^{-1} in T1 and T4 (1600 ppmv), respectively. Daily mean calculated values of HCO_3^- and CO_3^{2-} significantly differed between ambient and elevated pCO_2 treatments ($P < 0.01$). The aragonite saturation state (Ω_{Ar}) values were lower in T1 and T4 (1600 ppmv), and T2 and T5 (1000 ppmv) with mean ranges from 1.23 to 1.24, and 1.71 to 1.73, respectively, compared with ambient seawater (2.79 to 2.99).

3.2. Growth characteristics and tissue mineral content

After four weeks of exposure, the proportion of live thalli producing new segments and special growth rates (*SGR*) differed between both *Halimeda* species ($P < 0.01$). *H. cylindracea* had a significantly higher growth rate than *H. lacunalis* (Table 3). Elevated pCO_2 and irradiance had significant effects on growth characteristics of both species (Table 4), as the high irradiance intensity stimulated growth rates over time, whereas the low irradiance decreased growth rates over time at the three CO_2 concentration levels (Table 3). The *SGR* in elevated pCO_2 conditions decreased by 6.84%–86.70%, in contrast with those in ambient pCO_2 which increased by 3.39%–84.78% in high irradiance treatments. New segment production increased two to three-fold under high irradiance. $CaCO_3$ content varied between new (5–7 days) and mature (> 15 days) segments ($P < 0.01$). The percentage of $CaCO_3$ in new segments of both *Halimeda* species was significantly affected by pCO_2 and irradiance ($P < 0.01$) (Table 4). The $CaCO_3$ content of new segments was highest in the control pCO_2 treatment with high irradiance and lowest in the tissues of algae grown under elevated pCO_2 conditions with low irradiance. $CaCO_3$ content in mature segments varied between species ($P < 0.01$), but was not significantly altered by pCO_2 or/and irradiance (Table 4). Across all treatments, the $CaCO_3$ content of mature *H. cylindracea* and *H. lacunalis* ranged from 89.27% to 91.23% and 83.74% to 88.22%, respectively (Table 3).

3.3. Calcification rates

There were significant direct effects of elevated pCO_2 and irradiance intensities and their interactions on the net calcification rates (G_{net}) of the two *Halimeda* species in this study ($P < 0.01$) (Fig. 2). The G_{net} decreased by 51.78%–62.29% in two elevated pCO_2 (1000 ppmv and 1600 ppmv) treatments and increased by 29.61%–40.68% in high irradiance treatments. In the T1 treatment (1600 ppmv, 30 µmol photons·m^{-2}·s^{-1}), G_{net} showed a two-fold reduction compared with the T3 treatment (400 ppmv, 30 µmol photons·m^{-2}·s^{-1}). Highest G_{net} values were obtained in the T6 treatment (400 ppmv, 180 µmol photons·m^{-2}·s^{-1}), ranging from 0.98 to 1.31 µmol $CaCO_3$ g FW^{-1} h^{-1}. G_{net} values of these species exhibited relatively similar variations across the six treatments, with G_{net} decreasing in elevated pCO_2 conditions. In addition, the G_{net} of *H. cylindracea* was always higher than that of *H. lacunalis* under the same conditions ($P < 0.05$), which indicated a significant difference between the two species (Fig. 2).

3.4. Photosynthetic performance and pigment content

During the experimental period, the maximum quantum yield (F_v/F_m) of the two *Halimeda* species in the T6 treatment remained within the range 0.722 to 0.782 ($P > 0.05$). There was, however, a significant decrease (2.37%–28.91%) of F_v/F_m in T1 and T4 after 12 days under 1600 ppmv conditions ($P < 0.01$). After the first 12 days of the experimental period, exposure to elevated pCO_2 led to a potential upregulation of F_v/F_m, as shown in Fig. 3. The F_v/F_m values of the two *Halimeda* species in T4 were consistently higher (1.68%–6.92%) than those in T1 (Table 4). The variations in pigment content are shown in Fig. 4. There were no obvious changes in initial Chl-*a* and carotenoid contents among the treatments (Fig. 4). After four to eight days of experimental exposure, the Chl-*a* content in *H. cylindracea* and *H. lacunalis* tissues in T1 were 254.6 ± 26.4 µg g^{-1} FW and 107.2 ± 17.2 µg g^{-1}

Table 2
Measured seawater parameters and calculated carbon chemistry via CO2SYS in acidification and non-acidification treatments at two irradiance levels (mean ± SD, n = 3).

Treatments	pH	TA (µmol L^{-1})	CO_2 (µmol kg^{-1})	HCO_3^- (µmol kg^{-1})	CO_3^{2-} (µmol kg^{-1})	Ω_{Ar}
T1	7.61 ± 0.12	2434.5 ± 43.1	64.0 ± 3.1	2283.3 ± 47.8	63.1 ± 7.6	1.24 ± 0.21
T2	7.80 ± 0.09	2372.6 ± 45.6	30.7 ± 1.8	2102.3 ± 43.7	112.0 ± 13.2	1.73 ± 0.28
T3	8.12 ± 0.11	2168.4 ± 36.7	12.2 ± 0.7	1707.6 ± 39.6	187.2 ± 16.3	2.99 ± 0.32
T4	7.62 ± 0.08	2482.1 ± 49.4	65.9 ± 4.2	2327.7 ± 46.2	64.6 ± 6.3	1.23 ± 0.24
T5	7.79 ± 0.07	2339.5 ± 39.9	31.8 ± 1.2	2082.1 ± 44.6	106.4 ± 10.4	1.71 ± 0.27
T6	8.09 ± 0.10	2161.8 ± 43.2	13.2 ± 0.4	1726.3 ± 36.9	176.8 ± 14.5	2.79 ± 0.40

Table 3
Response variables of two *Halimeda* species in six treatments. Maximum new segment production rate was the percentage of individuals (mean ± SD, n = 5) with thalli that produced new segments. Mineral content of segments is shown as $CaCO_3$%.

Treatments		Max new seg. (%)	SGR (% d^{-1})	New seg. $CaCO_3$%	Mature seg. $CaCO_3$%
T1	*H. cylindracea*	9.16	0.123 ± 0.082	72.83 ± 1.21	89.27 ± 3.11
	H. lacunalis	3.12	0.057 ± 0.012	69.26 ± 2.12	86.20 ± 2.33
T2	*H. cylindracea*	10.11	0.119 ± 0.063	73.71 ± 1.01	90.25 ± 1.78
	H. lacunalis	2.89	0.063 ± 0.007	70.38 ± 3.61	87.28 ± 2.01
T3	*H. cylindracea*	15.36	0.192 ± 0.058	74.23 ± 2.19	91.22 ± 1.82
	H. lacunalis	2.62	0.087 ± 0.004	72.29 ± 2.61	88.22 ± 2.11
T4	*H. cylindracea*	19.23	0.213 ± 0.051	76.28 ± 4.01	90.73 ± 2.19
	H. lacunalis	3.96	0.059 ± 0.007	73.17 ± 2.62	83.74 ± 2.03
T5	*H. cylindracea*	26.77	0.782 ± 0.201	79.23 ± 1.62	91.23 ± 2.72
	H. lacunalis	4.88	0.103 ± 0.036	77.23 ± 2.83	86.72 ± 1.03
T6	*H. cylindracea*	38.41	1.601 ± 0.274	79.89 ± 3.37	91.23 ± 1.67
	H. lacunalis	6.99	0.098 ± 0.033	75.56 ± 3.66	85.83 ± 1.55

Table 4
Results of MANOVA tests, including *F*-ratios and *P* (95% confidence level), to analyze the effects of three pCO_2 levels crossed with two irradiance intensities on the physiological performance of *Halimeda cylindracea* (a) and *Halimeda lacunalis* (b).

Source of variations	Elevated pCO_2		Irradiance		Elevated pCO_2 * irradiance	
	F	P	F	P	F	P
(a) *Halimeda cylindracea*						
SGR	38.51	0.000	110.57	0.000	29.82	0.000
New seg $CaCO_3$%	1.70	0.024	17.65	0.001	0.39	0.686
Mature seg $CaCO_3$%	0.44	0.654	0.57	0.465	0.16	0.857
F_v/F_m	42.81	0.000	3.88	0.072	0.53	0.600
Chl *a*	6.34	0.013	15.46	0.002	0.35	0.714
Carotenoid	1.76	0.214	1.40	0.259	7.23	0.009
Proline	11.51	0.002	2.89	0.115	2.55	0.119
MDA	4.59	0.033	4.49	0.056	1.51	0.260
TC	2.28	0.045	0.48	0.501	0.16	0.851
TN	1.71	0.042	0.96	0.347	0.25	0.780
TP	0.04	0.965	0.53	0.479	0.25	0.782
(b) *Halimeda lacunalis*						
SGR	0.26	0.773	3.12	0.103	5.56	0.020
New seg $CaCO_3$%	2.15	0.049	11.23	0.006	0.08	0.926
Mature seg $CaCO_3$%	2.67	0.110	4.18	0.064	0.15	0.865
F_v/F_m	5.10	0.025	5.33	0.040	0.39	0.686
Chl *a*	28.82	0.000	15.33	0.002	18.88	0.000
Carotenoid	0.32	0.733	41.14	0.000	1.10	0.365
Proline	3.40	0.047	0.45	0.514	0.03	0.971
MDA	4.49	0.035	0.47	0.506	0.55	0.593
TC	0.94	0.047	4.40	0.058	0.60	0.565
TN	1.02	0.038	4.31	0.060	0.34	0.721
TP	0.59	0.571	1.37	0.264	1.00	0.397

FW, 7.75% and 61.25% lower than those in T6 ($P < 0.01$), respectively. At the end of the experimental period, the Chl-*a* content in both elevated pCO_2 treatments was significantly lower than T6 ($P < 0.01$) (Table 4). Carotenoid content was also affected by elevated pCO_2 and irradiance variations and were lowest during the first 4–12 days at 10.6–21.5 μg g^{-1} FW and 10.4–22.9 μg g^{-1} FW, respectively (Fig. 4). However, there was a significant increase (12.12%–57.45%) in carotenoid content in low irradiance conditions with levels of 25.1–26.4 μg g^{-1} FW and 22.7–35.3 μg g^{-1} FW, respectively.

3.5. Proline and MDA content

In the context of physiological responses, chemical compounds were measured every four days to evaluate abiotic stress levels. Under 180 μmol photons m^{-2} s^{-1} conditions, lower proline levels were detected among the two *Halimeda* species (Fig. 5). The average proline contents of *H. cylindracea* and *H. lacunalis* in the T6 treatment were 1.338 ± 0.19 μg ml^{-1} FW and 1.630 ± 0.13 μg ml^{-1} FW, respectively. The proline levels in T1 and T4 (1600 ppmv) were two- to four-fold higher than those in T6 (400 ppmv) during the first 4–12 days of the experiment ($P < 0.01$) (Table 4), after which proline accumulated in the two *Halimeda* species, and the levels in the six treatments declined by 22.11%–42.72% and 45.67%–72.80%, respectively (Fig. 5). A similar trend was observed in malondialdehyde (MDA) content. There were equal MDA levels in T6. Following treatment with CO_2 enrichment (1000–1600 ppmv), the MDA content in two *Halimeda* species was significantly higher (two to three-fold) than T6 (400 ppmv). From day 12 onwards MDA content decreased greatly and then remained stable in six treatments from days 24–28.

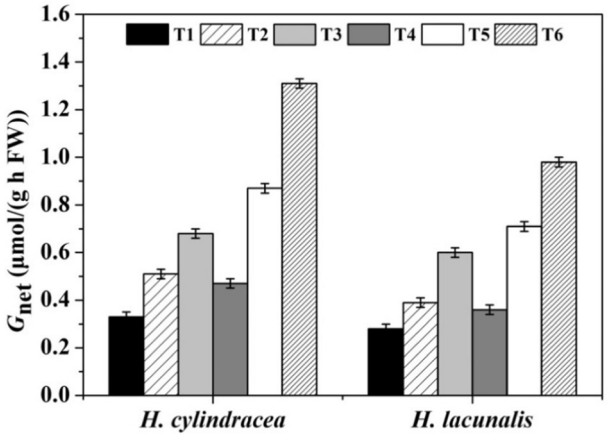

Fig. 2. Calcification rate (G_{net}) responses of two *Halimeda* species (mean ± SD, n = 3) in six pCO_2-irradiance treatments.

3.6. Tissue carbon, nitrogen and phosphorus content

Total organic carbon (TC_{org}) and total nitrogen (TN) of two *Halimeda* species were significantly higher in the CO_2 enrichment treatments than in the non-enrichment treatments ($P < 0.05$) regardless of irradiance levels (Fig. 6, Table 4); however, total phosphorus (TP) did not follow the same pattern (Fig. 6). The TC_{org} content in *H. cylindracea* ranged from 16.08% to 17.47% and was significantly higher than the TC_{org} content in *H. lacunalis*, which ranged from 14.49% to 15.39% across all treatments ($P < 0.01$). TC_{org} content was positively affected by elevated pCO_2 (Table 4). Similarly, the TN content in *H. cylindracea* were highest than those for *H. lacunalis*, with an average of 1.47 ± 0.28%, while the average TN content in *H. lacunalis*

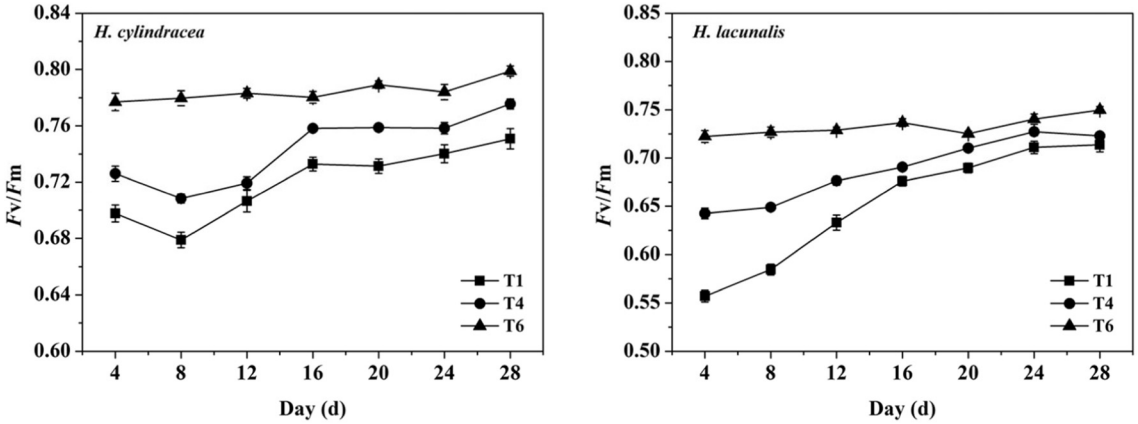

Fig. 3. Photosynthetic maximum quantum yield (F_v/F_m) of two *Halimeda* species (mean ± SD, n = 5) incubated in T1, T4 and T6 treatments during the four week experimental period. The selected treatments represented elevated pCO_2 and ambient pCO_2 from low (30 μmol photons m^{-2} s^{-1}) to high (180 μmol photons m^{-2} s^{-1}) irradiance conditions.

tissue was 0.85 ± 0.15%. TN content was higher in elevated pCO_2 treatments for both *Halimeda* species. Conversely, C_{org}: N ratios were lowest in *H. cylindracea* tissues under elevated pCO_2 conditions. During the experimental period, the average TP content for *H. cylindracea* and *H. lacunalis* was 0.033 ± 0.008% and 0.025 ± 0.009%, respectively (Fig. 6), with no significant difference observed with carbon enrichment and irradiance intensities (Table 4).

4. Discussion

Drastic shifts in seawater pH due to elevated pCO_2 are predicted to lower aragonite saturation (Ω_{Ar}), which may negatively affect the calcification of marine organisms, in particular calcifying macroalgae [9,27,44]. The results of present study demonstrate that elevated pCO_2 levels negatively influence the growth characteristics, photosynthesis, calcification and other physiological processes of calcifying macroalgae *H. cylindracea* and *H. lacunalis*, however, these negative effects can be mitigated to some extent by increased irradiance availability. The results of this study showed that the *SGR* of two *Halimeda* species were reduced by elevated pCO_2 levels in different irradiance environments, especially under 30 μmol photons m^{-2} s^{-1} conditions after 28 days of incubation (Table 3), which is in agreement with previous findings. Wei et al. [45] found that in CO_2 enriched conditions (pH 7.50–7.80), the growth rate of *Halimeda opuntia* was reduced by 48.29%–58.80%. Previous studies have also reported that when CO_2 concentrations in seawater increase from 360 μmol mol^{-1} (pH ~8.20) to 1000 μmol mol^{-1} (pH ~7.80), the fresh weight of the coralline red algae *Amphiroa* sp. declined by 40.01% after 73 days incubation [46].

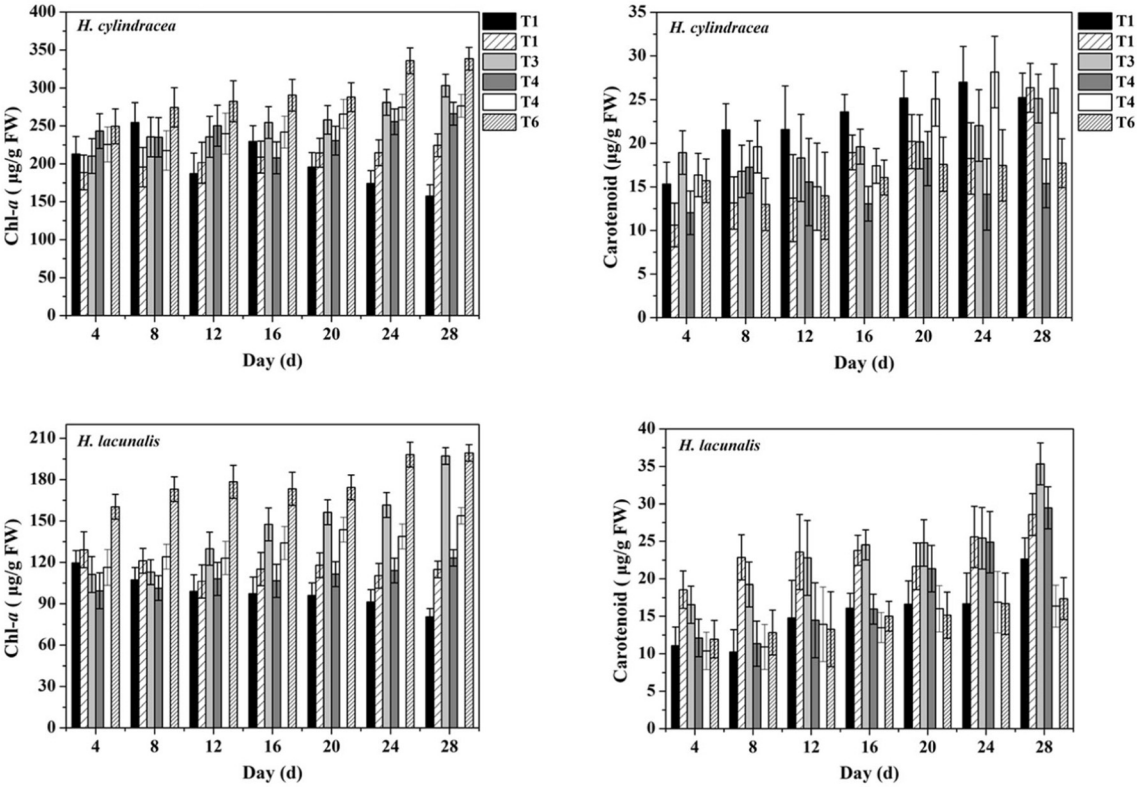

Fig. 4. Variations in pigment content of chlorophyll (Chl) *a* and carotenoid from tissues of two *Halimeda* species (mean ± SD, n = 3) in six pCO_2-irradiance treatments during the experimental period.

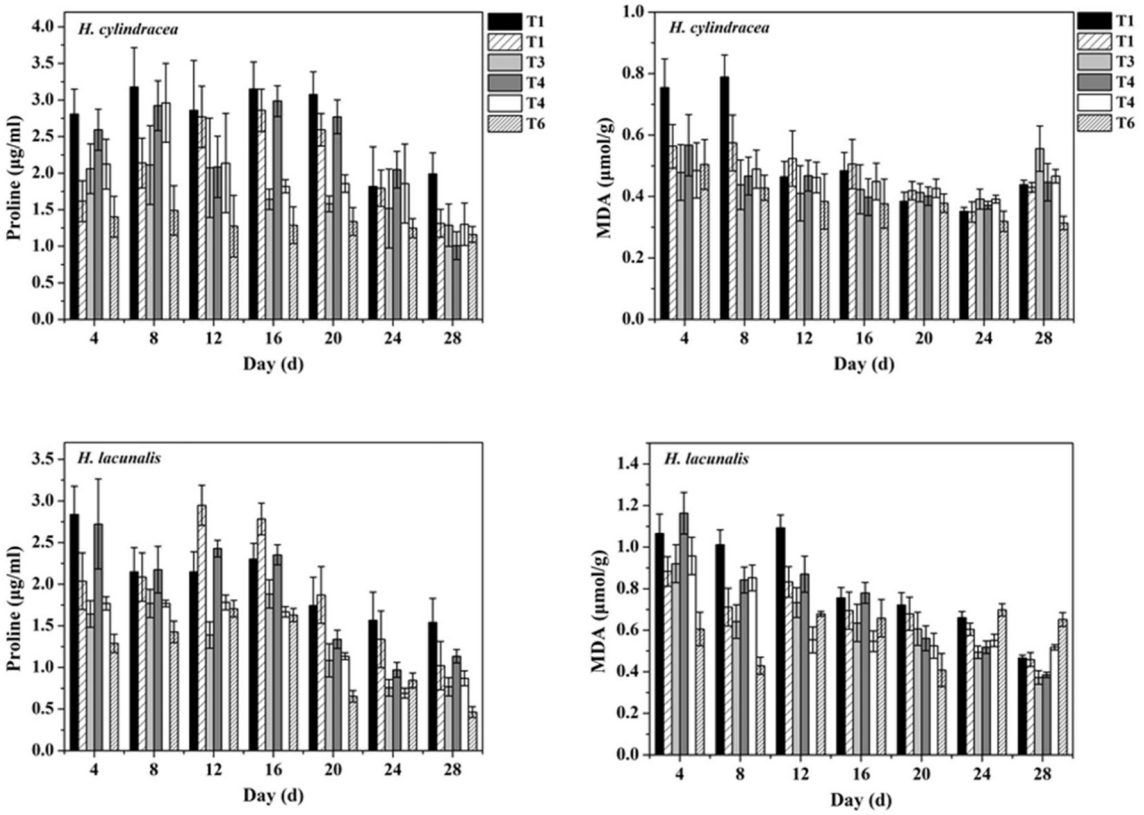

Fig. 5. Variations in proline and MDA content in the tissues of two *Halimeda* species tissues (mean ± SD, n = 3) in six pCO_2-irradiance treatments during the experimental period.

Notably, the two *Halimeda* species examined in this study maintained positive growth rates in seawater with increased irradiance availability. A similar response has also been observed in *Halimeda tuna*, which exhibited higher biomass at depths of 7 m than populations at depths of 21 m in the Florida Keys due to increased irradiance availability in shallower waters [47]. Teichberg et al. [19] found that the growth rates of *H. opuntia* were significantly higher at depths of 5 m compared with 15 m. According to the results of this study and previous reports, it seems that irradiance intensity and elevated pCO_2 significantly influence growth responses in most cases, even if irradiance or/and elevated pCO_2 related effects appear to be species-specific [19,25]. The *SGR* of *H. cylindracea* in the current study was significantly higher than the values reported by Hofmann et al. [48], due to low irradiance availability (50 μmol photons m^{-2} s^{-1}) and high shedding rates of old segments of *H. opuntia* with elevated pCO_2 conditions (1600 ppmv) in their experiment [48].

It is essential to gain a further understanding of how ocean acidification drives calcification differences among *Halimeda* species, since these algal fragments eventually form carbonate deposits that may be vulnerable to dissolution under elevated pCO_2 conditions [25,49,50]. The calcification of the two *Halimeda* species in this study was adversely affected by CO_2-induced declines in pH, which was in keeping with the findings of previous studies. Campbell et al. [51] noted that the calcification rates of *H. opuntia* and *Halimeda simulans* decreased by 15% and 50%, respectively, as a result of shifts in carbonate chemistry (pCO_2 = 2400 ppmv). Similarly, Comeau et al. [52] demonstrated that the calcification rate of *Halimeda minima* declined by 11.0% as pCO_2 doubled over ambient conditions. The researchers also suggested that decreased carbonate ion availability could inhibit $CaCO_3$ deposition [51,52]. Nevertheless, variable effects have been reported for other calcifying species, which exhibited relatively minor changes in response to shifts in elevated pCO_2 conditions. Ries et al. [53] found that the calcification rates of *Halimeda incrassata* declined only in the highest pCO_2 treatment (pCO_2 = 2593 ppmv, Ω_{Ar} = 0.9), while minor responses in Ω_{Ar} value were detected ranging from 1.8 to 3.1. Thus, it appears that such distinctions of pH-influenced calcification may depend on species-specific acclimation and organismal tolerances [34]. In the current study, irradiance availability likely played a significant role in regulating OA responses for *Halimeda* species. The results showed that increased irradiance elevated calcification by a similar magnitude to pH-induced declines in calcification, which suggested that OA and moderate increased irradiance influenced calcification in opposing directions, and the effects of increased irradiance availability offset the effects of low pH. Positive effects on calcification rate were observed in two *Halimeda* species under high irradiance intensity conditions, independent of elevated pCO_2 conditions, which suggested that increased irradiance availability may regulate and mitigate the effects of OA in these calcified macroalgae [19,54]. After exposure to the experimental treatments, the amount of $CaCO_3$ in the skeleton of new segments was lower in the elevated pCO_2 treatments than ambient pCO_2 conditions (Table 3), which demonstrated lower aragonite saturation state weakened skeletal mineralogy in *Halimeda* tissues and may result in these algae being more vulnerable and palatable for higher ecological niche grazers [9]. With CO_2 enrichment, the ratio of dissolved inorganic carbon components changed and Ω_{Ar} state decreased (Table 2), which may accelerate the dissolution of calcium carbonate structures in algal tissue [45]. Similar conclusions have been reached in other comparative studies, where calcifying species with high $CaCO_3$ content displayed increased sensitivity to OA [51], highlighting the importance of Ω_{Ar} state for skeletal mineralogy (e.g. segment ultrastructure and utricle organization) [12]. In addition, previous studies suggested that non-living *Halimeda* segments may lose mass more rapidly under elevated pCO_2 conditions, which may lead to a more rapid decrease in tropical carbonate accretions of *Halimeda* spp. skeletons [25,55]. Such a decrease of $CaCO_3$ content under elevated pCO_2 conditions would result in the relative contributions of *Halimeda* spp. skeletons to tropical

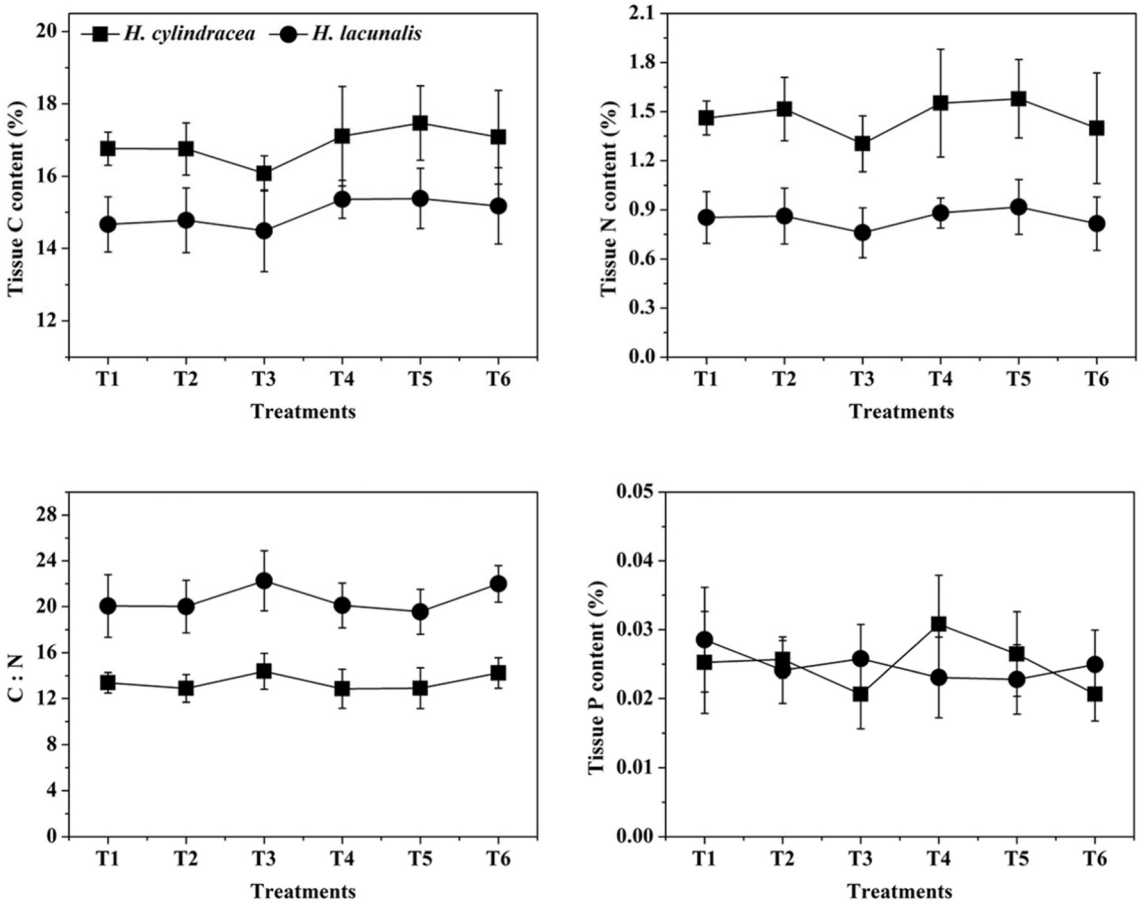

Fig. 6. Variations in tissue TC_{org}, TN, and TP content (%) and C_{org}: N ratio from the tissues of two *Halimeda* species (mean ± SD, n = 3) in six pCO_2-irradiance treatments during the experimental period.

carbonate sediment decreasing under predicted future ocean conditions [9,25].

Elevated pCO_2 in seawater is expected to enhance the growth of a diversity of marine phototrophs, such as seagrasses [56,57] and non-calcifying macroalgae [58], because of the increased availability of inorganic carbon sources for photosynthesis. However, based on the results of the current study (Fig. 4), a decline in Chl-*a* content in elevated pCO_2 treatments may reflect increases in light stress, indicating decreased chlorophyll accumulation or pigment degradation in these two *Halimeda* species [59]. Increasing carotenoid content with decreased Chl-*a* content is compatible with acclimation to high light stress as shown in Fig. 4 [19]. In this study, the variation in F_v/F_m of both species was similar, confirming that the different selected irradiance regimes exerted similar pressure on the photosynthetic apparatus of each *Halimeda* species. According to the results, changes in $CaCO_3$ content (Table 3) and/or in the internal nitrogen partitioning of the algal thallus (Fig. 6) in elevated pCO_2 treatments may affect the optical properties of the thalli and/or the antenna size of the photosystems [16,60]. The enhancement of F_v/F_m as the experiment progressed was likely driven by the increasing carotenoid content. This new organization of the photosynthetic machinery in the two *Halimeda* species implies that they are more vulnerable to light stress and elevated pCO_2, and thus, require higher carotenoid contents [16,60]. The results presented confirm that this new adjustment was appropriate for the new environmental conditions induced by the experimental treatments, as it allowed *Halimeda* to enhance photosystem II activity (increasing F_v/F_m as the experiment progressed). Notably, according to previous studies on the correlation between photosynthesis and calcification, algal calcification is energetically dependent on photosynthesis [61,62]. Photosynthesis can support a high fraction of the energetic costs of the bio-mineralization process [14]. Thus, in this study, it is proposed that high irradiance availability induced increases in photosynthesis enhanced calcification and compensated for declines in aragonite saturation state (Ω_{Ar}), which is in agreement with previous findings. Vásquez-Elizondo and Enríquez [16] pointed out that increasing light irradiance and photosynthesis could enhance coralline algal calcification rates, which is in keeping with the results of the current study. Peach et al. [25] also suggested that increased photosynthesis performance may ameliorate potential negative impacts on biological calcification processes in response to elevated pCO_2, which contributed to crystal formation and $CaCO_3$ precipitation [34].

Elevated pCO_2 is predicted to reduce growth processes on tropical reefs by decreasing the carbonate saturation state (Ω) [63,64]. Under environmental stress conditions, it is essential for plants to make various modifications to improve greater flexibility and plasticity, including multiple physiological and biochemical mechanisms [65,66]. Malondialdehyde (MDA), a secondary end product of oxidative lipid degradation, has become the choice for estimating lipid peroxidation in membrane and biological systems, which is formed through auto-oxidation and enzymatic degradation of polyunsaturated fatty acids in cells [42]. From days 4–12 of the experimental period, both *Halimeda* species, especially in T1 treatment (1600 ppmv), exhibited significantly higher lipid peroxidation, as revealed by high MDA levels, demonstrating that OA (elevated pCO_2) had a negative influence on physiological performance, which was consistent with the lower growth and calcification rates in T1. To cope with OA challenges, organic osmolytes, including proline, are secreted in abundance and work in protecting cellular structures, detoxifying enzymes and scavenging reactive

oxygen species (ROS) alone or in combination with other defense-related enzyme systems [66,67]. According to the results (Fig. 5), higher levels of proline in both *Halimeda* species were observed in elevated pCO_2 treatments, which conferred the integrity to maintain the functioning of the photosynthetic system and to protect membranes from damage [66,68]. As the experiment progressed, proline and MDA content decreased in response to OA stress (Fig. 5). It is proposed that these changes may contribute to the internal carbon and nitrogen partitioning of the algal thallus (Fig. 6) [16,60], which is in keeping with the results that showed gradually increasing F_v/F_m (Fig. 3) and carotenoid content (Fig. 4) for the two *Halimeda* species in elevated pCO_2 conditions compared with T6 treatment (400 ppmv, 180 μmol photons m^{-2} s^{-1}).

The observed differences in tissue organic carbon (TC_{org}) and nitrogen (TN) were also likely associated with physiological distinctions among treatments as shown in Table 4. In the current study, elevated pCO_2 caused an increase in tissue TC_{org} and TN accumulation. Similar conclusions have been reached in other comparative studies [9,69,70], which suggested that CO_2 enrichment had a positive effect on tissue organic carbon and nitrogen content. Although this study presents no characterization of any enzymatic activities relative to increase in tissue TC_{org} and TN, however, Hofmann et al. [9] have reported the similar conclusions and given the specific explanations. According to previous analysis, a possible reason for such shifts in TC_{org} and TN content may be due to the changes in metabolic enzyme-driven activities under elevated pCO_2 conditions [9,70–75]. Hofmann et al. [9] have reported that when the macroalgae *H. opuntia* were exposed to CO_2 enrichment, external carbonic anhydrase activity (eCAA) increased, while nitrate reductase activity (NRA) decreased. When NRA decreases, elevated CO_2 could enhance the efficiency of ammonium over nitrate, resulting in the formation of glutamine and subsequent repression of NRA [76]. Such a response would save energy due to the lower energy requirements of ammonium assimilation compared to nitrate, and allocate more energy to producing eCAA for regulating inorganic carbon and nitrogen uptake and assimilation [77,78]. Indeed, a positive correlation between high irradiance and high TC_{org} and TN levels was observed during the experimental period (Fig. 6), but was not statistically significant ($P > 0.05$) (Table 4). These values were consistent with the higher growth and calcification rates under high irradiance conditions.

In summary, the results of present study showed that elevated pCO_2 in seawater plays a critical role in influencing growth, calcification, photosynthetic and other aspects of the physiological performance of *H. cylindracea* and *H. lacunalis*. However, increased irradiance availability may serve to enhance metabolic performance, indicating its important role in mitigating the responses of primary producers from the *Halimeda* genus to OA in certain situations. Under elevated pCO_2 stress conditions, higher lipid peroxidation (MDA) levels were observed, which means that cell membranes were damaged. To protect cellular structure and metabolic stability, proline was largely secreted under high irradiance conditions to mitigate the negative influences of environmental stress. These positive results reported here can have profound consequences on *Halimeda* species, as moderate increases in irradiance availability can compromise the contribution of calcifying macroalgae to reef carbon budgets, reef cementation and the maintenance of reef biodiversity in response to OA.

CRediT authorship contribution statement

Zhangliang Wei: Investigation, Writing - original draft. **Chao Long:** Resources. **Fangfang Yang:** Conceptualization, Methodology. **Lijuan Long:** Conceptualization, Methodology. **Yuanzi Huo:** Formal analysis. **Dewen Ding:** Resources. **Jiahao Mo:** Resources.

Declaration of competing interest

1. No potential financial or other interests influenced the outcomes of this research.
2. No conflicts of interest, issues of consent, human or animal rights relating to this research have been identified.
3. This manuscript is approved by all authors for publication. The work described is original research that has not been published previously, and is not under consideration for publication elsewhere.

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References

[1] C.L. Sabine, R.A. Feely, The oceanic sink for carbon dioxide, in: D. Eds Reay, N. Hewitt, J. Grace, K. Smith (Eds.), Greenhouse Gas Sinks, CABI Publishing, Oxfordshire, 2007, pp. 31–49.
[2] J.C. Orr, V.J. Fabry, O. Aumont, et al., Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms, Nature 437 (2005) 681–686.
[3] V.J. Fabry, B.A. Seibel, R.A. Feely, J.C. Orr, Impacts of ocean acidification on marine fauna and ecosystem processes, ICES J. Mar. Sci. 65 (2008) 414–432.
[4] NOAA-CCGG, National Oceanographic and Atmospheric Administration Carbon Cycle Greenhouse Gases Group, Dr. Pieter Tans, NOAA/ESRL (www.esrl.noaa.gov/gmd/ccgg/trends/) and Dr. Ralph Keeling, Scripps Institution of Oceanography (http://scrippsco2.ucsd.edu/), (2012) (accessed May 2012).
[5] M. Koch, G. Bowes, C. Ross, X.H. Zhang, Climate change and ocean acidification effects on seagrasses and marine macroalgae, Glob. Chang. Biol. 19 (2013) 103–132.
[6] G.E. Hofmann, J.P. Barry, P.J. Edmunds, R.D. Gates, D.A. Hutchins, T. Klinger, M.A. Sewell, The effect of ocean acidification on calcifying organisms in marine ecosystems: an organism-to-ecosystem perspective, Annu. Rev. Ecol. Evol. Syst. 41 (2010) 127–147.
[7] K.R.N. Anthony, D.I. Kline, G. Díaz-Pulido, S. Dove, O. Hoegh-Guldberg, Ocean acidification causes bleaching and productivity loss in coral reef builders, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 17442–17446.
[8] U. Riebesell, I. Zondervan, B. Rost, P.D. Tortell, R.E. Zeebe, F.M.M. Morel, Reduced calcification of marine plankton in response to increased atmospheric CO_2, Nature 407 (2000) 364–367.
[9] L.C. Hofmann, J. Heiden, K. Bischof, M. Teichberg, Nutrient availability affects the response of the calcifying chlorophyte *Halimeda* opuntia (L.) J.V. Lamouroux to low pH, Planta 239 (2014) 231–242.
[10] I.B. Kuffner, A.J. Andersson, P.L. Jokiel, K.S. Rodgers, F.T. Mackenzie, Decreased abundance of crustose coralline algae due to ocean acidification, Nat. Geosci. 1 (2) (2008) 114–117.
[11] K.J. Kroeker, R.L. Kordas, R.N. Crim, G.G. Singh, Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms, Ecol. Lett. 13 (2010) 1419–1434.
[12] N.N. Price, S.L. Hamilton, J.S. Tootell, J.E. Smith, Species-specific consequences of ocean acidification for the calcareous tropical green algae *Halimeda*, Mar. Ecol. Prog. Ser. 440 (2011) 67–78.
[13] S. Martin, A. Charnoz, J.P. Gattuso, Photosynthesis, respiration and calcification in the Mediterranean crustose coralline alga *Lithophyllum Cabiochae* (Corallinales, Rhodophyta), Eur. J. Phycol. 48 (2) (2013) 163–172.
[14] F.W. Meyer, N. Schubert, K. Diele, M. Teichberg, C. Wild, S. Enríquez, Effect of inorganic and organic carbon enrichments (DIC and DOC) on the photosynthesis and calcification rates of two calcifying green algae from a Caribbean reef lagoon, PLoS One 11 (8) (2016) e0160268, , https://doi.org/10.1371/journal.pone.0160268.
[15] O. Hoegh-Guldberg, P.J. Mumby, A.J. Hooten, R.S. Steneck, P. Greenfield, E. Gomez, C.D. Harvell, P.F. Sale, A.J. Edwards, K. Caldeira, N. Knowlton, C.M. Eakin, R. Iglesias-Prieto, N. Muthiga, R.H. Bradbury, A. Dubi, M.E. Hatziolos, Coral reefs under rapid climate change and ocean acidification, Science 318 (2007) 1737–1742.
[16] R.M. Vásquez-Elizondo, S. Enríquez, Coralline algal physiology is more adversely affected by elevated temperature than reduced pH, Sci. Rep. 6 (2016) 19030.
[17] C.D. Hepburn, D.W. Pritchard, C.E. Cornwall, R.J. McLeod, J. Beardall, J.A. Raven, C.L. Hurd, Diversity of carbon use strategies in a kelp forest community: implications for a high CO_2 ocean, Glob. Chang. Biol. 17 (2011) 2488–2497.
[18] J.M. Guinotte, V.J. Fabry, Ocean acidification and its potential effects on marine ecosystems, Ann. N. Y. Acad. Sci. 1134 (2008) 320–342.
[19] M. Teichberg, A. Fricke, K. Bischol, Increased physiological performance of the calcifying green macroalga *Halimeda opuntia* in response to experimental nutrient enrichment on a Caribbean coral reef, Aquat. Bot. 104 (2013) 25–33.
[20] S.M. Stanley, J.B. Ries, L.A. Hardie, Increased production of calcite and slower growth for the major sediment-producing alga Halimeda as the Mg/Ca ratio of seawater is lowered to a "Calcite Sea" level, J. Sediment. Res. 80 (2010) 6–16.

[21] W.A. Nelson, Calcified macroalgae-critical to coastal ecosystems and vulnerable to change: a review, Mar. Freshw. Res. 60 (8) (2009) 787–801 (CSIRO Publishing).
[22] G.M. Amado-Filho, R.L. Moura, A.C. Bastos, L.T. Salgado, P.Y. Sumida, A.Z. Guth, R.B. Francini-Filho, et al., Rhodolith beds are major $CaCO_3$ bio-factories in the tropical South West Atlantic, PLoS One 7 (4) (2012) (Public library of science: e35171).
[23] C.E. Payri, *Halimeda* contribution to organic and inorganic production in a Tahitian reef system, Coral Reefs 6 (1988) 251–262.
[24] I.M. El-Manawy, M.A. Shafik, Morphological characterization of *Halimeda* (Lamouroux) from different biotopes on the Red Sea coral reefs of Egypt, Am. J. Agric. Environ. Sci. 3 (2008) 532–538.
[25] K.E. Peach, M.S. Koch, P.L. Blackwelder, C. Manfrino, Calcification and photophysiology responses to elevated pCO_2 in six *Halimeda* species from contrasting irradiance environments on Little Cayman Island reefs, J. Exp. Mar. Biol. Ecol. 486 (2017) 114–126.
[26] R.A. Feely, C.L. Sabine, J.M. Hernandez-Ayon, D. Ianson, B. Hales, Evidence for upwelling of corrosive "acidified" water onto the continental shelf, Science 320 (2008) 1490–1492.
[27] I.S. Semesi, J. Kangwe, M. Björk, Alterations in seawater pH and CO_2 affect calcification and photosynthesis in the tropical coralline alga, *Hydrolithon* sp. (Rhodophyta), Estuar. Coast. Shelf Sci. 84 (2009) 337–341.
[28] S.C. Barry, T.K. Frazer, C.A. Jacoby, Production and carbonate dynamics of *Halimeda incrassata* (Ellis) Lamouroux altered by *Thalassia testudinum* Banks and Soland ex König, J. Exp. Mar. Biol. Ecol. 444 (2013) 73–80.
[29] D.P. Manzello, Coral growth with thermal stress and ocean acidification: lessons from the eastern tropical Pacific, Coral Reefs 29 (2010) 749–758.
[30] S. Sinutok, R. Hill, M.A. Doblin, R. Wuhrer, P.J. Ralph, Warmer more acidic conditions cause decreased productivity and calcification in subtropical coral reef sediment-dwelling calcifiers, Limnol. Oceanogr. 56 (2011) 1200.
[31] B. Morton, G. Blackmore, South China Sea, Mar. Pollut. Bull. 42 (12) (2001) 1236–1263.
[32] L. Hillis-Colinvaux, Ecology and taxonomy of *Halimeda*: primary producer of coral reefs, Adv. Mar. Biol. 17 (1980) 1–327.
[33] D. Xu, C.E. Schaum, F. Lin, K. Sun, J.R. Munroe, X. Zhang, X. Fan, L. Teng, Y. Wang, Z. Zhang, N. Ye, Acclimation of bloom-forming and perennial seaweeds to elevated pCO_2 conserved across levels of environmental complexity, Glob. Chang. Biol. 23 (2017) 4828–4839.
[34] J.E. Campbell, J. Fisch, C. Langdon, V.J. Paul, Increased temperature mitigates the effects of ocean acidification in calcified green algae (*Halimeda* spp.), Coral Reefs 35 (2016) 357–368.
[35] K. Beach, L. Walters, P. Vroom, C. Smith, J. Coyer, C. Hunter, Variability in the ecophysiology of Halimeda spp. (Chlorophyta, Bryopsidales) on Conch Reef, Florida Keys, USA, J. Phycol. 39 (2003) 633–643.
[36] IPCC, Climate change 2013: the physical science basis, in: T.F. Stocker, D. Qin, G.K. Plattner, M. Tignor, S.K. Allen, Nauels A. Boschung, Y. Xia, V. Bex, P.M. Midgley (Eds.), Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 2013, p. 1535.
[37] K. Caldeira, M.E. Wickett, Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean, J. Geophys. Res. 110 (2005), https://doi.org/10.1029/2004JC002671. C09S04.
[38] D. Pierrot, E. Lewis, D.W.R. Wallace, MS Excel Program Developed for CO_2 Systems Calculations: ORNL/CDIAC-105a, Carbon Dioxide Information Analysis Center Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee, 2006.
[39] Z.L. Wei, Y.Z. Huo, Q. Liu, F.F. Yang, L.J. Long, H.S. Bi, C.L. Fan, P.M. He, A field scale evaluation of *Gracilaria lemaneiformis* co-cultured with *Crassostrea gigas* as a nutrient bioextraction strategy in Yantian Bay, China, Algal Res. 38 (2019) 101407.
[40] R.J. Ritchie, Universal chlorophyll equations for estimating chlorophylls a, b, c, and d and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents, Photosynthetica 46 (2008) 115–126.
[41] D.P. Shan, J.G. Huang, Y.T. Yang, Y.H. Guo, C.A. Wu, G.D. Yang, Z. Gao, C.C. Zheng, Cotton GhDREB1 increases plant tolerance to low temperature and is negatively regulated by gibberellic acid, New Phytol. 176 (2007) 70–81.
[42] D.M. Hodges, J.M. DeLong, C.F. Forney, R.K. Prange, Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds, Planta 207 (1999) 604–611.
[43] F. Koroleff, Determination of phosphorus, in: K. Grasshoff, M. Ehrhardt, F. Kremling (Eds.), Methods of Seawater Analysis, Weinheim, Verlag chemie, 1983, pp. 125–139.
[44] C.E. Cornwall, C.D. Hepburn, D. Pritchard, K.I. Currie, C.M. McGraw, K.A. Hunter, C.L. Hurd, Carbon-use strategies in macroalgae: differential responses to lowered pH and implications for ocean acidification, J. Phycol. 48 (2012) 137–144.
[45] Z.L. Wei, J.H. Mo, Q.J. Hu, R.F. Yan, C. Long, D.W. Ding, F.F. Yang, L.J. Long, The physiological performance of the calcifying green macroalga *Halimeda* species in response to ocean acidification with irradiance variability, Mar. Sci. Bull. 38 (05) (2019) 574–584 (In Chinese with English abstract).
[46] P. Li, H.H. Su, W.C. Guan, The effect of high CO_2 concentrations and UVR on growth and photochemical efficiency in *Amphiroa* sp. (Rhodophyta) and *Cladophora* sp. (Chlorophyta), Acta Hydrobiol. Sin. 38 (04) (2014) 636–641 (In Chinese with English abstract).
[47] J.E. Smith, C.M. Smith, P.S. Vroom, K.L. Beach, S. Miller, Nutrient and growth dynamics of *Halimeda tuna* on Conch Reef, Florida Keys: possible influence of internal tides on nutrient status and physiology, Limnol. Oceanogr. 49 (2004) 1923–1936.
[48] L.C. Hofmann, K. Bischof, C. Baggini, A. Johnson, K. Koop-Jakobsen, M. Teichberg, CO_2 and inorganic nutrient enrichment affect the performance of a calcifying green alga and its non-calcifying epiphyte, Oecologia 177 (2015) 1157–1169.
[49] K.E. Fabricius, C. Langdon, S. Uthicke, C. Humphrey, S. Noonan, G. De'ath, R. Okazaki, N. Muehllehner, M.S. Glas, J.M. Lough, Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations, Nat. Clim. Chang. 1 (2011) 165–169.
[50] A. Wizemann, F.W. Meyer, L.C. Hofmann, C. Wild, H. Westphal, Ocean acidification alters the calcareous microstructure of the green macro-alga *Halimeda opuntia*, Coral Reefs 34 (2015) 941–954.
[51] J. Campbell, J. Craft, N. Muehllehner, C. Langdon, V. Paul, Responses of calcifying algae (*Halimeda* spp.) to ocean acidification: implications for herbivores, Mar. Ecol. Prog. Ser. 514 (2014) 43–56.
[52] S. Comeau, P.J. Edmunds, N.B. Spindel, R.C. Carpenter, The responses of eight coral reef calcifiers to increasing partial pressure of CO_2 do not exhibit a tipping point, Limnol. Oceanogr. 58 (2013) 388–398.
[53] J.B. Ries, A.L. Cohen, D.C. McCorkle, Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification, Geology 37 (2009) 1131–1134.
[54] P.S. Vroom, C.M. Smith, J.A. Coyer, L.J. Walters, C.L. Hunter, K.S. Beach, J.E. Smith, Field biology of *Halimeda tuna* (Bryopsidales, Chlorophyta) across a depth gradient: comparative growth, survivorship, recruitment, and reproduction, Hydrobiologia 501 (2003) 149–166.
[55] A. Wizemann, F.W. Meyer, H. Westphal, A new model for the calcification of the green macro-alga *Halimeda opuntia* (Lamouroux), Coral Reefs 33 (2014) 951–964.
[56] F.T. Short, H.A. Neckles, The effects of global climate change on seagrasses, Aquat. Bot. 63 (1999) 169–196.
[57] S.L. Palacios, R.C. Zimmerman, Response of eelgrass *Zostera marina* to CO_2 enrichment: possible impacts of climate change and potential for remediation of coastal habitats, Mar. Ecol. Prog. Ser. 344 (2007) 1–13.
[58] K. Gao, Y. Aruga, K. Asada, M. Kiyohara, Influence of enhanced CO_2 on growth and photosynthesis of the red algae *Gracilaria* sp. and *G. chilensis*, J. Appl. Phycol. 5 (1993) 563–571.
[59] S. Sinutok, R. Hill, M.A. Doblin, M. Kühl, P.J. Ralph, Microenvironmental changes support evidence of photosynthesis and calcification inhibition in *Halimeda* under ocean acidification and warming, Coral Reefs 31 (2012) 1201–1213.
[60] R.M. Vásquez-Elizondo, S. Enríquez, Light absorption in coralline algae (Rhodophyta): a morphological and functional approach to understanding species distribution in a coral reef lagoon, Front. Mar. Sci. 4 (2017) 297.
[61] J.R.M. Chisholm, Calcification by crustose coralline algae on the northern Great Barrier Reef, Australia, Limnol. Oceanogr. 45 (2000) 1476–1484.
[62] J.R.M. Chisholm, Primary productivity of reef-building crustose coralline algae, Limnol. Oceanogr. 48 (2003) 1376–1387.
[63] P.L. Jokiel, Predicting the impact of ocean acidification on coral reefs: evaluating the assumptions involved, ICES J. Mar. Sci. (2015), https://doi.org/10.1093/icesjms/fsv091.
[64] R.A. Dixon, N.L. Paiva, Stress-induced phenylpropanoid metabolism, Plant Cell 7 (1995) 1085–1097.
[65] M.R. Hemm, S.D. Rider, J. Ogas, D.J. Murry, C. Chapple, Light induces phenylpropanoid metabolism in Arabidopsis roots, Plant J. 38 (2004) 765–778.
[66] L. Xiong, K.S. Schumaker, J.K. Zhu, Cell signaling during cold, drought, and salt stress, Plant Cell 14 (2002) S165–S183.
[67] D.P.S. Verma, Osmotic stress tolerance in plants: role of praline and sulfur metabolisms, in: K. Shinozaki, K. Yamaguchi-Shinozaki (Eds.), Molecular Responses to Cold, Drought, Heat and Salt Stress in Higher Plants, R.G. Landes Company, Austin, TX, 1999, pp. 153–168.
[68] Y.G. Sun, B. Wang, S.H. Jin, X.X. Qu, Y.J. Li, B.K. Hou, Ectopic expression of Arabidopsis glycosyltransferase *UGT85A5* enhances salt stress tolerance in tobacco, PLoS One 8 (3) (2013) e59924, https://doi.org/10.1371/journal.pone.005992.
[69] B.B. Chen, D.H. Zou, H. Du, Z.W. Ji, Carbon and nitrogen accumulation in the economic seaweed *Gracilaria lemaneiformis* affected by ocean acidification and increasing temperature, Aquaculture 482 (2018) 176–182.
[70] B.B. Chen, D.H. Zou, Y.F. Yang, Increased iron availability resulting from increased CO_2 enhances carbon and nitrogen metabolism in the economical marine red macroalga *Pyropia haitanensis* (Rhodophyta), Chemosphere 173 (2017) 444–451.
[71] M. Geiger, V. Haake, F. Ludewig, U. Sonnewald, M. Stitt, The nitrate and ammonium nitrate supply have a major influence on the response of photosynthesis, carbon metabolism, nitrogen metabolism and growth to elevated carbon dioxide in tobacco, Plant Cell Environ. 22 (1999) 1177–1199.
[72] A. Alexandre, J. Silva, P. Buapet, M. Björk, R. Santos, Effects of CO_2 enrichment on photosynthesis, growth, and nitrogen metabolism of the seagrass *Zostera noltii*, Ecol. Evol. 2 (2012) 2625–2635.
[73] F.J. Gordillo, F.X. Niell, F.L. Figueroa, Non-photosynthetic enhancement of growth by high CO_2 level in the nitrophilic seaweed *Ulva rigida* C. Agardh (Chlorophyta), Planta 213 (2001) 64–70.
[74] F.J. Gordillo, J. Aguilera, C. Jiménez, The response of nutrient assimilation and biochemical composition of Arctic seaweeds to a nutrient input in summer, J. Exp. Bot. 57 (2006) 2661–2671.
[75] L.C. Hofmann, G. Yildiz, D. Hanelt, K. Bischof, Physiological responses of the calcifying rhodophyte, *Corallina officinalis* (L.), to future CO_2 levels, Mar. Biol. 159 (2012) 783–792.
[76] M. Stitt, A. Krapp, The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background, Plant Cell Environ. 22 (1999) 583–621.
[77] M. Losada, M.G. Guerrero, The Photosynthetic Reduction of Nitrate and Its Regulation. Photosynthesis in Relation to Model Systems, Elsevier, Amsterdam, 1979, pp. 365–408.
[78] P.J. Syrett, Nitrogen metabolism of microalgae, Can. Bull. Fish. Aquat. Sci. 210 (1981) 182–210.