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Impacts of large-scale aquaculture activities on the seawater carbonate system and air–sea CO_2 flux in a subtropical mariculture bay, southern China

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ABSTRACT: In this study, the variations of the seawater carbonate system parameters and air–sea CO_2 flux (F_{CO_2}) of Shen'ao Bay, a typical subtropical aquaculture bay located in China, were investigated in spring 2016 (March to May). Parameters related to the seawater carbonate system and F_{CO_2} were measured monthly in 3 different aquaculture areas (fish, oyster and seaweed) and in a non-culture area near the bay mouth. The results showed that the seawater carbonate system was markedly influenced by the biological processes of the culture species. Total alkalinity was significantly lower in the oyster area compared with the fish and seaweed areas, mainly because of the calcification process of oysters. Dissolved inorganic carbon (DIC) and CO_2 partial pressure (pCO$_2$) were highest in the fish area, followed by the oyster and non-culture areas, and lowest in the seaweed area. Oysters and fish can have indirect influences on DIC and pCO$_2$by releasing nutrients, which facilitate the growth of seaweed and phytoplankton and therefore promote photosynthetic CO_2 fixation. For these reasons, Shen'ao Bay acts as a potential CO_2 sink in spring, with an average F_{CO_2} ranging from −1.2 to −4.8 mmol m^{-2} d^{-1}. CO_2 fixation in the seaweed area was the largest contributor to CO_2 flux, accounting for ca. 58% of the total CO_2 sink capacity of the entire bay. These results suggest that the carbonate system and F_{CO_2} of Shen'ao Bay were significantly affected by large-scale mariculture activities. A higher CO_2 sink capacity could be acquired by extending the culture area of seaweed.

KEY WORDS: Carbonate chemistry · Air–sea CO_2 flux · F_{CO_2} · Seaweed culture · Oyster culture · Shen'ao Bay

1. INTRODUCTION

Mariculture is developing rapidly in coastal waters and is one of the fastest-growing food-producing sectors in the world. Mariculture production comprised 28.7 million t in 2016, accounting for approximately 40% of global aquaculture production (FAO 2018). The rapid expansion in the number of aquaculture species in coastal waters has contributed to an increased release of dissolved inorganic carbon (DIC) into aquatic ecosystems. Intensive-culture animals (e.g. fish and oysters) act as carbon dioxide (CO_2) generators through respiratory processes taking place within inner bays with slow flow velocity (Jiang et al. 2015, Morris & Humphreys 2019, Han et al. 2017, 2020). This results in higher seawater bicarbonate (HCO_3^-) and hydrogen ion (H^+) concentrations, in combination with a lower carbonate (CO_3^{2-}) concentration, leading to a decreased surface ocean pH (Caldeira & Wickett 2003, Orr et al. 2005). However, whether shellfish aquaculture acts as a CO_2 source or sink is still being debated. One view is that farmed bivalves can take up larger amounts of DIC through calcification than the amount released into seawater by

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respiration, resulting in lower total alkalinity (TA), HCO_3^- and CO_3^{2-} due to calcium carbonate ($CaCO_3$) precipitation (Li et al. 2021). Another view is that TA and the buffering capacity of carbonate chemistry in aquaculture waters are reduced by the rapid calcification processes of fast-growing calcifying organisms (Mos et al. 2015); lower TA decreases the relative calcite saturation state for a given partial pressure of CO_2 (pCO_2) (Mos et al. 2015), causing the release of CO_2 into the atmosphere (Fine et al. 2017).

Conversely, for seaweed, photosynthetic fixation of dissolved CO_2 leads to a concurrent decrease in DIC and pCO_2 and to an increase in pH (Zou et al. 2004, Jiang et al. 2014, Han et al. 2013, 2017, 2020). Although respiration of seaweed also releases CO_2 into seawater, the photosynthesis rate is higher than the respiration rate, and the released CO_2 is balanced by that taken up through photosynthesis (Zhang et al. 2012). Seaweed species that are commercially profitable, such as *Saccharina japonica* and *Gracilaria lemaneiformis*, have been widely cultured in China for over 30 yr. The photosynthetic CO_2 fixation rates of seaweed could be depressed in seawater with elevated pH, especially in the presence of high biomass and limited seawater exchange. For example, *G. lemaneiformis* exhibited high affinity for DIC and carbon-saturated maximum photosynthesis in seawater with a pH of 8.0 or lower, whereas the DIC affinity and the photosynthetic ability to use HCO_3^- were dramatically reduced in seawater with a pH > 8.0 (Zou et al. 2004).

Furthermore, pCO_2 is sensitive to TA, pH, salinity and temperature conditions; thus any mariculture activity that alters these parameters could affect seawater pCO_2 and air–sea CO_2 exchange. In addition to these direct influences, the release of ammonium (NH_4^+) increases TA, while the nitrification of NH_4^+ and remineralization of organic particles (e.g. animal feces and feed residue) decrease TA (Schlesinger 1997, Wolf-Gladrow et al. 2007). Overall, the uptake of nitrate (NO_3^-) and NH_4^+ by seaweed modifies TA (Brewer & Goldman 1976, Goldman & Brewer 1980). These findings suggest that the interactions between mariculture and the seawater carbonate system are very complex and that the analysis of their comprehensive effects should be based on species- and site-specific investigations.

Previous studies have investigated the effects of aquaculture fish, shellfish, seaweed and/or their combination on the seawater carbonate system, mostly based on the physiological characteristics of cultivated organisms either under controlled conditions such as in respiratory bottles or mesocosms (Han et al. 2013, 2017, 2020, Jiang et al. 2014, Fang et al. 2020), or in the open ocean (e.g. seaweed beds [Delille et al. 2000], seagrass beds [Buapet et al. 2013, Hendriks et al. 2014, Challener et al. 2016] and coral reefs [DeCarlo et al. 2017, Lønborg et al. 2019]). However, to date there has been limited research on the effects of large-scale aquaculture on the seawater carbonate system, air–sea CO_2 flux (F_{CO_2}) and underlying biogeochemical mechanisms in coastal aquaculture waters (Han et al. 2017).

Shen'ao Bay, located in the northern South China Sea, is a typical subtropical drowned valley bay. It has been exploited for aquaculture for more than 40 yr and is one of the largest aquaculture production sites in China, with suspended longline cultures of oysters and seaweed and finfish floating cages throughout the bay. To date, the bay-scale influences produced by intensive aquaculture on the seawater carbonate system and air–sea F_{CO_2} in subtropical bays remain unclear. In this study, the carbonate system characteristics of surface seawater in different cultivation areas were investigated, and direct influences of physiological processes and the indirect influences of hydrographic factors were examined in order to assess the impact of large-scale aquaculture activities on the seawater carbonate system and air–sea F_{CO_2}, as well as to further understand the interactions between aquaculture and the marine environment at a bay scale.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in Shen'ao Bay, Nan'ao Island, Shantou City, Guangdong Province, China, from March to May 2016. The area is a 13.3 km^2 coastal embayment (23° 27'–23° 29' N, 117° 04'–117° 07' E) with large-scale mariculture since the 1980s (Fig. 1). The bay is surrounded on 3 sides by mountains, has no surface runoff input and is affected by northeasterly winds in spring, which cause waters outside the bay to flow into the bay through a wide north-facing mouth that meets Zhelin Bay (see Section 2.2). Water depth ranges from 1 to 10 m, with an average depth of 4.3 m. The bay contains suspended longline cultures of Pacific oyster *Crassostrea gigas* and seaweed *Gracilaria lemaneiformis* and floating cages of Hong Kong grouper *Epinephelus akaara* and yellow grouper *E. awoara*. Fish cultivation occurs mainly between the eastern edge of the bay and Lieyu Island, oysters are cultivated at the bottom of the bay, and seaweed cultivation is mostly located in

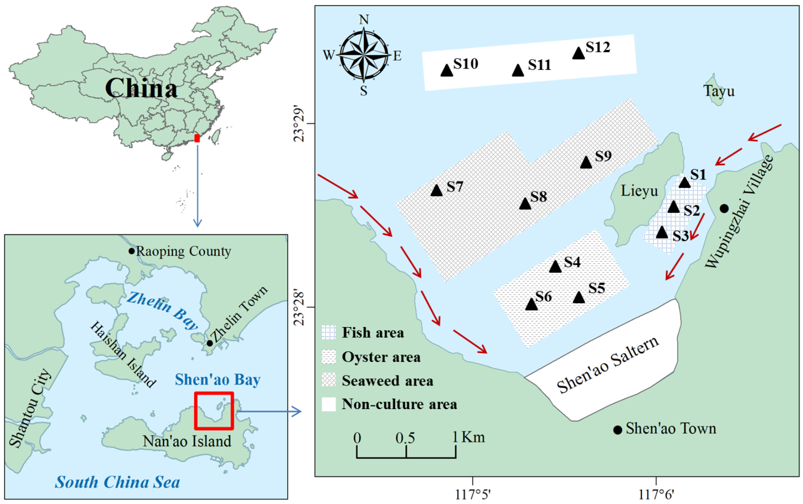

Fig. 1. Sampling stations (▲) from March to May in Shen'ao Bay, China: S1–S3 in the fish culture area, S4–S6 in the oyster culture area, S7–S9 in the seaweed culture area, and S10–S12 in the non-culture area. The red arrows represent the direction of the current

the central area. The 3 aquaculture areas have relatively independent positions in the bay.

2.2. Experimental design and sampling procedure

The selected station grid included 9 stations placed in the fish cage area (S1–S3, referred to as fish area), oyster raft areas (S4–S6, oyster area) and seaweed raft area (S7–S9, seaweed area). Three additional stations (S10–S12), where no culture activities were practiced and which were located near the bay mouth, were chosen as reference sites (non-culture area). The distance between the non-culture area and the closest seaweed area was ca. 1.5 km, and that between the non-culture area and Zhelin Bay mouth ca. 6 km. Zhenlin Bay is a large fish cage culture location in China. However, southward currents are a recurring event in spring and, as Shen'ao Bay is characterized by a 'V' shape, with its mouth opening northwards toward Zhelin Bay, Zhelin Bay waters are forced southwards and enter Shen'ao Bay. Thus, non-culture areas in Shen'ao Bay during spring may also be impacted by water coming from Zhelin Bay. However, it was difficult to find non-culture stations to be used as reference areas for Shen'ao Bay because of the high intensity of aquaculture activities affecting the entire bay. The stations in each area were laid out in a grid, and the distance between adjacent stations in the non-culture, fish, oyster and seaweed areas was ca. 0.6, 0.2, 0.5 and 0.8 km, respectively. The size of each culture area was calculated based on the GPS positioning data recorded at peripheral stations, and the non-culture, fish, oyster and seaweed areas measured ca. 2, 1, 2 and 5 km^2, respectively.

Three monthly survey cruises were conducted in spring 2016, from March to May. The culture period of *G. lemaneiformis* is from late November to May, harvest is no later than the end of May; after that the water temperature is too high for this species. During this period, the water temperature is also suitable for the cultured fish and oysters in the bay. Their growth and metabolism rates are the highest in these months of the year, so their physiological and ecological influences on the seawater carbonate system and air–sea F_{CO_2} are also most evident at this time.

The irregular semidiurnal tidal current in this bay can cause temporal variations in the seawater chemistry of the same station. To minimize the effects of tidal currents on sampling, the collection of samples took place each month between 10:00 and 14:00 h on the same day of the lunar tidal cycle. Each month, one surface water sample at each station was collected at a depth of 0.5 m with a 5 l acid-cleaned erect plexiglass sampler. For each sample, a 500 ml subsample for TA analysis was immediately filtered using a Whatman GF/F filter and filled with a 0.02% volume-saturated $HgCl_2$ solution; it was then sealed and stored in a cooler. Two 1 l subsamples were filtered through 2 separate Whatman GF/F filters; the filters were used for the determination of chlorophyll *a* (chl *a*) and for particulate organic matter (POM) analysis, and the filtrate was used for dissolved inorganic nutrient concentration analysis, including NO_3^-, NH_4^+, nitrite (NO_2^-) and phosphate (PO_4^{3-}). All Whatman GF/F filters were pre-combusted at 450°C for 4 h. Before filtering, the sampled water was pre-filtered through a 100 µm sieve to remove zooplankton.

Sea surface salinity (SSS) and sea surface temperature (SST) were measured at a depth of 0.5 m using a YSI meter (YSI Professional Plus 6600, Yellow Springs Instrument Company). The accuracy of SSS and SST measurements was ±0.1 and ±0.15°C, respectively; pH was measured at a depth of 0.5 m using a pH meter (Thermo Scientific Orion 320P-01, Thermo Electron) calibrated on the US National Bureau of Standards scale. The precision of pH measurements was ±0.01 pH units. SSS, SST and pH were measured twice at each station, and the average values were used in the analysis. In each subsample, TA was measured in triplicate by Gran titration with 0.1 M HCl, using an alkalinity titrator (AS-AlK2, Apollo SciTech). The accuracy of the TA measurements was determined using the Certified Reference Material (Batch 158, A. G. Dickson, Scripps Institution of Oceanography, California, USA). The reproducibility of TA values was better than ±5 µmol kg^{-1}. DIC, HCO_3^-, CO_3^{2-}, CO_2 and pCO_2 were obtained from SSS, SST, pH and TA using the CO2_SYS_XLS calculation program (Pierrot et al. 2006). Uncertainties in the measurements of pH and TA may result in probable errors of ±4 µmol kg^{-1} and ±2 µatm in the computation of DIC and pCO_2, respectively (Millero 2007).

The concentrations of NO_3^-, NH_4^+, NO_2^- and PO_4^{3-} were determined using standard spectrophotometric methods. The filters used for the determination of POM were dried at 60°C until constant weight was reached, and then the filter weight losses were measured after an ignition phase of 4 h at 450°C. Chl *a* was extracted from a 10 ml solution of 90% acetone following 24 h in darkness at 4°C (Parsons et al. 1984), and its concentration was determined with a Turner Design 10 fluorometer.

Air–sea F_{CO_2} was calculated using the following equation: $F_{CO_2} = k \times \alpha \times \Delta pCO_2$, where k (cm h^{-1}) is the gas transfer velocity of CO_2. We computed k using the parameterization given by Wanninkhof (2014), which uses short-term winds $k = 0.251 \times u_{10}^2 / (Sc/660)^{1/2}$, where u_{10} is the wind speed at a height of 10 m from the water surface level (m s^{-1}), Sc is the Schmidt number of CO_2 at *in situ* temperature and salinity, which has a 20% uncertainty. 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 values of atmospheric pCO_2 were downloaded from www.cmdl.noaa.gov (Climate and Meteorological Diagnostics Laboratory, NOAA) and corrected for water vapor pressure (Takahashi et al. 2002). Positive magnitudes of F_{CO_2} indicate a flux from water to air and vice versa.

2.3. Statistical analysis

Since the samples were collected at the same station each month, we adopted a sampling design with dependent repeated measures. In this context, a 2-way repeated-measures ANOVA (rmANOVA) with 4 sampling areas and 3 sampling months was performed to test the separated effects of areas, months and their interaction on the seawater carbonate system parameters and hydrographic factors. The rmANOVA assumption of sphericity was evaluated using Mauchly's criterion. The probabilities of the interactive effect of area and month on the seawater carbonate system parameters and some hydrographic parameters (pH, NO_2^-, PO_4^{3-} and POM) were higher than the Bonferroni-corrected level of p, suggesting that the interactions were not significant. Subsequently, the data from the above-mentioned parameters were analyzed by two 1-way ANOVAs, one examining the effect of area combined across all months and the other examining the effect of month across all areas. The residual parameters with the interactive effect of area and month factors were further analyzed with a 1-way ANOVA to test all 12 treatments (4 areas × 3 months). The LSD multiple comparisons test was used to determine the difference among areas and months.

Tests for homogeneity and normality were run using Levene's test and the Kolmogorov-Smirnov test prior to the ANOVA. All parameters in each area and each month were normally distributed and had equal variances. Correlation analysis (Pearson's correlation coefficients) was used to test the relationships between carbonate system parameters and hydrographic factors. Given the high number of tests conducted within 2-way rmANOVA and correlation analysis, p-values were adjusted by applying a Bonferroni correction to the number of analyzed parameters to reduce the risk of a Type I error. Sixteen parameters affected by areas and months were adjusted at 0.003125 (0.05/16; number of 2-way rmANOVAs performed), and the carbonate system correlations with environment were adjusted at 0.001111 (0.05/[5 carbonate system parameters × 9 hydrographic factors]). The above calculations and statistical analyses were performed in SPSS 19.0 for Windows.

Principal component analysis (PCA), performed using the package 'vegan' in R (R Core Team 2016), was used to identify the key variables with the highest influence on seawater environmental characteristics. Before conducting the PCA, all parameters were scale standardized with zero mean and unit variance, so that they all had equal weight in the analysis.

3. RESULTS

3.1. SSS and SST

SSS in Shen'ao Bay during spring ranged from 25.7 to 31.7. There were significant differences in SSS among different areas and months (2-way rmANOVA, $p < 0.003125$). SSS in April and May was significantly lower than that in March (1-way ANOVA, $p < 0.05$). This was mainly due to the heavy rains that occurred in April and May. The overall variation trend of SSS increased moving from the outside to the inside of the bay, and the SSS in the fish area was significantly higher than that in the other areas (1-way ANOVA, $p < 0.05$) (Fig. 2A). Significant (2-way rmANOVA, $p < 0.003125$) differences in SST were found between months rather than between areas. SST in Shen'ao Bay gradually increased from 16.4°C in March to 25.9°C in May (Fig. 2B).

3.2. pH, nutrients, POM and chl *a*

pH values were significantly different among areas and months (2-way rmANOVA, $p < 0.003125$). Over the 3-mo period (March to May), seawater pH values were significantly higher in April than in the other 2 months (1-way ANOVA, $p < 0.05$), and they were significantly higher in the seaweed area than in the other 3 areas for the entire period (March to May) (1-way ANOVA, $p < 0.05$) (Fig. 3A).

Dissolved inorganic nitrogen (DIN) in Shen'ao Bay was mainly composed of NO_3^- (ca. 70%) and NH_4^+ (ca. 20%). There were significant differences in NO_3^- and NH_4^+ concentrations among areas, months and their interactions; the PO_4^{3-} concentration was significantly different only between months (2-way rmANOVA, $p < 0.003125$). The average concentrations of NO_3^-, NH_4^+ and PO_4^{3-} were lowest in April and highest in March. In March and May, they were significantly higher in the fish, oyster and non-culture areas than in the seaweed area (1-way ANOVA, $p < 0.05$) (Fig. 3B,C,E).

POM concentrations were significantly different among areas and months (2-way rmANOVA, $p < 0.003125$). The lowest and highest POM concentrations were recorded in March and April, respectively. POM concentration in the seaweed area was the lowest, and it was significantly lower than in the other 3 areas (1-way ANOVA, $p < 0.05$), which did not show any significant differences between themselves (one-way ANOVA, $p > 0.05$) (Fig. 3F).

Chl *a* concentration presented significant differences among areas, months and their interaction (2-way rmANOVA, $p < 0.003125$). In April and May, chl *a* concentration in the non-culture and seaweed areas showed the highest and lowest values, respectively, and was significantly higher and lower than in the other areas, respectively (1-way ANOVA, $p < 0.05$). Chl *a* concentrations were significantly different between all months in the non-culture, fish and oyster areas; whereas in the seaweed area, the difference was significant only between March and May (1-way ANOVA, $p < 0.05$) (Fig. 3G).

3.3. Carbonate system parameters

Seawater carbonate system parameters presented significant differences among areas and months (2-way rmANOVA, $p < 0.003125$). TA in the oyster area was significantly lower than that in the fish and seaweed areas (1-way ANOVA, $p < 0.05$) (Fig. 4A). The highest and lowest DIC concentrations were observed in March and April, respectively. DIC concentration in the fish area was significantly higher than that in the other areas throughout the study period, while DIC concentration in the seaweed area in March and April was significantly lower than that in the other areas (1-way ANOVA, $p < 0.05$) (Fig. 4B). The calculated HCO_3^- and CO_2 concentrations showed a variation pattern similar to that of DIC, while CO_3^{2-} showed an opposite pattern (Fig. 4C–E).

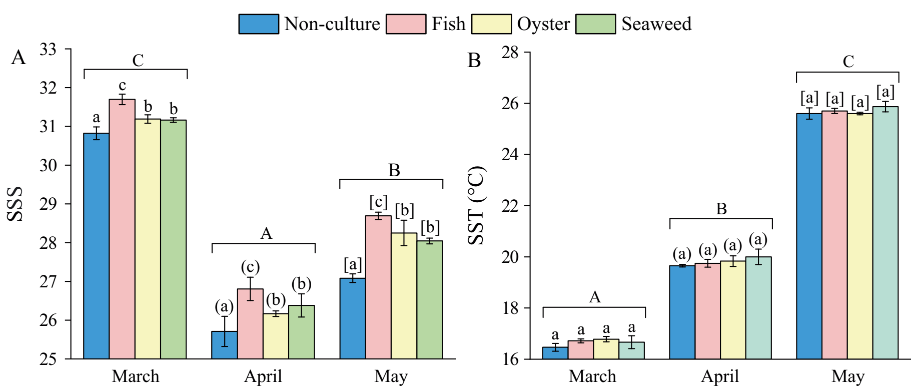

Fig. 2. (A) Sea surface salinity (SSS) and (B) sea surface temperature (SST) in different areas of Shen'ao Bay from March to May 2016. Different lowercase letters, without, in round and in square brackets above the columns represent statistically different results in March, April and May, respectively. Different uppercase letters above the columns represent statistically different results among areas in March, April and May. Error bars show ±SD

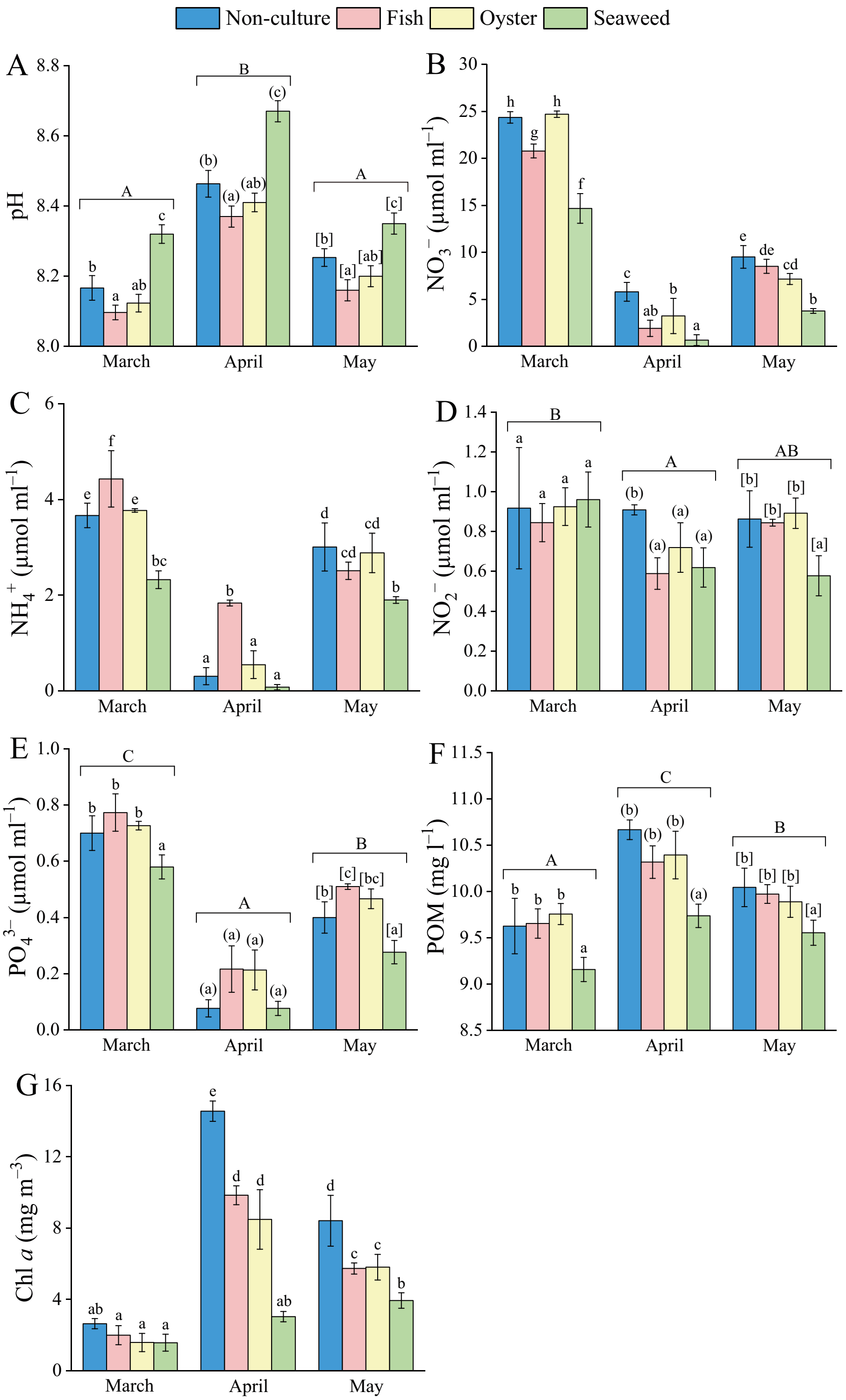

Fig. 3. (A) pH and concentrations of (B) nitrate (NO_3^-), (C) ammonium (NH_4^+), (D) nitrite (NO_2^-), (E) phosphate (PO_4^{3-}), (F) particulate organic matter (POM) and (G) chl *a* in different culture areas of Shen'ao Bay from March to May 2016. For pH, NO_2^-, PO_4^{3-} and POM, different lowercase letters without, in round and in square brackets above the columns represent statistically different results in March, April and May, respectively. Different uppercase letters above the columns represent statistically different results among areas in March, April and May. For NO_3^-, NH_4^+ and chl *a*, different lowercase letters above the columns represent statistically different results among areas and months. Error bars show ±SD

3.4. Factors influencing the seawater carbonate system

Pearson's correlation analysis was conducted to determine the correlations existing between seawater carbonate system parameters and hydrographic factors. For example, seawater carbonate system parameters were significantly affected by SSS ($p < 0.001111$; Table 1). In order to examine the contribution of hydrographic factors to the variations of the seawater carbonate system and remove the contribution derived from SSS changes, the seawater carbonate system parameters were normalized to a constant salinity of 35 as nTA, nDIC, $nHCO_3^-$, nCO_3^{2-} and nCO_2 (e.g. nTA = TA × 35/SSS [salinity *in situ*]) (Millero et al. 1998). Subsequently, the correlations between the normalized seawater carbonate system parameters and hydrographic factors were estimated by a Pearson's correlation analysis. After salinity normalization, nTA, nCO_3^{2-} and nCO_2 were significantly correlated with salinity; there was no significant correlation between salinity and nDIC and $nHCO_3^-$ (Table 2). Area had a significant effect on the distribution of SSS values (Fig. 2A), mainly because of river inputs flowing into Zhelin Bay and domestic sewage. Thus, salinity was not a relevant factor to be used to make comparisons in this study. The key factors were related to physiological activities such as photosynthesis in seaweed, respiration and excretion in fish and oysters and calcification in oysters. Pearson's correlation analysis showed that the distribution profiles of nTA were significantly affected by pH, nutrients (NO_3^-, NH_4^+ and PO_4^{3-}), POM and chl *a*; nDIC had a strong positive correlation with

POM and chl *a*; and $nHCO_3^-$, nCO_3^{2-} and nCO_2 were significantly correlated with pH and nutrients (NO_3^-, NH_4^+ and PO_4^{3-}) ($p < 0.001111$, Table 2).

Seawater spatial characteristics were further clarified by PCA, in which the first 2 principal components (PCs) accounted for 84.8% of the total variability. Variability along the first axis was mainly explained by a decrease in nCO_2 and $nHCO_3^-$ in the order of seaweed area, oyster area and fish area, and a concomitant increase in pH and nCO_3^{2-} (Fig. 5). PC1 was associated with variables such as nutrients, nCO_2, pH, nCO_3^{2-} and nTA, showing the direct relationship of C, N and P with the physiological metabolic activities of the different culture species. PC2 mainly represents the dependence on nDIC, $nHCO_3^-$, POM and chl *a*, showing the indirect influence of culture species on the seawater carbonate system.

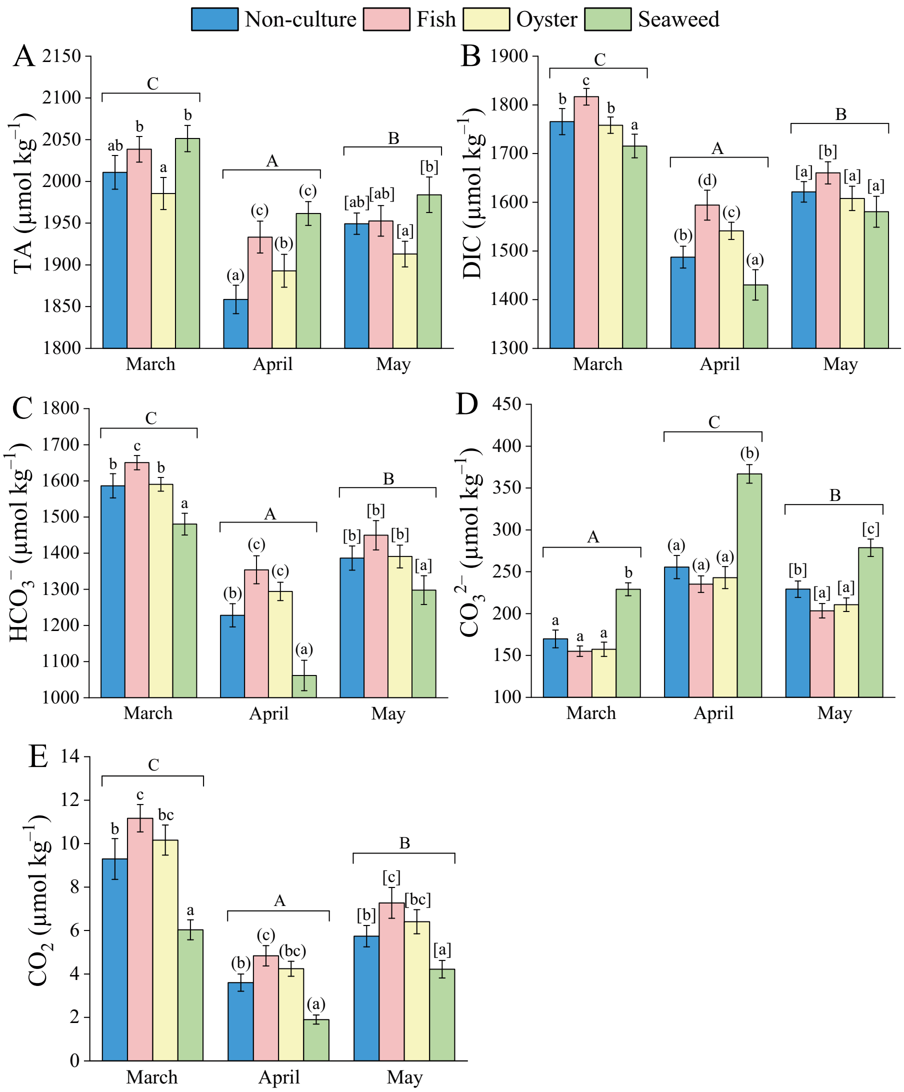

Fig. 4. Variations of the carbonate system parameters (A) total alkalinity (TA), (B) dissolved inorganic carbon (DIC), (C) bicarbonate (HCO_3^-), (D) carbonate (CO_3^{2-}) and (E) CO_2 in different areas of Shen'ao Bay from March to May 2016. Different lowercase letters without, in round and in square brackets above the columns represent statistically different results in March, April and May, respectively. Different uppercase letters above the columns represent statistically different results among areas in March, April and May. Error bars show ±SD

3.5. *p*CO_2 and air–sea F_{CO_2}

pCO_2 and air–sea F_{CO_2} were affected by both area and month (2-way rmANOVA, $p < 0.003125$). From March to May, atmospheric *p*CO_2 values ranged from 407.7 to 409.6 µatm, and the *p*CO_2 values in the surface water of Shen'ao Bay ranged from 56.2 to 309.2 µatm, which was lower than the atmospheric *p*CO_2, indicating that the bay was acting as a sink for atmospheric CO_2. The calculated air–sea F_{CO_2} ranged from –1.2 to –4.8 mmol m^{-2} d^{-1}, with the lowest and highest CO_2 sink capacities observed in the fish and seaweed areas, respectively. The CO_2 sink capacities of the 4 areas were highest in April and lowest in March, with the seaweed area value being significantly higher than that of the other areas (1-way ANOVA, $p < 0.05$) (Fig. 6).

4. DISCUSSION

There were significant differences in the seawater carbonate system parameters and air–sea F_{CO_2} between areas and over months in Shen'ao Bay, suggesting that mariculture activities modify the seawater carbonate system at the bay scale. The presence of culture species can affect the seawater carbonate system directly and indirectly. The photosynthesis of *Gracilaria lemaneiformis* absorbed CO_2 from the seawater, leading to decreased DIC, HCO_3^- and *p*CO_2, and increased pH and CO_3^{2-} in the seaweed area.

Table 1. Relationship between carbonate system parameters and hydrographic factors using the Pearson correlation test. TA: total alkalinity; DIC: dissolved inorganic carbon; HCO_3^-: bicarbonate; CO_3^{2-}: carbonate; CO_2: carbon dioxide; SSS: sea surface salinity; SST: sea surface temperature; NO_3^-: nitrate; NH_4^+: ammonium; NO_2^-: nitrite; PO_4^{3-}: phosphate; POM: particulate organic matter. Values in **bold** represent significant influences, and significance was defined as the Bonferroni-corrected level of $p < 0.00111$ (5 carbonate system parameters × 9 hydrographic factors, at the 0.05 level). β is the Pearson correlation coefficient; p is probability

Variable	TA		DIC		HCO_3^-		CO_3^{2-}		CO_2	
	β	p	β	p	β	p	β	p	β	p
SSS	0.819	**<0.001**	0.913	**<0.001**	0.866	**<0.001**	−0.705	**<0.001**	0.852	**<0.001**
SST	−0.393	0.018	−0.396	0.017	−0.369	0.027	0.286	0.090	−0.371	0.026
pH	−0.405	0.014	−0.886	**<0.001**	−0.929	**<0.001**	0.942	**<0.001**	−0.916	**<0.001**
NO_3^-	0.600	**<0.001**	0.869	**<0.001**	0.865	**<0.001**	−0.792	**<0.001**	0.887	**<0.001**
NH_4^+	0.619	**<0.001**	0.903	**<0.001**	0.898	**<0.001**	−0.818	**<0.001**	0.895	**<0.001**
NO_2^-	0.096	0.577	0.390	0.019	0.431	0.009	−0.481	0.003	0.419	0.011
PO_4^{3-}	0.679	**<0.001**	0.941	**<0.001**	0.930	**<0.001**	−0.835	**<0.001**	0.926	**<0.001**
POM	−0.847	**<0.001**	−0.510	**0.001**	−0.394	0.017	0.127	0.459	−0.351	0.036
Chl *a*	−0.829	**<0.001**	−0.577	**<0.001**	−0.474	0.003	0.231	0.175	−0.508	0.002

Table 2. As in Table 1, but showing the relationship between normalized (indicated by the letter 'n' before the parameter) carbonate system parameters and hydrographic factors using the Pearson correlation test

Variable	nTA		nDIC		$nHCO_3^-$		nCO_3^{2-}		nCO_2	
	β	p	β	p	β	p	β	p	β	p
SSS	−0.946	**<0.001**	−0.319	0.058	0.406	0.014	−0.801	**<0.001**	0.797	**<0.001**
SST	0.417	0.011	0.237	0.164	−0.096	0.579	0.311	0.065	−0.306	0.069
pH	0.811	**<0.001**	−0.271	0.111	−0.840	**<0.001**	0.953	**<0.001**	−0.941	**<0.001**
NO_3^-	−0.890	**<0.001**	−0.133	0.438	0.533	**0.001**	−0.836	**<0.001**	0.852	**<0.001**
NH_4^+	−0.809	**<0.001**	0.092	0.594	0.674	**<0.001**	−0.861	**<0.001**	0.891	**<0.001**
NO_2^-	−0.508	0.002	−0.064	0.711	0.320	0.057	−0.488	0.003	0.418	0.011
PO_4^{3-}	−0.916	**<0.001**	−0.066	0.702	0.612	**<0.001**	−0.894	**<0.001**	0.904	**<0.001**
POM	0.521	**0.001**	0.528	**0.001**	0.097	0.573	0.266	0.117	−0.280	0.098
Chl *a*	0.633	**<0.001**	0.570	**<0.001**	0.056	0.745	0.357	0.033	−0.434	0.008

Conversely, the respiration of a large number of fish and oysters released CO_2 into seawater, following the reaction $CH_2O + O_2 \rightarrow CO_2 + H_2O \rightarrow HCO_3^- + H^+ \rightarrow CO_3^{2-} + 2H^+$, causing an increase in seawater DIC, HCO_3^- and pCO_2, and a decrease in pH, which did not occur in the non-culture and seaweed areas, particularly in April.

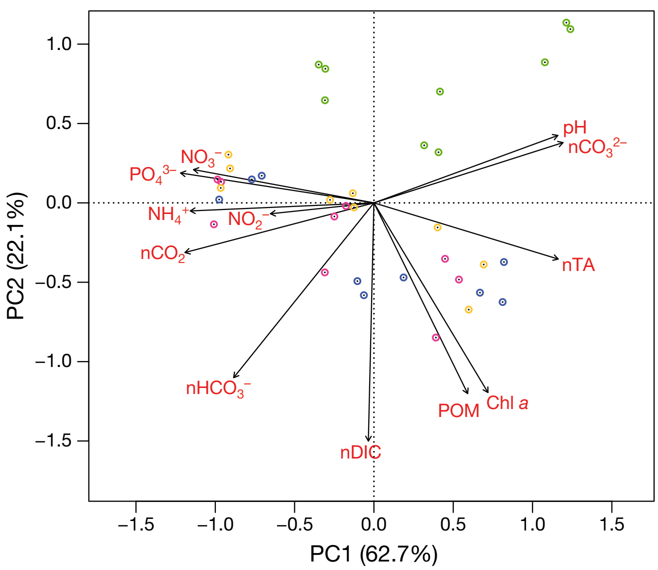

Fig. 5. Ordination diagram displaying the first (PC1) and second (PC2) axes of a principal component analysis (PCA). Loadings of the environmental variables are displayed as arrows, with the scheme for the variation of environmental factors in each area depending on the scores of the first 2 principal components. nTA: normalized total alkalinity; nDIC: normalized dissolved inorganic carbon; $nHCO_3^-$: normalized bicarbonate, nCO_3^{2-}: normalized carbonate; nCO_2: normalized carbon dioxide; NO_3^-: nitrate; NH_4^+: ammonia; NO_2^-: nitrite; PO_4^{3-}: phosphate; POM: particulate organic matter

TA is a measure of the acid neutralization capacity of seawater. This parameter is particularly useful because it can be used to calculate the concentrations of various chemical species characterizing the carbonate system (e.g. CO_2, HCO_3^- and CO_3^{2-}). Animal respiration and seaweed photosynthesis can influence DIC concentration by either producing or

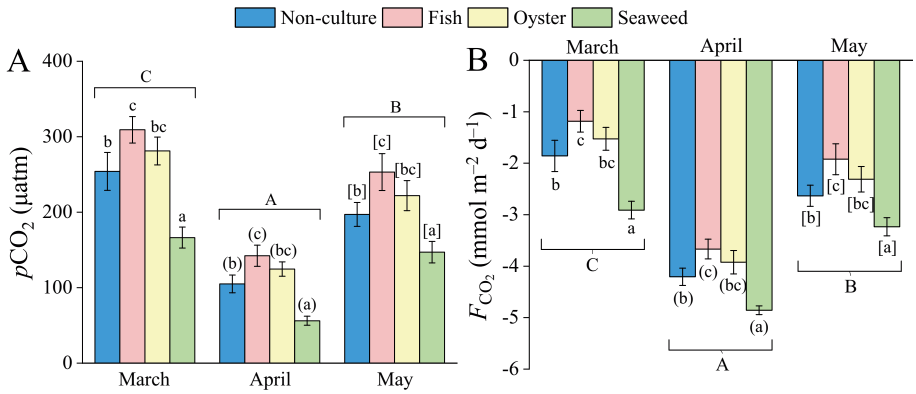

Fig. 6. Variations of the carbonate system parameters (A) partial pressure of CO_2 (pCO_2) and (B) air–sea CO_2 flux (F_{CO_2}) in different areas of Shen'ao Bay from March to May 2016. Different lowercase letters without, in round and in square brackets above the columns represent statistically different results in March, April and May, respectively. Different uppercase letters above the columns represent statistically different results among areas in March, April and May. Error bars show ±SD

absorbing CO_2, but have almost no effect on TA (Redfield et al. 1963). Oyster calcification processes, via the reaction known as $Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$, lead to higher CO_2 concentrations and lower TA. However, DIC concentration in the oyster area was significantly lower than that in the fish area. A decreased DIC has also been reported in bivalve farms area in Sanggou Bay (Li et al. 2021). The ratio of released CO_2/precipitated $CaCO_3$ during calcification was largely dependent on the buffering capacity of the surrounding seawater (Frankignoulle et al. 1995), and was estimated by calculating the temperature as $0.8 - 8.3/10^3 \times$ SST (Frankignoulle et al. 1994); in the present study, it ranged from 0.58 to 0.67. Previous studies have shown that oysters can fix larger amounts of DIC by calcification than the amount released into seawater through respiration (Li et al. 2021). The reduced DIC concentration in the oyster area should result in a relatively low pCO_2 level. However, the oyster area did not contribute to a significant reduction in pCO_2 and F_{CO_2} when compared with the non-culture area. Thus, whether oyster calcification favors the fixation of CO_2 from the atmosphere needs to be further investigated.

In addition to these direct influences, culture species can also exert indirect influences, through excretion or absorption of nutrients, and thus further influence phytoplankton production, which has a similar function to that of seaweed. Pearson correlation analysis results (Table 2) showed a significant relationship between the carbonate system and nutrients. In particular, it showed that the close relationship between C, N and P was regulated by different biological processes.

Because seaweed and phytoplankton compete for nutrients and carbon sources, the large biomass of *G. lemaneiformis* depresses phytoplankton production in the seaweed area. For this reason, the lowest chl *a* concentration was recorded in this area in April and May. This was probably also due to the water temperature during this period (20–26°C) being suitable for the growth of *G. lemaneiformis* (Yang et al. 2006, Zou & Gao 2013). The competition for nutrients from the seaweed decreased the biomass of phytoplankton; whereas in the colder March conditions, the growth of *G. lemaneiformis* was lower than that in April and May. This could be a possible reason for the higher chl *a* found in the seaweed area in March. Furthermore, the exhaustion of gaseous CO_2 in seawater limited the growth of both seaweed and phytoplankton. In such conditions, seaweed and phytoplankton absorbed HCO_3^- using the proton pump mechanism and carbonic anhydrase (Tortell et al. 1997), causing a significantly lower HCO_3^- concentration in the seaweed area. For fish and oysters, the excretion of dissolved nutrients leads to high chl *a* and intense primary productivity (Ho et al. 2010, Gong et al. 2011). Higher phytoplankton consumes more CO_2, and this could be a possible explanation for the lower pCO_2 observed in these areas. However, this was inconsistent with what was observed in the bivalve culture area of Sanggou Bay — another intensive aquaculture bay specializing in scallops and oysters in northern China — where pCO_2 ranged from 427 to 862 µatm (Li et al. 2021) and the lower average annual chl *a* concentration ranged from 0.44 to 6.89 µg l^{-1} (Jiang et al. 2017).

The possible influence of culturing *G. lemaneiformis* on the seawater carbonate system is not negligible, since this species reaches a large biomass volume in Shen'ao Bay during spring. Based on the nutrient-H^+-compensation principle and TA expression, the assimilation of 1 mol of NO_3^- during the photosynthetic process of *G. lemaneiformis* results in an increase of alkalinity by 1 mol (Wolf-Gladrow et al. 2007). Thus, the massive NO_3^- uptake by cultivated *G. lemaneiformis* could increase TA, as observed in the seaweed area.

NH_4^+ is the primary N excretion product in fish and bivalves. Under aerobic conditions, NH_4^+ is ultimately oxidized into NO_3^-, as defined by the equation $NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$, resulting in a decrease in TA of 2 mol per mol of NO_3^- formed (Schlesinger 1997). Therefore, nitrification of NH_4^+ produces an increase in NO_3^- concentration, coupled with a decrease in TA in areas subjected to animal aquaculture (Schlesinger 1997, Wolf-Gladrow et al. 2007). At the same time, uneaten feed, feces from fish, and feces and pseudofeces from oysters are released into the water column (Giles et al. 2006), resulting in higher amounts of POM compared with seaweed areas. Remineralization of POM can introduce a considerable amount of nitrogen and phosphate into the marine environment around aquaculture farms (Kim et al. 2019, Qi et al. 2019). As a consequence, this process may modify seawater TA and its distribution depending on the form of reactive nitrogen produced. In Shen'ao Bay, NO_3^- accounts for 70% of total DIN. It mainly derives from NH_4^+ nitrification and remineralization of POM. A release of 1 mol of NO_3^- results in a 1 mol decrease in TA (Wolf-Gladrow et al. 2007). Therefore, this could be an indirect influence of fish and oysters on the decreased TA observed in their respective areas.

It is noteworthy that NO_3^- concentration in the non-culture area was also very high. A possible reason for it is that this area is located near the mouth of Shen'ao Bay, which is about 6 km away from Zhelin Bay (Fig. 1). The level of NO_3^- was high in Zhelin Bay due to the intensive fish cage culture present in that bay. A portion of the NO_3^- could have diffused from there to the non-culture area in Shen'ao Bay through seawater exchange driven by tides and northeasterly monsoon winds in early March (Du et al. 2010).

Both fish and oysters release CO_2 into seawater, but they also excrete dissolved inorganic nutrients (e.g. NH_4^+), which stimulate intense phytoplankton productivity. Phytoplankton, in turn, consumes CO_2 and HCO_3^-, thus counterbalancing the CO_2 released by fish and oysters. Consequently, the negative values of F_{CO_2} observed in the 4 areas indicate that the entire Shen'ao Bay acted as a net importer of CO_2 in spring, as the CO_2 absorbed was greater than that released. Conversely, in the bivalve farming area of Sanggou Bay, F_{CO_2} in May was 8.90 ± 3.47 mmol m^{-2} d^{-1}, indicating that the area acted as a net source of CO_2 for the atmosphere (Li et al. 2021). This is probably correlated with primary production, due to the lower chl *a* concentration of that bay in spring (0.83 ± 0.45 µg l^{-1}) (Jiang et al. 2017) compared with what was observed in Shen'ao Bay (5.65 ± 3.85 µg l^{-1}). This hypothesis was, to some extent, confirmed by observations in Jiaozhou Bay, another mariculture bay in northern China, located at a similar latitude as Sanggou Bay. In Jiaozhou Bay, it was found that approximately half of the bay area shifted from being a CO_2 source to being a sink following a considerable increase in primary production (Li et al. 2017). Although seaweeds release both particulate and dissolved organic carbon, comprising about 43.5% of seaweed production, they can be buried into sediments or exported to the deep sea, thus acting also as CO_2 sinks (Duarte et al. 2017, Wu et al. 2020). Therefore, it is inferred that in Shen'ao Bay, the seaweed aquaculture area represents the strongest CO_2 sink, with an approximately 25–60% greater capacity than the other areas. These results clearly demonstrated that seaweed cultivation could be an effective method to promote CO_2 sequestration from the atmosphere. Based on the extent of the 4 areas studied and the CO_2 flux at the air–sea interface, the total CO_2 absorption of Shen'ao Bay was estimated at ca. 12 Mt C per month from March to May. CO_2 absorption values observed separately in non-culture, fish, oyster and seaweed areas were ca. 6.3, 2.5, 5.6 and 19.8 Mt C, respectively. CO_2 fixation in the seaweed area was the largest contributor to the CO_2 flux, accounting for ca. 58% of the total CO_2 sink capacity of the entire bay.

5. CONCLUSIONS

The results of the present study suggest that the seawater carbonate system and air–sea CO_2 flux of Shen'ao Bay are strongly influenced by bay-scale mariculture activities. A higher CO_2 sink capacity could be acquired by extending the culture of seaweed such as *G. lemaneiformis*.

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