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Interactive effects of oyster and seaweed on seawater dissolved inorganic carbon systems: implications for integrated multi-trophic aquaculture

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ABSTRACT: We examined the separate effect of Portuguese oyster *Crassostrea angulata* and the interactive effects of oyster and red seaweed *Gracilaria lemaneiformis* on seawater dissolved inorganic carbon (DIC) systems and the air–sea CO_2 flux (F_{CO2}) in Daya Bay, southern China. Respiration and calcification rates of oysters were measured and the effects of oyster aquaculture on marine DIC systems were evaluated. The interactive effects on seawater DIC and air–sea F_{CO2} were examined using mesocosms containing oyster and seaweed assemblages. Results showed populations of *C. angulata* cultured in Daya Bay sequestered ca. 258 g C m^{-2} yr^{-1} for shell formation, whereas the CO_2 released due to respiration and calcification was 349 and 153 g C m^{-2} yr^{-1}, respectively. This indicates that oyster cultivation in Daya Bay is a CO_2 generator, favoring the escape of CO_2 into the atmosphere. DIC, HCO_3^- and CO_2 concentrations and the partial pressure of CO_2 in oyster–seaweed co-cultured mesocosms were significantly lower than the oyster monoculture mesocosm. These results indicated that *G. lemaneiformis* effectively absorbs the CO_2 released by oysters. The negative values of air–sea F_{CO2} in the co-culture groups represent a CO_2 sink from the atmosphere to the sea. These results demonstrated that there could be an interspecies mutual benefit for both *C. angulata* and *G. lemaneiformis* in the integrated culture system. Considering that photosynthesis of seaweed is carbon limited, we suggest that the 2 species are co-cultured at a ratio of ca. 4:1 (based on fresh weight) for efficient utilization of DIC in seawater by *G. lemaneiformis*, and further to increase the ocean CO_2 sink.

KEY WORDS: *Crassostrea angulata* · *Gracilaria lemaneiformis* · Daya Bay · Dissolved inorganic carbon · Integrated multi-trophic aquaculture · IMTA · Air–sea CO_2 flux

INTRODUCTION

Calcification of aquatic animals such as shellfish (e.g. oyster, scallop, and clam) is also a source of CO_2 (Chauvaud et al. 2003, Martin et al. 2006, Mistri & Munari 2013, Munari et al. 2013, Jiang et al. 2014). Shellfish utilize carbon in 2 ways. First, they consume organic carbon to sustain their growth and metabolism, following the reaction $CH_2O + O_2 \rightarrow CO_2 + H_2O$. Second, they use HCO_3^- from seawater to generate $CaCO_3$ shells, based on the reaction $Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 + CO_2 + H_2O$. These 2 processes both lead to net CO_2 production in ocean waters. Second, shellfish secrete calcium carbonate ($CaCO_3$) to form their skeletal material. This process acts as a marine biological pump by removing CO_2 from circu-

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lation and storing carbon in the ocean (Lerman & Mackenzie 2005). In fact, the ratio of released CO_2/precipitated $CaCO_3$ is largely dependent on the buffering capacity of the surrounding seawater, such that in some marine ecosystems, the ratio could be ca. 0.6 (Frankignoulle et al. 1995). It is reasonable that the buffering capacity of seawater might vary significantly among different waters with variations in pH, alkalinity, salinity, and temperature (Millero 1995, Lerman & Mackenzie 2005, Dickson 2010, Mackenzie & Andersson 2013)

Seaweeds (e.g. *Saccharina*, *Gracilaria*) are intricately involved as primary producers in coastal ecosystems. They assimilate inorganic carbon either via diffusion (for CO_2), or active uptake of HCO_3^- using carbon-concentration mechanisms. During photosynthesis, these mechanisms result in an increase in seawater pH and a drop in seawater CO_2 partial pressure (pCO_2) (Han et al. 2013). Therefore, seaweed could induce a significant shift in seawater dissolved inorganic carbon (DIC) systems according to the following formula: $CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-}$. Therefore, seaweed may exert a significant impact on the DIC buffering capacity of seawater.

As mentioned above, both shellfish and seaweed can change the seawater DIC system and the buffering capacity. One implication is a complex interspecies interaction between shellfish and seaweed in co-cultured systems. For example, CO_2 or HCO_3^- can become a major limiting factor affecting the photosynthetic rates and aquaculture production of seaweed, particularly when they are grown under conditions of high biomass densities and reduced seawater motion (Zou et al. 2004). Likewise, the alteration in DIC speciation can cause responses in calcifying organisms (e.g. oyster), thereby potentially affecting their growth and physiological functions (Ho & Carpenter 2017, Scanes et al. 2017). Thus, the interaction between shellfish and seaweed and their combined effect on DIC partitioning and cycling still needs to be investigated using an ecosystem approach. Similarly, few studies have been conducted to elucidate the influence of integrated aquaculture of shellfish and seaweed on variations in DIC systems, as well as the air–sea CO_2 flux. Furthermore, the optimum culture ratio in co-culture systems for obtaining the largest CO_2 sink is not known.

Shellfish and seaweed mariculture in the coastal waters of China has been growing rapidly over the past 3 decades; they are by far the largest and most well-known aquaculture industries in the world, with an annual production of ca. 13.6×10^6 t and 2.1×10^6 t, accounting for ca. 72.4% and 11.1% of the total mariculture production in China, respectively (China Bureau of Fisheries 2016). In most coastal waters, shellfish and seaweed are co-cultured, using suspended longlines as the main cultivation method. In fact, they usually dominate an entire bay, such as in Sanggou Bay (Fang et al. 2016) and in Daya Bay (Yu et al. 2014) where this study was conducted.

In the present study, we conducted an *in situ* mesocosm experiment to measure the calcification and respiration rates of the Portuguese oyster *Crassostrea angulata* to evaluate its effect on marine DIC systems. In addition, the role of the red seaweed *Gracilaria lemaneiformis* was assayed for elimination of CO_2 in seawater. Subsequently, we investigated the impact of co-culture interactions on DIC systems and the air–sea CO_2 flux using different ratios. The results from this study will be useful in evaluating the effects of large-scale coastal aquaculture of oysters and seaweed on the marine CO_2 budget with the hope of finding methods of carbon removal from coastal waters.

MATERIALS AND METHODS

Study site

Daya Bay, located in Guangdong Province, southern China, is a 600 km^2 semi-enclosed embayment in the northeast South China Sea (Fig. 1). The average water depth is 10 m (range: 6–20 m). The annual mean air temperature is 22°C. The minimum sea surface temperature occurs in winter (15°C) and the maximum in summer and fall (30°C). The bay is one of the most intensive culture areas in China. The Portuguese oyster *Crassostrea angulata* is the main cultured bivalve species. Suspension aquaculture of *C. angulata* has been practiced for over 3 decades, with an estimated standing stock of 6.6×10^4 t in 2016. The seaweed *Gracilaria lemaneiformis* is another important cultured species, with a production of ca. 27×10^4 t in China in 2015 (China Bureau of Fisheries 2016).

Estimation of calcification and respiration rate of *C. angulata*

Experimental *C. angulata* were collected from Daya Bay in April 2016 and taken to the laboratory in a temperature-controlled case within 1 h. After arrival, animals were disinfected, and any visible fouling organisms on shell surfaces were cleaned by

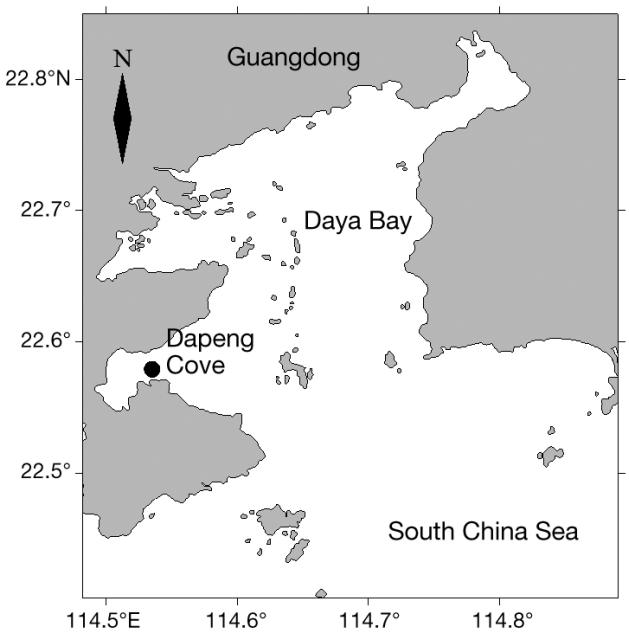

Fig. 1. Location of the study area (●) in Daya Bay

washing with filtered seawater. Approximately 30 oysters of similar sizes (7–8 cm shell height) were acclimatized to laboratory conditions for 1 wk in a 50 l tank with aerated seawater. During acclimation, seawater was changed once per day and oysters were fed daily with 2×10^4 cell ml^{-1} *Chaetoceros* sp. at 08:00 h. During the trial, temperature (T), salinity (S), and pH were 22 ± 1°C, 30.8 ± 0.1, and 8.03 ± 0.02, respectively.

At the end of the acclimatization period, the oysters were placed in closed 20 l transparent polyethylene plastic mesocosms filled with seawater. The mesocosms were hung from a suspended longline so that the experimental oysters were at a depth of ca. 2 m, corresponding to the routine culture depth for oysters. A factorial design was used to test the effects of 3 stocking densities: i.e. low, medium, and high (ca. 1, 5, and 10 g (fresh weight, FW) oyster l^{-1}, respectively) on water pH, dissolved oxygen (DO), total alkalinity (TA), DIC, and carbonate ion (CO_3^{2-}) concentrations. Water samples were taken at 0 and 4 h. T and S were measured using a multi-parameter water quality meter (YSI Professional Plus 6600, Yellow Springs Instrument Company). pH was measured using a pH meter (Thermo Scientific Orion 320P-01, Thermo Electron Corporation) calibrated on the US National Bureau of Standards scale. The precision of pH measurements was ±0.01 pH units. Oxygen concentrations were determined by the Winkler method (Strickland & Parson 1972). TA was measured using an 848 Titrino plus automatic titrator (Metrohm) on 100 ml GF/F filtered samples. The accuracy of measurements was ±2 µmol l^{-1}. DIC and CO_3^{2-} concentrations were computed from T, S, pH, and TA using the CO_2_SYS_XLS calculation program (Pierrot et al. 2006). The dissociation constants for carbonic acid (K_1, K_2) were from Mehrbach et al. (1973) as refitted by Dickson & Millero (1987), the dissociation constant for bisulfate ion (K_{HSO4}) was obtained from Dickson (1990), and the dissociation constant for boric acid (K_B) was from Uppstrom (1974).

CO_2 respiratory rate (R; µmol FW g^{-1} h^{-1}) can be expressed as follows:

$$\Delta DIC = DIC_f - DIC_i \quad (1)$$

$$R = \frac{(2\Delta DIC + TA_i - TA_f) \times V}{2 \times t \times M} \quad (2)$$

where ΔDIC is the net change in DIC concentration (µmol l^{-1}), which was caused by the interaction between calcification and respiration. DIC_i and DIC_f were the initial and final DIC concentration (µmol l^{-1}), respectively. V is the incubation chamber volume (l), t is the experimental time (h), and M is the fresh weight of experimental oyster (g). TA_i and TA_f were the initial and final TA concentration (µmol l^{-1}), respectively.

For $CaCO_3$ production, 30 oysters were sampled, and the dry weight (DW) of oyster shell was determined by drying at 80°C till constant weight (±0.01 g). Bivalve shells largely consist (95%) of $CaCO_3$, and the remaining 5% are made up by magnesium, β-chitin, and various glycoproteins (Goulletquer & Wolowicz 1989). Shell DWs were corrected accordingly. The calcimass (g $CaCO_3$ m^{-2}) was estimated by the shell DWs per m^2 and its $CaCO_3$ content. Dry tissue weight was calculated for each individual using the ash-free dry weight (AFDW) method: oyster soft tissue was dried at 80°C (72 h) and then ashed at 500°C (4 h), with tissue weight computed as the difference between the 2 weights.

The CO_2 released from *C. angulata* respiration was estimated using: (1) the relation established by Schwinghamer et al. (1986): $\log_{10} R = 0.367 + 0.993 \log_{10} P$, where P and R are production and respiration (kcal m^{-2} yr^{-1}), respectively; (2) the 22.79 J mg AFDW^{-1} conversion factor for bivalve energetic content (Rumohr et al. 1987); (3) the conversion 1 J = 0.239 cal (Peters 1983); and (4) the conversion 1 g C = 11.4 kcal (Chauvaud et al. 2003).

The monthly ratio of CO_2 released to $CaCO_3$ precipitated (Ψ) was estimated as a function of the water temperature, measured with a YSI meter. CO_2 fluxes due to calcification were calculated using a Ψ value

estimated by computing the temperature according to: $\Psi = 0.8–8.3 \times 10^{-3} T$, where T is the water temperature (°C), and assuming pCO_2 = 350 µatm (Fankignoulle et al. 1994).

Deployment of *in situ* mesocosm experiment

The *in situ* mesocosm experiment was carried out from 20 to 22 April 2016 in Daya Bay (22° 34′ N, 114° 32′ E) (Fig. 1). The cylindrical mesocosms (1 m diameter × 1.5 m height) were made from transparent polyethylene plastic and were hung on suspended longlines with the top ca. 2.0 m below the water surface. Fifteen cylindrical mesocosms (450 l) were deployed over 24 h periods and consisted of 5 treatments each with 3 replicates (Table 1). One treatment with only seawater served as the control (C), the second treatment contained only oysters (oyster only, O), and the other 3 treatments were co-culture systems, with 3 oyster:seaweed ratios, i.e. 8:1, 4:1, and 2:1 (based on FW of oyster and seaweed, referred to as OS_8:1, OS_4:1 and OS_2:1, respectively). After filling with natural seawater, the mouths of the mesocosms were tied using ropes. A pipe was placed in the tied site and maintained for 24 h to keep DIC concentration in equilibrium with air due to water mixing with air. Oysters and seaweed were coiled into 100-cm-long ropes and placed in the mesocosms. The ropes were suspended using thin ropes and tied to the mouth of the mesocosm, such that the seaweed thalli were positioned vertically around the oysters. The mesocosms were immobilized using a set of ropes connected top-side to a float and submerged under the water surface.

The experiment began at 10:00 h and lasted for 24 h. Water T, S, DO, pH, and TA were measured at the beginning and end of the experiment. Parameters for the seawater DIC system and aqueous pCO_2 were calculated by the CO2_SYS_XLS calculation program (Pierrot et al. 2006).

The sea–air CO_2 fluxes (F_{CO2}) were calculated based on the following equation: $F_{CO2} = k \times \alpha \times \Delta pCO_2$, where k (cm h^{-1}) is the gas exchange coefficient of CO_2. We computed k using the parameterization given by Wanninkhof & McGillis (1999) that uses short-term winds $k = 0.0283 u_{10}^3 (Sc/660)^{-1/2}$. u_{10} stands for the wind speed at a 10 m height from the water surface level (m s^{-1}) and Sc is the Schmidt number calculated according to the relationship proposed by Wanninkhof (1992). α (mol kg^{-1} atm^{-1}) is the solubility coefficient of CO_2 calculated after Weiss (1974). ΔpCO_2 is the pCO_2 difference between surface seawater and the atmosphere. In this study, the value of atmospheric pCO_2 was downloaded from www.cmdl.noaa.gov (National Oceanographic and Atmospheric Administration, NOAA, Climate and Meteorological Diagnostics Laboratory) and corrected for water vapor pressure (Takahashi et al. 2002). Positive magnitudes of F_{CO2} indicate a flux from water to air and vice versa.

Table 1. Overview of the co-culture systems in the 5 treatments, with oysters and seaweed retained in mesocosms (g fresh weight per mesocosm, mean ± SD)

Treatment (abbreviation)	Oyster	Seaweed
Control (C)	–	–
Oyster (O)	4402 ± 141	–
Oyster:seaweed 8:1 (OS_8:1)	4418 ± 126	583 ± 27
Oyster:seaweed 4:1 (OS_4:1)	4750 ± 102	1103 ± 68
Oyster:seaweed 2:1 (OS_2:1)	4631 ± 150	2210 ± 115

The net oxygen production rate by *G. lemaneiformis* in co-culture systems was determined based on the DO concentrations in experimental mesocosms, as:

$$\text{Net oxygen production rate } (\mu\text{mol } O_2\ g^{-1}\ FW\ h^{-1}) = (C_{os} - C_o) \times V/FW/t, \quad (3)$$

where C_{os} and C_o are the DO concentrations (µmol l^{-1}) of oyster–seaweed co-culture mesocosms and oyster-only mesocosm, respectively, at the end of the experiment. V is the volume of mesocosms (l), FW is the fresh weight of *G. lemaneiformis* (g), and t is the duration of the experiment (h).

Statistical analysis

Data were analyzed by 1-way ANOVA. All data were graphically assessed for normality and homogeneity of residuals (Faraway 2002). When overall differences were significant at the 0.05 level, Tukey's HSD multiple range test was used to compare the mean values of individual groups. Data are reported as means ± SE (n = 3). All statistical tests were performed using SPSS 17.0 for Windows.

RESULTS

Calcification and respiration rates of *Crassostrea angulata*

As shown in Fig. 2, after a 4 h incubation, the seawater pH, TA, and CO_3^{2-} concentrations in the closed

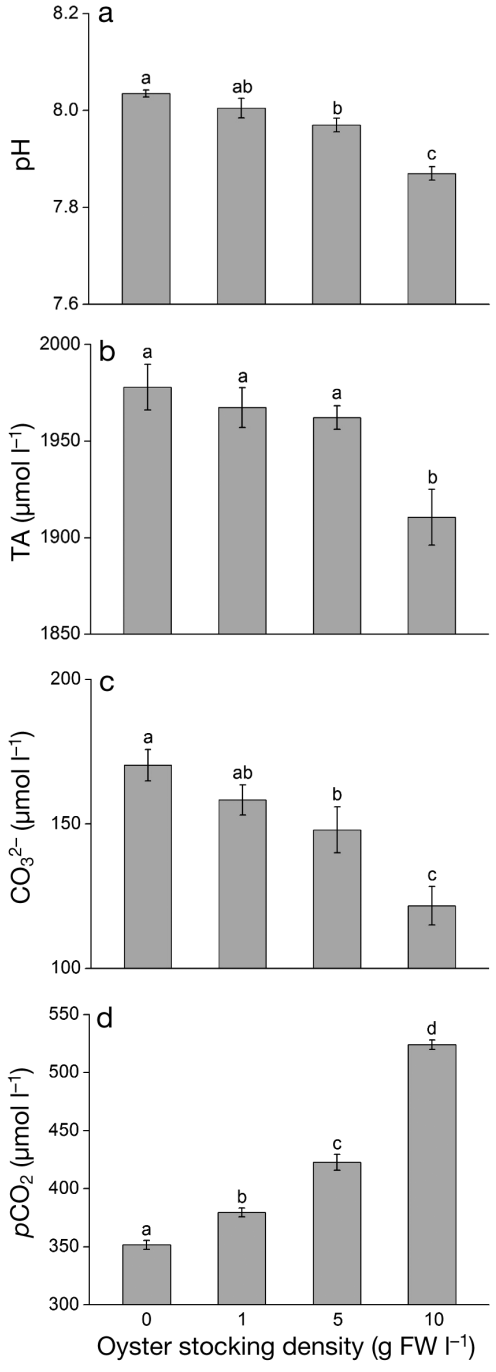

Fig. 2. Effects of Portuguese oyster *Crassostrea angulata* stocking density on (a) pH, (b) total alkalinity (TA), (c) CO_3^{2-}, and (d) pCO_2 of seawater in the closed mesocosm systems. Bars with different lowercase letters are significantly different (ANOVA, $p < 0.05$)

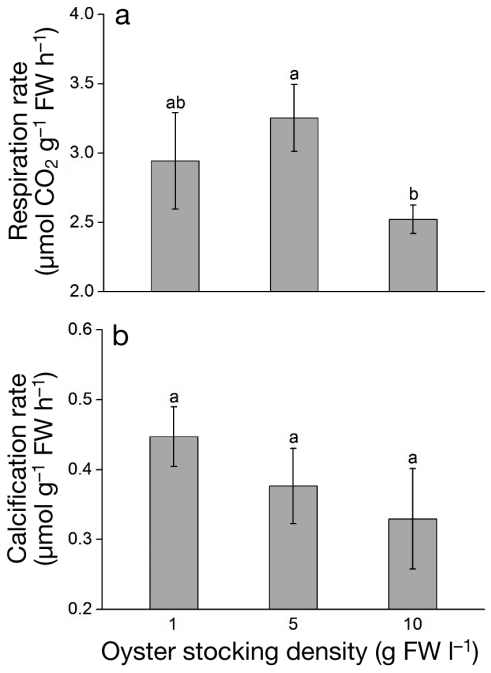

Fig. 3. (a) Respiration and (b) calcification rate of the Portuguese oyster *Crassostrea angulata* at different stocking densities. Bars with different lowercase letters are significantly different (ANOVA, $p < 0.05$)

mesocosms gradually decreased with increasing oyster stocking density. Values in the highest stocking density group were significantly lower than the other groups ($p < 0.05$) (Fig. 2a–c). The pCO_2 followed the converse general pattern, and values were significantly different between each treatment ($p < 0.05$) (Fig. 2d).

The lowest and highest respiration rates of oysters were found in the high- and medium-density groups, respectively, and were significantly different ($p < 0.05$) (Fig. 3a). Calcification rates of oysters decreased with increasing stocking density, but no significant difference occurred ($p > 0.05$) (Fig. 3b).

The total $CaCO_3$ production by the *C. angulata* population in Daya Bay was estimated to be ca. 2150 g $CaCO_3$ m^{-2} yr^{-1}. *C. angulata* sequestered 258 g C m^{-2} yr^{-1} for shell formation. The ratio of CO_2 released to $CaCO_3$ precipitated (Ψ) ranged from 0.54 to 0.65 and varied monthly with water temperature variation in Daya Bay (Table 2). CO_2 released due to calcification and respiration was 153 and 349 g C m^{-2} yr^{-1}, respectively.

Variations of seawater pH and TA in different mesocosms

As shown in Fig. 4a, the seawater pH differed significantly among different mesocosms ($p < 0.05$). pH was lowest in the oyster-only treatment, significantly lower than that of the control and the oyster–seaweed co-culture groups ($p < 0.05$). pH gradually increased

Table 2. Mean monthly water temperature (T, °C) and corresponding molar ratio (Ψ) in Daya Bay

Month	T	Ψ
January	18.3	0.65
February	18.2	0.65
March	18.7	0.64
April	22.4	0.61
May	24.5	0.59
June	29.0	0.56
July	31.3	0.54
August	29.7	0.55
September	29.5	0.55
October	28.3	0.56
November	27.6	0.57
December	19.2	0.64
Mean ± SD	24.7 ± 5.1	0.59 ± 0.04

with increasing seaweed density in co-culture treatments, and was significantly higher than that of the control ($p < 0.05$). TA was highest in the control, significantly higher than that of the other groups ($p < 0.05$) (Fig. 4b). TA values in OS_4:1 and OS_2:1 co-culture groups were significantly lower than that of the other groups ($p < 0.05$) (Fig. 4b).

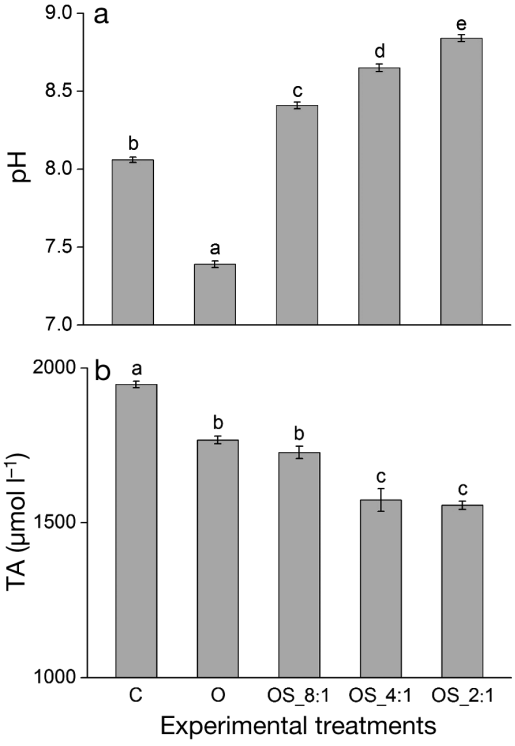

Fig. 4 (a) Seawater pH and (b) total alkalinity (TA) of oyster *Crassostrea angulata* monoculture and oyster–seaweed *Gracilaria lemaneiformis* co-culture mesocosms. C: control; O: oyster only; OS_8:1, OS_4:1, and OS_2:1: oyster co-cultured with seaweed at ratios of 8:1, 4:1, and 2:1, respectively. Bars with different lowercase letters are significantly different (ANOVA, $p < 0.05$)

Variations of seawater DIC systems and pCO_2 in different mesocosms

After 24 h incubation, DIC, HCO_3^-, and CO_2 concentrations and pCO_2 showed similar trends among treatments (Fig. 5a,b,d,e). The highest values were found in the oyster-only group, and then continuously decreased with increasing seaweed density in co-culture groups. Co-culture with seaweed lead to a significant decrease of CO_2 concentration and pCO_2 ($p < 0.05$). The degree of reduction was positively correlated with the seaweed density (Fig. 5d,e). CO_3^{2-} concentration followed the converse general pattern to HCO_3^- concentrations (Fig. 5c).

Variations of air–sea CO_2 flux in different mesocosms

The air–sea CO_2 flux (F_{CO2}) in the oyster-only treatment group had a high and positive value (110.4 ± 10.5 mmol m^{-2} d^{-1}), representing a CO_2 source to the atmosphere, and was significantly higher than in the other groups ($p < 0.05$). In contrast, the negative values (from −[8.4 ± 0.7] to −[33.6 ± 4.0] mmol m^{-2} d^{-1}) in the control and co-culture groups represent a CO_2 sink from the atmosphere to the sea, where the degree of CO_2 sink was proportional to seaweed stocking density; there was no significant difference in F_{CO2} between the oyster–seaweed (OS_8:1) co-culture and the control ($p < 0.05$), but the F_{CO2} values in OS_4:1 and OS_2:1 groups were significantly lower than that in the control ($p > 0.05$) (Fig. 6).

Oxygen production rate of *Gracilaria lemaneiformis* in co-culture mesocosms

In oyster–seaweed co-culture mesocosms, although the DO was mainly produced by *G. lemaneiformis*, the phytoplankton also produced some oxygen. Therefore, the oxygen concentration in oyster–seaweed co-culture mesocosms (Fig. 7) was refitted by the oxygen concentration in the control and oyster-only mesocosms. The net oxygen production rates in the low- (OS_8:1) and medium- (OS_4:1) seaweed-density treatments were significantly ($p < 0.05$) higher than that in the high-seaweed-density group (OS_2:1).

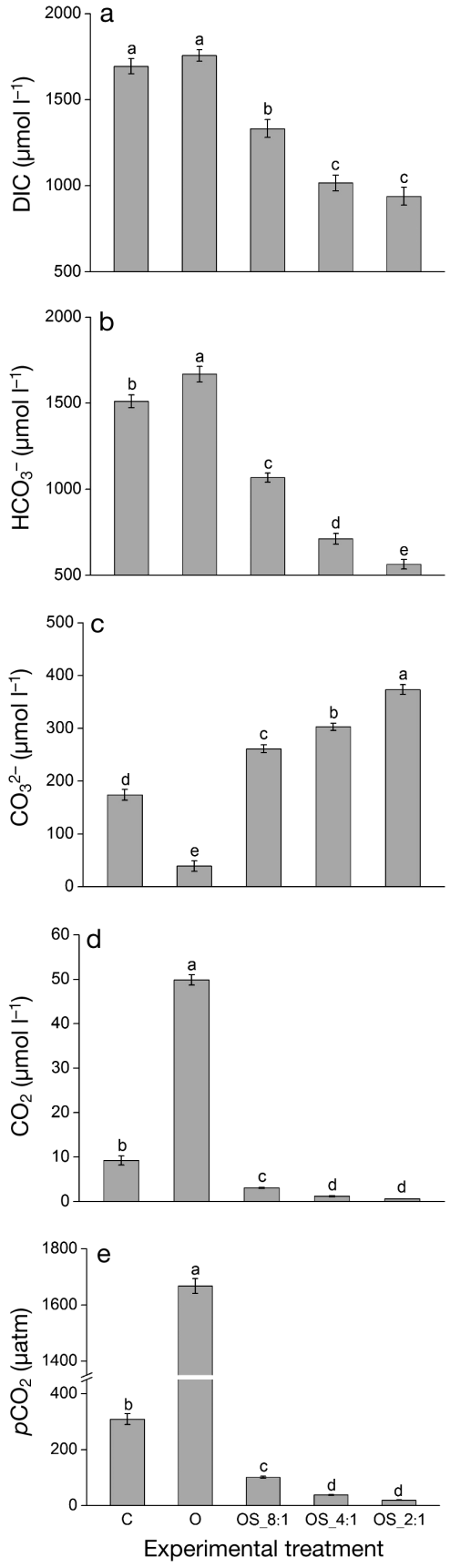

Fig. 5 Seawater concentrations of (a) dissolved inorganic carbon (DIC), (b) HCO_3^-, (c) CO_3^{2-}, (d) aqueous CO_2, and (e) pCO_2 in oyster *Crassostrea angulata* monocultures and oyster–seaweed *Gracilaria lemaneiformis* co-culture mesocosms (see Table 1 for treatments). Bars with different lowercase letters are significantly different (ANOVA, $p < 0.05$)

DISCUSSION

The results of the present study indicated that the oyster *Crassostrea angulata* cultivated in Daya Bay seems to be a CO_2 generator, as pCO_2 increased in oyster-only culture mesocosms. Oyster harvesting sequesters ca. 258 g C m^{-2} yr^{-1} due to shell formation in Daya Bay. In contrast, the CO_2 fluxes due to respiration and calcification were ca. 349 and 153 g C m^{-2} yr^{-1}, respectively, accounting for 69.5% and 30.5% of the total CO_2 fluxes (502 g C m^{-2} yr^{-1}), respectively.

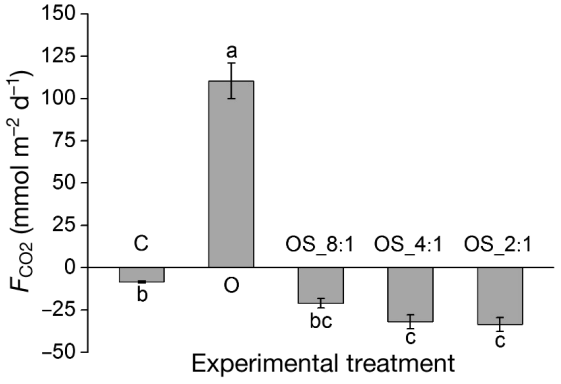

Fig. 6. Variations in air–sea CO_2 flux (F_{CO2}) among different experimental mesocosms. C: control; O: oyster only; OS_8:1, OS_4:1, and OS_2:1: oyster co-cultured with seaweed at ratios of 8:1, 4:1, and 2:1, respectively. Bars with different lowercase letters are significantly different (ANOVA, $p < 0.05$)

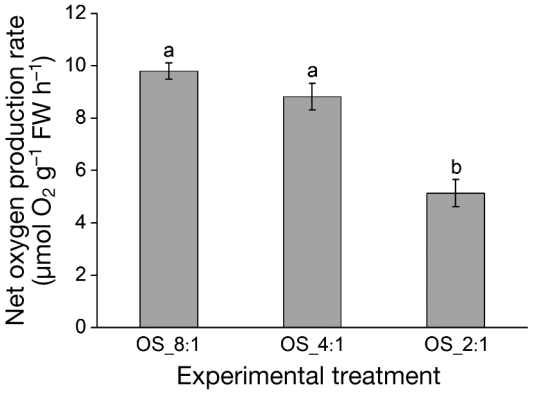

Fig. 7. Net oxygen production rates of seaweed *Gracilaria lemaneiformis* in oyster *Crassostrea angulata*–seaweed co-culture mesocosms (see Table 1 for treatments). Bars with different lowercase letters are significantly different (ANOVA, $p < 0.05$)

This result indicated that total carbon fluxes were mainly influenced by respiration, but the contribution of calcification was not negligible. Based on the balance between $CaCO_3$ sequestration and CO_2 release, the *C. angulata* populations in Daya Bay increase CO_2 release to the atmosphere in coastal ecosystems. Moreover, our measurements may have underestimated the overall contribution of *C. angulata* to CO_2 fluxes, since we have not considered the rate of carbonate dissolution of shells that remained in the system after oyster death.

During the 4 h incubation, the DO concentrations in all mesocosms were above 4 mg l^{-1}. This level was not likely to induce stress to the oysters (Diaz & Rosenberg 2008). The reduced respiration rate by oysters in the high-density group (Fig. 3a) might be a strategy to cope with variability in seawater pH and the ability to adapt to seawater acidification (Guppy & Withers 1999, Langenbuch & Pörtner 2004).

Seawater CO_3^{2-} can affect the ability of calcifying organisms to precipitate $CaCO_3$ (Gazeau et al. 2007, Zhang et al. 2011, Dineshram et al. 2013, Li et al. 2013, Mos et al. 2015, McGrath et al. 2016). However, in the present experiment, no significant differences in calcification rate were found among the different density treatments (Fig. 3b), although the CO_3^{2-} was lower in the high-density group (Fig. 2c). This might indicate that the CO_3^{2-} deficiency stress was not severe enough to depress calcification. Therefore, further studies with longer incubation times and/or larger biomass of oysters are needed to produce more severe acidification stress.

According to the calcification rate and culture density of oysters, the mean $CaCO_3$ production by *C. angulata* population in Daya Bay is ca. 2150 g $CaCO_3$ m^{-2} yr^{-1}. This is higher than that of the oyster *Crassostrea gigas* (134 g $CaCO_3$ m^{-2} yr^{-1}) in Brest Bay (Lejart et al. 2012). Varying results could be due to species-specific differences. Lejart et al. (2012) studied the natural populations of *C. gigas*, which inhabit the intertidal zone going through 14 h of underwater respiration and calcification, and 10 h aerial respiration each day with the changing tides. However, *C. angulata* in Daya Bay were cultured under constant immersion conditions. Therefore, *C. angulata* has a longer period of calcification to produce higher amounts of $CaCO_3$.

CO_2 released during $CaCO_3$ precipitation of oysters in Daya Bay represented about 30.5% of the total CO_2 production. This result was consistent with previous findings, e.g. 30% for *Ophiothrix fragilis* (Migné et al. 1998), 33% for *Potamocorbula amurensis* (Chauvaud et al. 2003), and 23–26% for *Acrocnida brachiata* (Davoult et al. 2009) in the eastern English Channel. Therefore, although total carbon fluxes were mainly influenced by underwater respiration, there is a contribution from calcification that should not be neglected.

Gracilaria lemaneiformis can use both CO_2 and HCO_3^- for photosynthesis. In the oyster–seaweed co-culture mesocosm, the HCO_3^- concentration was significantly higher in the OS_4:1 group than in the OS_2:1 group, but there was no significant difference in CO_2 concentration (Fig. 5). This phenomenon was consistent with the findings of Raven et al. (2014) and Axelsson et al. (2000), who reported that in seawater of pH 8.0 and above, the principal species of DIC in the medium is HCO_3^-, but the active transport of HCO_3^- needs higher energy than passive CO_2 diffusion. Hence, it is reasonable that *G. lemaneiformis* has a higher affinity for CO_2 than HCO_3^-, which lead to a preferential CO_2 exhaust over HCO_3^-.

In the present study, calcification and respiration by oysters occurred over the duration of 24 h in the closed mesocosm system, while photosynthesis by *G. lemaneiformis* occurred only during the daytime when there is light. The CO_2:CO_3^{2-} ratio and pH of the seawater in the co-culture system would depend on the balance between the photosynthesis rate by the seaweed and the respiration rate and calcification rate of the oysters (Menéndez et al. 2001, Zhang et al. 2012). Seawater pCO_2 and CO_2:CO_3^{2-} ratios decreased in all oyster–seaweed co-culture systems, indicating that there was stronger CO_2 uptake by *G. lemaneiformis* than CO_2 release from *C. angulata*, leading to a net uptake of CO_2 from the atmosphere into the seawater. Meanwhile, we found that the net oxygen production rate of *G. lemaneiformis* in the OS_2:1 treatment was significantly decreased compared with that in the OS_8:1 and OS_4:1 groups (Fig. 7). As the primary production of seaweed is carbon limited, the carbon-saturated maximum photosynthesis of *G. lemaneiformis* would drastically reduce when it was 'starved' of DIC (Han et al. 2013). Since the numbers of oysters among the 3 treatments were almost the same, the decreased oxygen production rate i.e. the photosynthesis rate of *G. lemaneiformis* in the OS_2:1 group was probably due to a carbon limitation. Thus, there could be an evident interspecies mutual benefit for both *C. angulata* and *G. lemaneiformis* in the co-culture system. Based on the results of the present study, we suggest that the 2 species are co-cultured at a ratio of ca. 4:1 (fresh weight) for efficient utilization of seawater DIC by *G. lemaneiformis*, and further to increase the ocean CO_2 sink.

In conclusion, the physiological activities of *C. angulata* lead to a shift in the seawater DIC system equilibria towards higher CO_2, lower pH, and lower CO_3^{2-} concentration, and subsequently are affected by this shift. Seaweed *G. lemaneiformis* could act as an efficient sink for CO_2. Incorporation of seaweed into oyster aquaculture can be helpful in eliminating DIC release from *C. angulata*. There could be complex interspecies effects between *C. angulata* and *G. lemaneiformis*. The beneficial effects of an integrated multi-trophic aquaculture system on seawater carbon budget and air–sea CO_2 fluxes should be determined based on an ecosystem approach.

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