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Carbon dioxide fixation by the seaweed *Gracilaria lemaneiformis* in integrated multi-trophic aquaculture with the scallop *Chlamys farreri* in Sanggou Bay, China

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Abstract The red alga *Gracilaria lemaneiformis* was cultivated with the scallop *Chlamys farreri* in an integrated multi-trophic aquaculture (IMTA) system for 42 h at Sanggou Bay, located in north China. Variation in inorganic carbon in the IMTA system was determined. The experiment included three treatments each with three replicates and three scallop monoculture systems as controls. Scallop density (399.1 ± 7.85 g per microcosm) remained the same in all treatments while seaweed density differed. The seaweed density was set at three levels (treatments 1, 2, 3) with thallus wet weights of 125.3 ± 4.72 g, 252.3 ± 7.50 g, and 378.7 ± 6.51 g per microcosm, respectively. This produced bivalve to seaweed wet weight ratios of 1:0.31, 1:0.63, and 1:0.96 for treatments 1, 2, and 3, respectively. In control groups, continuous dissolution of carbon dioxide (CO_2) produced by scallops into the seawater not only caused an ongoing increase in partial pressure of CO_2 (pCO_2), 5.5 times higher than that of natural seawater, but also acidified seawater by 0.8 units after 42 h of culture. However, in all seaweed-scallop groups, the higher the algal density, the more CO_2 was absorbed; pCO_2 was lowest in treatment 3. The results suggest that a ratio of bivalve to seaweed less than 1:0.96 may produce an even stronger CO_2 sink. Overall, the integrated culture of seaweed and scallop could provide an efficient and environmentally friendly means to reduce CO_2 emissions from bivalve mariculture.

Keywords Carbon dioxide fixation · *Gracilaria lemaneiformis* · *Chlamys farreri* · Integrated multi-trophic aquaculture (IMTA)

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Introduction

The ocean has been recognized as the largest carbon reservoir on annual to millennial timescales, and the ocean carbon cycle exhibits significant spatial and temporal variability (Takahashi et al. 2002). According to recent inverse model estimates, observational proxies, and numerical simulations, the net uptake of carbon dioxide (CO_2) by the ocean is estimated as 2 Gt C year^{-1}, about 36 % of total annual CO_2 emissions (Fasham et al. 2001). Although coastal regions make up less than 10 % of the ocean by surface area, the rapid development of intensive aquaculture (e.g., fish, shrimp, bivalve, and algae) in coastal areas has greatly affected the net global air-sea CO_2 exchange. The activities of aquaculture organisms cause a substantial impact on the carbon cycle in coastal waters. Bivalves are a common and economically important group of species, which are important in the cycling of substances and energy transportation in coastal areas. High filtration rates and intense biodeposition of organic matter to the underlying sediments by filter-feeding bivalves are a major pathway bringing CO_2 into the ocean. Marine phytoplankton sequester dissolved CO_2 to produce particulate organic carbon (POC) through photosynthesis and then was fed by marine bivalves. Some POC, occurring as both living organisms and as nonliving particles, penetrates the pycnocline as the export production of carbon. Therefore, the net formation of organic carbon by biogeochemical processes of filter-feeding bivalves can be a significant net sink for CO_2. However, bivalves can also significantly influence CO_2 concentration in seawater through their respiration and calcification processes (Chauvaud et al. 2003). Respiration induces CO_2 release by aerobic oxidation of organic carbon, whereas calcification mainly results in shifts in the seawater carbonate equilibrium through the generation of dissolved CO_2 (Copin Montégut and Copin Montégut 1999). Thus, the respiration and calcification processes of bivalves releasing CO_2 into the seawater can be net carbon sources (Chauvaud et al. 2003; Golléty et al. 2008; Martin et al. 2006). As there are vast differences within and between coastal filter-feeding bivalve cultivation areas, it cannot be said that all such regions are net sinks of carbon, weak, or strong.

Marine macroalgae have been shown to utilize dissolved inorganic carbon (DIC) from the surrounding seawater (Smith and Bidwell 1989; Raven 1991) for photosynthesis and growth, resulting in a decrease in the DIC concentration of seawater and a drop in the partial pressure of carbon dioxide (pCO_2) below atmospheric levels. Dissolved inorganic carbon (DIC) is depleted during the day and can become a major limiting factor affecting the photosynthetic rates and aquaculture production of macroalgae growing at high biomass densities and reduced seawater motion (Zou et al. 2004). Given these factors, polyculture of shellfish and seaweed may make more efficient use of the net global air-sea CO_2 exchange than monoculture of either shellfish or macroalgae. China has a long history of integrated multi-trophic aquaculture (IMTA) of filter-feeding bivalves and seaweeds (Fang et al. 1996; Hawkins et al. 2002; Wang et al. 1993; Mao et al. 2009; Nunes et al. 2003; Zhang and Wang 1985; Yang et al. 2000; 2005; Zhou et al. 2006). However, such studies have focused on whether the dissolved nitrogen and phosphorus from bivalves are efficiently taken up by seaweeds. There have been few studies examining variations in air-sea CO_2 exchange and inorganic carbon source and sinks in IMTA systems of filter-feeding bivalves and seaweeds.

In this study, decoupling of DIC by *Gracilaria lemaneiformis* was determined in an IMTA system with the scallop *Chlamys farreri*. The objective of this study was to (1) determine whether *G. lemaneiformis* can efficiently fix dissolved CO_2 produced by *C. farreri*; and (2) evaluate the optimum ratio of scallop to seaweed for IMTA systems.

Materials and methods

A field microcosm experiment was conducted in closed systems at Sanggou Bay, located in north China. The experiment was performed from 9 to 11 August 2011 (42 h). Both the seaweed *G. lemaneiformis* and scallop *C. farreri* were collected from the Xunshan Fisheries Corporation, one of the biggest sea kelp culture operations in China. Using the long-line culture method, *G. lemaneiformis* and *C. farreri* were cultivated in 12 transparent polyethylene plastic bags (1 m^3, 1 m diameter, 1.5 m height) enwrapped by nets, as shown in Fig. 1. The scallops were attached to a plastic disk 25 cm in diameter, 1 cm thick, with some round holes in it. Each disk contained 40–50 scallops. The seaweed thalli (15 cm length) were coiled into 50-cm-long ropes. The plastic disk and ropes were suspended using thin ropes and tied to the mouth of the plastic bag, such that the seaweed thalli were positioned vertically at the scallops. All polyethylene plastic bags were closed, and no water was exchanged.

The experiment included three treatments each with three replicates. Three scallop monoculture microcosms served as controls. Scallops (9.59 g wet weight, 10.0 cm shell height) were of almost the same density in all treatments (ANOVA, $p > 0.05$) while seaweed density differed with 125, 252 g, and 379 g per microcosm, respectively (Table 1).

Seawater used in this experiment was natural seawater. Water temperature, salinity, irradiance, pH, and dissolved oxygen (DO) concentration were measured at 10:00 A.M. and 16:00 P.M. every day by using a water analyzer (YSI, Professional Plus, Yellow Springs, USA), Li-250 model illuminometer, and pH meter (Thermo Orion, 9107BNMD, Thermo Electron Corporation, USA), respectively. During the experiment, the temperature ranged from 20.4 to 21.3 °C; pH ranged from 7.39 to 8.46; salinity ranged from 31.8 to 32.2 ppt; irradiance ranged from 10 to 20 μmol photons m^{-2} s^{-1}; and DO concentration ranged from 1.55 to 10.78 mg L^{-1}.

Samples for total alkalinity (TA) measurements were immediately filtered using Whatman GF/F. The filtrate was mixed with 0.02 % volume saturated solution of $HgCl_2$ solution, and then stored in sealed 150-mL borosilicate bottles at 4 °C, in a dark location (Dickson and Goyet 1994). Total alkalinity (TA) was measured using an 848 Titrino plus automatic titrator (Metrohm, Switzerland). Parameters for the seawater carbonate system

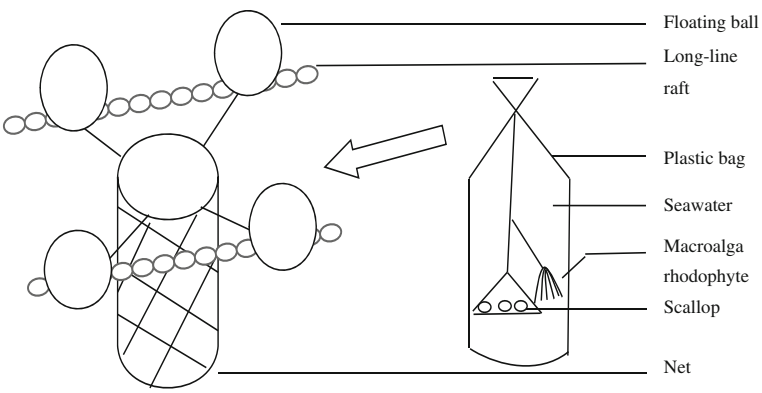

Fig. 1 Closed microcosm facility fixed to the long-line system

Table 1 Density of *C. farreri* and *G. lemaneiformis* in the different treatments (wet weight, g per microcosm)

Group	Scallop	Seaweed	Ratio
Control	400.6 ± 8.43	0	
Treatment 1	397.9 ± 7.17	125.3 ± 4.72	1:0.31
Treatment 2	401.4 ± 8.59	252.3 ± 7.50	1:0.63
Treatment 3	396.5 ± 10.89	378.7 ± 6.51	1:0.96

were calculated from pH, total alkalinity, temperature, and salinity according to the method of Dickson and Goyet (1994).

$$\mathrm{CA} = \mathrm{TA} - \mathrm{B}_T \times \frac{K'B}{aH^+ + K'B} \tag{1}$$

where CA and B_T represent the carbonate alkalinity and concentration of boric acid in seawater, respectively (Millero et al. 1998). a_H^+ is approximated as hydrogen ion concentration in seawater. $K'B$ is the dissociation constants for boric acid (Dickson 1990).

$$\mathrm{DIC} = \mathrm{CA} \times + \frac{aH^+ + K'2 + \frac{[a_H^+]^2}{K_1'}}{a_H^+ + 2 \times K_2'} \tag{2}$$

$$[\mathrm{HCO3}^-] = \mathrm{CA} \times \frac{a_H^+}{a_H^+ + 2 \times K_2'} \tag{3}$$

$$\left[\mathrm{CO}_3^{2-}\right] = \mathrm{CA} \times \frac{K_2'}{a_H^+ + 2 \times K_2'} \tag{4}$$

$$[\mathrm{CO}_2(T)] = \mathrm{CA} \times \frac{[a_H^+]^2}{K_1' \times (a_H^+ + 2 \times K_2')} \tag{5}$$

$$p\mathrm{CO}_2 = \frac{[\mathrm{CO}_{2(\mathrm{T})}]}{\alpha} \tag{6}$$

where $K'1$ and $K'2$ represent the temperature and salinity dependent the first and second dissociation constants for carbonic acid, respectively (Mehrbach et al. 1973). α is the CO_2 solubility constant (Weiss 1974).

Differences between treatments were tested using one-way analysis of variance (ANOVA) with SPSS version 19.0 for Microsoft Windows.

Results

Variation of pH in different IMTA treatments

pH of the control group decreased continuously from 8.24 to 7.41. On the other hand, pH in the treatment groups initially decreased slightly during the darkness (from D1 16:00 to D2 10:00 and D2 16:00 to D3 10:00 h), and increased during the daytime (D2 10:00 to D2 16:00 h), such as to 7.99, 8.06, and 8.20 in the end of the experiment, for treatments 1, 2,

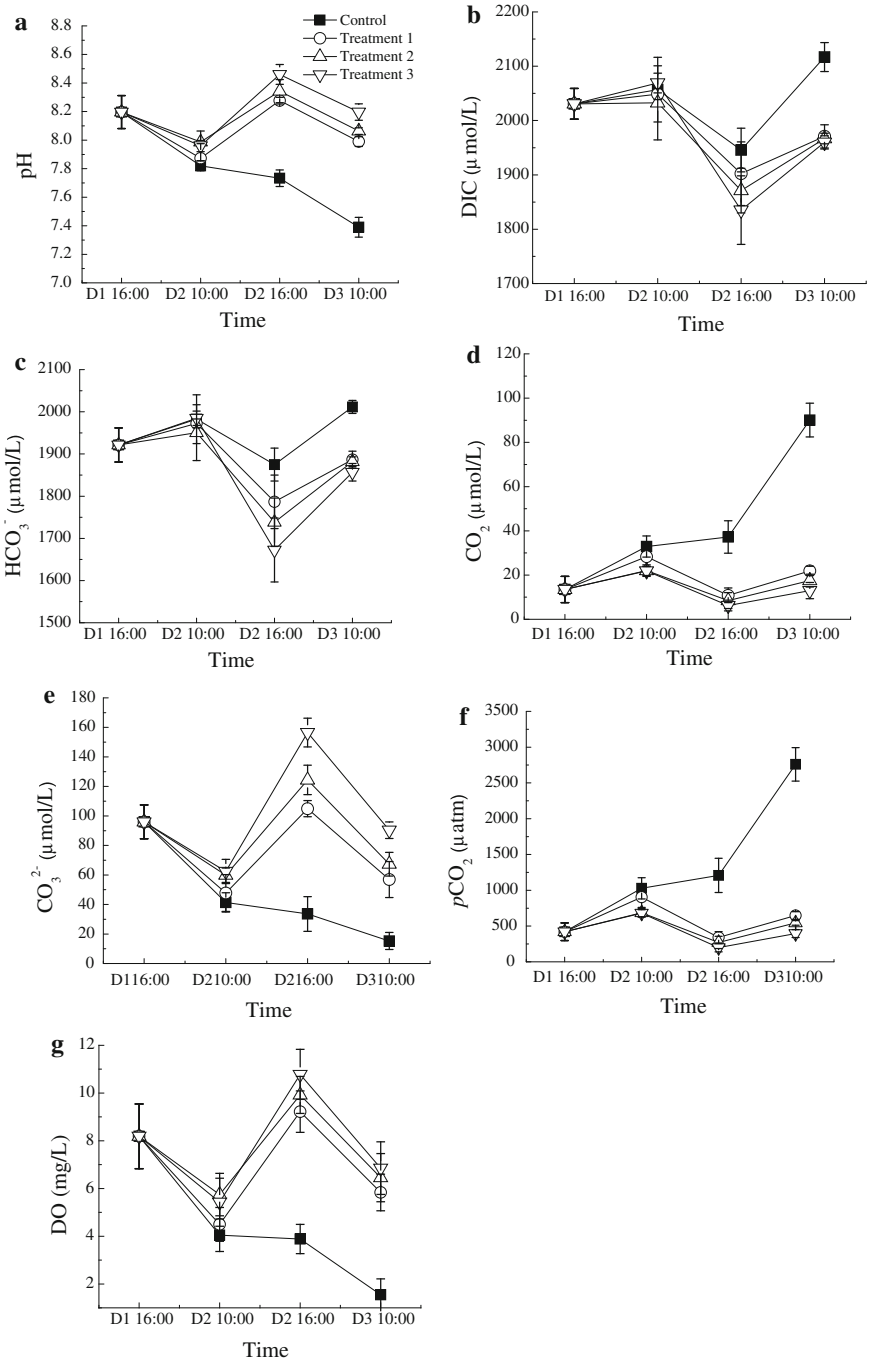

Fig. 2 Variations of the seawater parameters in different IMTA treatments: **a** pH, **b** DIC concentrations, **c** HCO_3^- concentrations, **d** CO_2 concentrations, **e** CO_3^{2-} concentrations, **f** pCO_2, **g** DO concentrations (*D1*, *D2*, and *D3* indicate the first day, second day, and third day of experiment, respectively). Each data point is the mean of three replicates, obtained in separate experiments, with SD values shown as *vertical bars*

and 3, respectively (Fig. 2a). ANOVA indicated that there were significant differences between the control group and any of the treatments ($p < 0.05$).

Variation of CO_2 system in different IMTA treatments

During the daytime, DIC and HCO_3^- concentrations decreased rapidly with increasing algal density reaching a minimum in treatment 3, while during the darkness, DIC, and HCO_3^- concentrations of all groups increased rapidly reaching a maximum in the control (2,117 μM for DIC concentration, and 2,012 μM for HCO_3^- concentration) (Fig. 2b, c). ANOVA indicated that DIC and HCO_3^- concentrations of the control were significant higher than those for any of the treatments after 42 h of culture ($p < 0.05$); there were no significant differences among treatments ($p > 0.05$).

In the control group, variation in CO_2 concentration differed from DIC and HCO_3^- concentrations; CO_2 concentration increased continuously from 13 to 90 μM at the end of the experiment. However, in the treatments, CO_2 concentrations decreased gradually with increasing algal density during the light daytime conditions and increased during the darkness (Fig. 2d). ANOVA indicated that there were no significant differences between the treatments, except between treatments 1 and 3, during day and night.

CO_3^{2-} concentration of the control decreased continuously from 96 to 15 μM, while CO_3^{2-} concentration in the treatment groups exhibited an opposite trend to that of DIC and HCO_3^- concentrations: decreasing rapidly during darkness and increasing sharply during light daytime conditions (Fig. 2e). ANOVA indicated significant differences among all groups ($p < 0.05$).

Variation of pCO_2 in different IMTA treatments

pCO_2 of the control group increased continuously during the course of experiment from 421 to 2,759 μatm, while that of the treatment groups showed daily variation with the lower levels after the dark period and the higher levels after the light period. At the end of the experiment, pCO_2 of the treatments decreased gradually with increasing algal density reaching 648, 545, and 396 μatm, for treatments 1, 2, and 3, respectively (Fig. 2f). ANOVA indicated that there were significant differences between the treatments ($p < 0.05$).

Variation of DO concentration in different IMTA treatments

Variation of DO concentration in the different IMTA systems showed a similar trend with that of pH. DO concentration of the control group decreased continuously from 8.06 to 1.55 mg L^{-1}. DO concentrations of the treatment groups showed an opposite trend with DIC concentration. DO concentrations increased during the daytime and decreased during the night time. DO concentration reached to 5.83, 6.45, and 6.85 mg L^{-1} for treatments 1, 2, and 3, respectively, at the end of the experiment (Fig. 2g). ANOVA indicated that there were significant differences between the treatments ($p < 0.05$).

Discussion

The mariculture of seaweeds and filter-feeding shellfish plays an important role in the coastal carbon cycle (Tang et al. 2011). Seaweeds transform DIC into organic carbon by

photosynthesis, and filter-feeding shellfish absorb POC during their feeding activity. Through the calcification process, a quantity of carbon is embedded into the shells of bivalves as $CaCO_3$. Therefore, a considerable mass of carbon can be removed from the ocean through harvesting. However, shellfish also continuously release CO_2 to seawater through respiration and calcification processes. Respiration induces CO_2 release through the aerobic oxidation of organic carbon, whereas calcification mainly results in shifts in the seawater carbonate equilibrium through the generation of dissolved CO_2 (Copin Montégut and Copin Montégut 1999). Therefore, mariculture significantly influences CO_2 concentration in seawater. As shown in Fig. 2d, CO_2 concentration of the control microcosm increased constantly during the experiment owing to the respiration and calcification processes of scallops. Continuous dissolution of CO_2 from scallops into the seawater caused an ongoing increase in pCO_2 to 5.5 times higher than that of natural seawater (Fig. 2f). Thus, regions with high scallop density in the absence of macroalgae may be an appreciable net carbon source. In addition, the seawater was acidified by 0.8 units after 42 h of scallop culture (Fig. 2a), which could have a negative impact on calcification and respiration of the scallops. If ocean pH reached 7.3, calcification rate would decrease to approximately 0, meaning that *C. farreri* would be unable to generate shell. Such a change is likely to be fatal for *C.farreri* (Zhang et al. 2011).

In seaweed-scallop microcosms, initial DIC decrease during the day is a result of the removal of inorganic carbon for photosynthesis exceeding the rate of CO_2 dissolution. DIC increase at the beginning of the dark period is a result of the continuing release of CO_2 (from respiration of seaweed and scallop and calcification of scallop) restoring the equilibrium. The rate of CO_2 dissolution in the closed system was estimated from the linear gradient of DIC with increase in seaweed density during the night (Fig. 2b). The integrated total of the rate of CO_2 dissolution and the rate of DIC decrease should yield the rate of photosynthetic carbon removal during the day. DIC reduction efficiency estimated in this way generally increased with algal density in the treatment groups, and the maximum reduction efficiency was 11.29 %. However, DIC reduction efficiency in the control group was only 5.40 %. Therefore, seaweeds can utilize DIC for photosynthesis, which can decrease the pCO_2 in seawater (Tang et al. 2011). Our experiment showed that not only was pCO_2 at D2 16:00 lower than that at D1 16:00, but also D3 10:00 was lower than D2 10:00 (Fig. 2f). In all seaweed-scallop groups, the higher the algal density, the more CO_2 was absorbed by the seaweed. Although pH increase can occur during the day from removal of CO_2 alone, this change should follow the hydration rate of CO_2 (about 1 $\mu M\ h^{-1}$ over the pH range 5–10.5) (Shelp and Canvin 1980). In the present study, in spite of a continuous influx of CO_2 from scallops, CO_2 was reduced during the daytime period at rates of 2.5–3.2 $\mu M\ h^{-1}$ in the seaweed-scallop groups. These values exceeded the hydration rate for CO_2 up to 3.2 times. As a result, pCO_2 drops below atmospheric levels and seaweed-scallop microcosms can become a net sink of CO_2, compared with the scallop microcosm.

On the other hand, increasing pCO_2 in seawater may enhance marine primary production (Hein and Sand-Jensen 1997; Schippers et al. 2004). Owing to the high solubility of CO_2 in seawater, DIC is rarely considered limiting for macroalgae. However, photosynthesis of *G. lemaneiformis* increased with increased DIC in the seaweed-scallop microcosms. This indicated that photosynthetic inorganic carbon uptake in this species is limited by the inorganic carbon source (about 2 mM DIC in air-equilibrated seawater) (Zou et al. 2004). When atmospheric CO_2 concentration increases, part of the increased CO_2 dissolves in seawater, forming carbonic acid, which in turn dissociates to form bicarbonate and then carbonate, reaching a new equilibrium (Gao et al. 1993). Therefore,

elevated pCO_2 and increased DIC in seawater can increase the photosynthesis and productivity of *G. lemaneiformis* (Xu and Gao 2010). Although high DIC uptake rates are achieved by supplying the seaweed culture with high areal loads of DIC, reduction efficiency may be low, and a large fraction of DIC remains in the water. Thus, perhaps a seaweed culture should be "starved"–supplied with a low areal load of DIC to achieve high DIC reduction efficiency, as to achieve high nutrient reduction efficiency to decrease the load of nutrients (Mao et al. 2009), which supports low seaweed areal yields with low protein content (Buschmann et al. 1994). In an integrated bivalve farm, it is therefore necessary to optimize the aerial DIC load to the seaweed, to reach acceptable levels of both uptake of DIC and decrease in pCO_2. In this study, the bivalve and seaweed wet weight ratio of treatments 1, 2, and 3 was 1:0.31, 1:0.62, and 1:0.96, respectively (Table 1). With a ratio from 1:0.31 to 1:0.96, DIC concentration and pCO_2 in the seaweed-scallop microcosms were considerably lower than that in the control microcosm, indicating high DIC and pCO_2 reduction efficiency. Furthermore, pCO_2 was lowest in treatment 3 suggesting that if the ratio of bivalve to seaweed was less than 1:0.96 a stronger CO_2 sink might result.

In summary, this study demonstrated that the red alga *G. lemaneiformis* has high inorganic carbon uptake efficiency, and its IMTA with the bivalve *C. farreri* could be an effective and environmentally friendly method to reduce CO_2 emission from bivalve culture. Further research is required to find the optimal ratios of seaweed and bivalve for the strongest CO_2 sink after harvesting.

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