



Article Experimental Study of the Environmental Effects of Summertime Cocultures of Seaweed Gracilaria lemaneiformis (Rhodophyta) and Japanese Scallop Patinopecten yessoensis in Sanggou Bay, China

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Abstract: The shellfish-algae mode of integrated multitrophic aquaculture (IMTA) is a sustainable aquaculture method that benefits the environment and the carbon cycle. However, most current shellfish-algae aquaculture modes are based on the expansion of kelp aquaculture. Due to the low tolerance of kelp to high temperatures, integrated shellfish-algae aquaculture areas often become shellfish monocultures in summer, which may lead to both high mortality rate of shellfish and to economic loss while causing serious environmental harm via eutrophication, decreases in dissolved oxygen (DO), and decreases in pH. In this study, we investigated the effects of different ratios of seaweed (Gracilaria lemaneiformis), which is tolerant of high temperatures, to Japanese scallop (Patinopecten yessoensis) on water quality and environmental parameters. A two-day small-scale enclosure water body experiment was conducted in Sanggou Bay (Shandong, China) in August 2019. The results demonstrated that culturing shellfish alone significantly affected pH, DO, eutrophication, and other environmental indicators, as well as the carbonate system. The negative environmental impact of the shellfish-algae aquaculture system was much smaller. However, too high a proportion of algae might consume excessive amounts of dissolved inorganic nitrogen (DIN) and nutrients, while too low a proportion of algae might not fully absorb the nutrients released by the cultured shellfish, in turn leading to an increased risk of eutrophication. The shellfish-algae aquaculture system not only improved the inorganic carbon system, but also the organic carbon system. At the end of the experiment, all the parameters of the inorganic carbon system had decreased significantly, while all the parameters of the organic carbon system had increased significantly. The results of this study illustrate the need to include macroalgae rotations in summer, and that an appropriate ratio of shellfish to algae is necessary to achieve a sustainable aquaculture system. Moreover, this research has also confirmed the importance of the future and related research in the actual production, which will provide useful information to guide governmental strategies for summer aquaculture rotations and insight into the controversy concerning whether aquaculture is a carbon source or sink.

Keywords: integrated multitrophic aquaculture (IMTA); Gracilaria lemaneiformis; Patinopecten yessoensis; Sanggou Bay

1. Introduction

The development of aquaculture is essential to meet growing human food requirements. However, aquaculture can also significantly enrich waters with organic matter and nutrients [1], thus making them more susceptible to eutrophication and which restricts



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). the broadscale application of aquaculture techniques. In order to overcome these problems, there is a growing global interest in the integrated multitrophic aquaculture (IMTA) mode [2]. IMTA cocultivates species of different trophic levels and ecological niches with complementary functions to achieve a healthy and sustainable aquaculture system [3]. The shellfish–algae IMTA mode has proven to be the only economical solution for recycling wasted nutrients in open water [2,4]. Additionally, cultured shellfish and algae play an important role in the coastal biocarbon cycle [5]. Carbon can be removed from the coastal ecosystem through the harvesting of shellfish and algae [6], thereby increasing the carbon sequestration capacity of marginal shelf seas, accelerating the absorption of atmospheric CO_2 by the ocean, and alleviating the global greenhouse effect. Therefore, shellfish–algae farming is an efficient and economically important mode of environmentally friendly aquaculture that benefits the environment and the coastal carbon cycle [6,7]. However, most studies on the role of shellfish–algae culture in the carbon cycle have focused on the inorganic carbon cycle, and few have considered its impact on organic carbon [7,8].

At present, the most successful shellfish–algae IMTA modes have been based on the expansion of kelp culture [9]. However, due to the intolerance of kelp to high temperatures, many shellfish-algae IMTA areas in northern China have large areas where only shellfish are being cultured for up to four months during summer. For example, Sanggou Bay is a key shellfish-algae IMTA farming base in North China that produces about 80,000 tons of kelp annually [10]. After the summer kelp is harvested, about 1/6 of the kelp farming area will be used to cultivate another macroalgae species, namely the seasonal rotational aquaculture of macroalgae. The annual production of these rotated algae accounts for approximately 1/8 of the kelp yield (data from local government). The physiological activities of shellfish without active algae may stress the natural ecosystems of the aquaculture areas [11]. Additionally, the stratification of seawater caused by freshwater input and high temperatures make eutrophication more likely in summer [12,13]. The combination of multiple environmental stressors such as temperature and eutrophication may also lead to a higher incidence of aquatic diseases in summer [14–16]. Japanese scallop (*Patinopecten* yessoensis) is a scallop that has become a major aquaculture species in northern China, covering the coastal area from Liaoning Province to Shandong Province. Recently, massive die-offs of Japanese scallop have been observed in Sanggou Bay during summer. This may be attributed to both extreme high temperatures and the absence of macroalgal cultures in summer [2,17].

Seaweed (*Gracilaria lemaneiformis*) is an algae with a high commercial value and a strong bioremediation effect [17–19] which grows fast, has a short growth cycle, and can be harvested in 2–3 months. This species can also adapt well to high temperature environments, so a rotation strategy that includes seaweed in summer can bring significant economic and environmental benefits. The physiological activity of this species in summer may also improve the conditions for the shellfish and reduce the frequency of summer shellfish die-offs. Therefore, in this study the effects of different Japanese scallop/seaweed culture modes on the marine environment and the local ecosystem were investigated in Sanggou Bay during summer. The objectives of this study were to identify the different environmental and ecological impacts of different culture modes, and to explore the benefits and feasibility of rotations that include seaweed in summer. We evaluated the impacts of shellfish–algae IMTA cultures on the marine environment and carbon cycle, identified any biologically mutual benefits, and illustrated the advantages of the IMTA culture mode. These results can be used as a reference for sustainable aquaculture activities in different regions.

2. Materials and Methods

2.1. Experiment Design and Management

A relatively closed ecosystem was constructed with water that simulated the water quality in Sanggou Bay (see Figure 1). Seaweed and Japanese scallop (wet weight ~9.5 g, shell height ~5 cm) obtained from Shandong Rongcheng Xunshan Group were used as

the IMTA culture components in this study, and the experiments were carried out using a multifunctional experimental enclosure device patented by the State Intellectual Property Office of China, as shown in Figure 2. The enclosure bag had a diameter of 1 m, a height of 2 m, and a volume of about 1.5 m³ filled with seawater. The Japanese scallops were scrubbed with a brush and placed on a plastic tray and the seaweed were suspended by a rope, then both were put into the enclosure bag which was filled with seawater and sealed by expelling all the air. The enclosures were fixed to a floating raft on the coastal sea surface. Four experimental groups and one blank control group were established, with three replicates in each group. The experiment was conducted from 18:00 on August 13, 2019 (48 h). The biomasses of the different experimental groups are shown in Table 1. Sampling was conducted at 12 h intervals. The sampling times were set to coincide with the local sunrise and sunset times.



Figure 1. The study site.



Figure 2. Experimental device: 1. Plexiglas barrel; 2. experimental bag–polyethylene material; 3. sinker stone; 4. air bag; 5. inflatable valve; 6. tie belt; 7. buckle ring–a; 8. sealing cap; 9. buckle ring–b; 10–11. locking buckle; 12. device for drainage; 13. rubber plugs; 14. rubber pads.

Mode	Japanese Scallop (g)	Seaweed (g)	Ratios
Control (0#)	0	0	/
Group 1 (1#)	400.3 ± 2.4	0	/
Group 2 (2#)	0	399.7 ± 3.22	/
Group 3 (3#)	401.3 ± 2.59	400.4 ± 7.52	1:1
Group 4 (4#)	398.87 ± 8.98	639.08 ± 6.15	1:1.6

Table 1. Density of Japanese scallop and seaweed in the different treatments.

2.2. Sample Analysis

The dissolved oxygen (DO), salinity (S), pH, and temperature (T) were determined in situ using a water analyzer (YSI, Professional Plus, Yellow Spring, OH, USA). An amount of 1000 mL water samples were collected from each replicate at each sampling time, and filtered with a filter (Nalgene, Rochester, NY, USA). A total of 200 mL of each water sample was filtered through a Whatman GF/F filter membrane precombusted at 450 °C for 5 h. The filtrate was passed through a total organic carbon analyzer to determine dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC), and the filter membrane was acidified and analyzed using an elemental analyzer (Elementar EL, Langenselbold, Germany) to measure the particulate organic carbon (POC) in the water. The partial pressure of carbon dioxide (pCO_2) and the components of the carbonate system were calculated using the CO₂SYS software, and the total alkalinity (TA) required for the calculation was measured after filtering with Whatman GF/F membranes using an 848 Titrino plus automatic titrator (Metrohm AG, Herisau, Switzerland). A total of 500 mL of each water sample was filtered with acetate membranes of three aperture sizes (20, 2, and 0.45 μ m) sequentially. The filter membranes were used to measure chlorophyll-a (Chl-a) contained via fluorometric analysis by particles of different sizes, and the sum of the three groups of Chl-a was taken as the total Chl-a concentration. The filtrate was used for the assay of nutrients, which were assayed according to the chemical methods specified in the Chinese National Standards.

2.3. Data Analysis

Data were analyzed using SPSS 19.0 statistical software. Two factor repeated ANOVA was used to detect the interactions between different experimental treatments and experimental times for each parameter. One-way ANOVA was used to detect the differences between different experimental times within the same experimental group, where p < 0.05 indicated a significant difference.

3. Results

None of the shellfish or algae died during the experiment. The water temperature and salinity varied within relatively small ranges, from 22.27 °C to 24.51 °C and 31.8 to 32.0, respectively, in all experimental groups.

3.1. Changes of pH, DO, and TA in Different Culture Modes

When the shellfish were cultured alone it resulted in acidification of the water and a decrease in both DO and TA (Figure 3a–c). The effect of the experimental treatment group and time on pH, DO, and TA were significant, and their interaction was also significant (p < 0.05; Table 2). By the end of the experiment the pH, DO, and TA values had decreased in group 1, which had shellfish only, but had significantly increased in the other three experimental groups (p < 0.05). The DO showed significant diurnal variation and was significantly higher during the day (p < 0.05, one-way ANOVA).



Figure 3. (a) The pH of modes at different times. The different letters indicate a significant difference of pH within mode at different detecting times. Means \pm SE (n = 3). (b) The DO of modes at different times. The different letters indicate a significant difference of DO within mode at different detecting times. Means \pm SE (n = 3). (c) The TA of modes at different times. The different letters indicate a significant different detecting times. Means \pm SE (n = 3). (c) The TA of modes at different times. The different letters indicate a significant difference of TA within mode at different detecting times. Means \pm SE (n = 3).

	Source	Sum of Squares df		Mean Squares	F	p	
	Mode	239.505	4	59.876	1373.056	0.000	
DO	Time	120.056	4	30.014	688.265	0.000	
	Mode \times Time	228.045	16	14.253	326.839	0.000	
	Mode	1.266	4	0.316	222.854	0.000	
pН	Time	0.409	4	0.102	72.051	0.000	
	$Mode \times Time$	0.672	16	0.042	29.575	0.000	
	Mode	36,383.561	4	9095.890	7517.265	0.000	
TA	Time	4551.065	4	1137.766	940.303	0.000	
	Mode \times Time	45,434.731	16	2839.671	2346.835	0.000	

Table 2. Two-way repeated ANOVA results of DO, pH, TA in the seawater of modes at different sampling times.

3.2. Variation of Chl-a and Components in Different Culture Modes

The concentration of Chl-*a* showed an overall increasing trend, with the smallest increase being in the shellfish only culture group. According to the two factor ANOVA, total Chl-*a* was significantly affected by the treatment and time, as well as the interaction between the two factors (p < 0.05; Table 3). The total Chl-*a* of all groups showed initial increasing trends and then decreasing trends (Figure 4a), and the total Chl-*a* of group 1 at the end of the experiment was not significantly different from that at the start (p > 0.05). The total Chl-*a* of the other groups were all significantly higher at the end than that at the beginning of the experiment (p < 0.05).

Table 3. Two-way repeated ANOVA results of Chl-a in the seawater of modes at different sampling times.

Source		Sum of Squares	df Mean Squares		F	p
	Mode	21.224	4	5.306	124.961	0.000
Chl-a	Time	249.662	4	62.415	1469.937	0.000
	$Mode\timesTime$	30.099	16	1.881	44.303	0.000

The structural compositions of Chl-*a* throughout the experiment for each group are shown in Figure 4b. The composition of Chl-*a* in the enclosure system was categorized by the particle size. At the beginning of the experiment the contribution of nanophytoplankton (2–20 μ m) was the largest, accounting for 86.66% of the Chl-*a*, while microphytoplankton (>20 μ m) and picophytoplankton (0.45–2 μ m) contributed 4.95% and 8.39%, respectively. At the end of the experiment, the contribution of nanophytoplankton to Chl-*a* decreased to 66.28% and 58.25% in the control and experimental group 1, respectively, but increased to 93.68% in group 2. The contributions of Chl-*a* by different particle sizes in experimental group 3 and group 4 did not significantly change during the experiment (*p* > 0.05).

3.3. Changes in DIC, pCO₂, and the Carbonate System in Different Aquaculture Modes

There were significant differences in DIC, pCO_2 , and the carbonate system (HCO₃⁻, CO_3^{2-} , and CO_2) between groups and times, and the interaction between the two factors was also significant (p < 0.05; Table 4). The DIC showed the same trend in all groups, with significant decreases during the day and increases at night (p < 0.05, one-way ANOVA). The DIC was significantly lower at the end of the experiment than at the beginning in all groups (p < 0.05; Figure 5a). Among the carbonate system components, HCO₃⁻ and CO_3^{2-} were significantly lower (p < 0.05) at the end of the experiment than that at the beginning and exhibited diurnal fluctuations (Figure 5c). The changes in CO_2 and pCO_2 were consistent with each other. The diurnal variation of CO_2 and pCO_2 was obvious in group 1, lower during the day and higher at night (p < 0.05), and their concentrations were significantly higher at the end of the experiment than at the beginning. The CO_2 and pCO_2 of the other groups were significantly lower after the end of the experiment than at the beginning of the experiment (p < 0.05). The trends in CO_2 and pCO_2 in groups 3 and 4

were the same and they exhibited a significant diurnal pattern (p < 0.05). At the end of the experiment, the CO₂ and pCO₂ in group 4 were significantly lower than in group 3 but not significantly different from those in group 2 (Figure 5b,c).

Table 4. Two-way repeated ANOVA results of DIC, *p*CO₂, and carbonate system in the seawater of modes at different sampling times.

	Source	Sum of Squares	df	Mean Squares	F	р
	Mode	215,895.095	4	53,973.774	10,709.048	0.000
DIC	Time	7,725,702.666	4	1,931,425.667	383,218.162	0.000
	$Mode\timesTime$	198,111.298	16	12,381.956	2456.730	0.000
	Mode	1,664,176.577	4	416,044.144	80,420.904	0.000
pCO_2	Time	307,442.537	4	76,860.634	14,857.081	0.000
	$Mode\timesTime$	823,088.365	16	51,443.023	9943.883	0.000
	Mode	250,760.705	4	62,690.176	2072.403	0.000
HCO_3^-	Time	6,416,415.953	4	1,604,103.988	53,028.231	0.000
-	$Mode\timesTime$	190,286.215	16	11,892.888	393.153	0.000
	Mode	5749.88	4	1437.475	47.520	0.000
CO_{3}^{2-}	Time	66,728.177	4	16,682.044	551.473	0.000
-	$Mode\timesTime$	11,856.723	16	741.045	24.497	0.000
CO ₂	Mode	1929.804	4	482.451	15.949	0.000
	Time	433.633	4	108.408	3.584	0.012
	$Mode\timesTime$	1073.704	16	67.107	2.218	0.016

3.4. POC and DOC in Different Culture Modes

The POC and the DOC were significantly dependent on the treatment and time, whose interaction was also significant (p < 0.05; Table 5). At the end of the experiment, the POC of group 1 was significantly lower than before the experiment, while the POC of the remaining groups were all significantly higher (p < 0.05; Figure 6a). The change of the DOC was similar to that of the POC. After the experiment, the DOC of all groups was significantly higher than that at the beginning (p < 0.05), except for group 1. Furthermore, the DOC of different groups was significantly different (p < 0.05; Figure 6b).

Table 5. Two-way repeated ANOVA results of POC and DOC in the seawater of modes at different sampling times.

Source		Sum of Squares	df	Mean Squares	F	р	
	Mode	48,740.061	4	12,185.015	50.286	0.000	
DOC	Time	125,626.612	4	31,406.653	129.611	0.000	
	$Mode\timesTime$	76,374.501	16	4773.406	19.699	0.000	
	Mode	1.599	4	0.400	53.790	0.000	
POC	Time	1.057	4	0.264	35.548	0.000	
	Mode \times Time	1.219	16	0.076	10.248	0.000	





Figure 4. (a) The Chl-*a* of modes at different times. The different letters indicate a significant difference of pH within mode at different detecting times. Means \pm SE (*n* = 3). (b) The size fraction of Chl-*a* of modes at different times.

(b)

3.5. Variation of Nutrients in Different Culture Modes

The nutrient concentrations and structural changes are shown in Table 6. At the end of the experiment, the concentration of dissolved inorganic nitrogen (DIN) was significantly higher in group 1, which had shellfish only, than in the other experimental groups, and it had also significantly increased over the course of the experiment (p < 0.05). Group 1 also had significantly higher phosphate (PO₄) than the other groups at the end of the experiment (p < 0.05), but it was not significantly different from the beginning of the experiment (p > 0.05). The concentration of silicate (SiO₄) had also significantly decreased in all experimental groups containing shellfish. The nutrients in the other groups all showed decreasing trends, with DIN and phosphate decreasing the most in the group 2, which had algae only.



(b)

Figure 5. Cont.



Figure 5. (a) The DIC of modes at different times. The different letters indicate a significant difference of DIC within mode at different detecting times. Means \pm SE (n = 3). (b) The fluctuations of pCO_2 in modes at different times. The different letters indicate a significant difference of pCO_2 within mode at different detecting times. Means \pm SE (n = 3). (c) The variation of carbonate system in modes at different times: (ci) HCO₃⁻; (cii) CO₃²⁻; (ciii) CO₂. The different letters indicate a significant difference of HCO₃⁻, CO₃²⁻, and CO₂ within modes at different detecting times. Means \pm SE (n = 3).



(a) 1200 а a a bbbb 1000 b_cbbb_ a d сассс c_c_dd DOC (µmol/L) 800 □ 0# □ 1# 600 ⊠ 2# 400 ⊞ 3# 200 ፼ 4# 0 D1 18:00 D2 6:00 D2 18:00 D3 6:00 D3 18:00 Time

(**b**)

Figure 6. (a) The POC of modes at different times. The different letters indicate a significant difference of POC within modes at different detecting times. Means \pm SE (n = 3). (b) The DOC of modes at different times. The different letters indicate a significant difference of DOC within modes at different detecting times. Means \pm SE (n = 3).

Mode	Time	SiO ₄	DIN	PO ₄	Si:P	N:P	Si:N
All gourps	Initial	14.88 ± 2.35	16.04 ± 1.32	1.57 ± 0.27	9.48	10.22	0.93
0#	Final	14.32 ± 1.22	3.85 ± 0.87	0.48 ± 0.12	29.83	8.02	3.72
1#	Final	6.56 ± 1.01	18.86 ± 2.21	1.32 ± 0.24	4.97	14.29	0.35
2#	Final	14.77 ± 1.25	2.37 ± 1.06	0.30 ± 0.11	36.93	7.9	6.23
3#	Final	8.21 ± 0.97	9.64 ± 1.13	0.49 ± 0.25	16.76	19.67	0.60
4#	Final	8.94 ± 1.33	8.9 ± 1.21	0.34 ± 0.10	26.29	26.17	0.69

Table 6. The nutrient concentrations and structural changes in the seawater of modes at the beginning and at the end of the experiment.

4. Discussion

In summer, when much of the kelp is harvested under high temperatures, the main issue of shellfish culture becomes the impact of the substances released and excreted during their growth process on the aquatic environment. In our study, the pH and DO were significantly reduced (p < 0.05) by the end of the experiment in group 1, where shellfish were cultured alone, and the DO concentration had dropped to below 5 mg/L. The increase in DIN concentration also demonstrated how shellfish cultures can exacerbate the risk of eutrophication. Zhang [20] has reported that shellfish were unable to form shells at pH 7.3, at DO levels of 4–5 mg/L where the growth of fish was inhibited and some even died, and at DO of 3–4 mg/L where the mass mortality of crustaceans can occur [21–23]. Therefore, if the shellfish culture scale is large, mismanagement may easily bring harm to the environment in semienclosed areas or in areas with poor hydrodynamics. Therefore, as during summer the common cocultivated algae, the species of kelp, cannot be grown due to high water temperature, it is necessary to use macroalgal rotation strategies for cocultures to avoid the negative effects of shellfish monocultures.

In the shellfish–algae IMTA areas, after the summer kelp is harvested, the kelp farming area can be used to cultivate seaweed, which can improve environmental parameters and fully absorb the nutrients in the water, thereby alleviating the environmental pressure caused by the shellfish culture [24–28]. However, only the appropriate ratio of shellfish to algae can achieve an environmentally friendly aquaculture system [7]. Generally, it seems that the greater the proportion of algae, the greater the benefit to environmental indicators (e.g., pH and DO) [2]. However, in low nutrient areas, or areas with poor water exchange, cultivation with too large a proportion of algae may lead to the excessive consumption of nutrients such as DIC, N, and P [29]. The same conclusion was reached in our study. At the end of the experiment, the DIC of group 2 with only algae was significantly reduced and the DIN was nearing the threshold of 2 μ mol/L at which point N becomes a limiting factor for phytoplankton growth [30,31]. In contrast, at the end of the experiment in the shellfish–algae IMTA mode group, N:P:Si did not deviate from the Redfield ratio (N:P:Si = 16:1:16) [32] so that there was no potential limiting nutrient, thus demonstrating that the shellfish–algae mode had little to no negative influence on the ambient nutrient structure.

Chl-*a* reflects the biomass of the phytoplankton, which is the main food source for shellfish. The phytoplankton community faces downward controlling effects due to shell-fish ingestion and upward controlling effects due to competition with macroalgae for nutrients during growth [33,34]. In our study, when the ratio of shellfish to algae was 1:1, the concentration of Chl-*a* at end of the experiment was significantly higher than that in other experimental groups. This was because shellfish have phytoplankton size preferences and actively select the size class on which they feed, and Japanese scallop mainly feeds on nanophytoplankton (2–20 μ m) [35,36]. This meant that the growth of microphytoplankton (>20 μ m) and picophytoplankton (0.45–2 μ m) were not impeded in this shellfish–algae system. In order to avoid eutrophication and food shortages for shellfish, the Chl-*a* concentration should be maintained at a stable level. Furthermore, it appeared that in the actual culture process if the proportion of algae is too low the upward controlling effect of

macroalgae on nutrient competition was not strong, which could lead to overpopulating by picophytoplankton and microphytoplankton.

Under high temperature conditions the respiration rate of scallops and CO_2 production increased but the IMTA mode was able to effectively absorb the nutrients released by the shellfish and thereby prevent the accumulation of CO_2 produced by respiration. In the shellfish–algae IMTA mode, 70% of the CO₂ released by shellfish can be absorbed by algae [8], making the aquaculture system a net carbon sink [7]. Han [37] reported that when the Chlamys farreri to seaweed ratio exceeded 1:0.31, the inorganic carbon system in the water was improved. Likewise, this study found that the DIC, carbonate system components, and the pCO_2 were all significantly reduced in the shellfish–algae polyculture group. We also found that the greater the proportion of algae, the more significant the impact on the inorganic carbon cycle. It should be noted that cultivation with too large a proportion of algae might lead to the excessive consumption of nutrients, and that the pursuit of improving the inorganic carbon system of the water body needs to be tempered by the awareness of other environmental parameters. Our study verified the importance of the appropriate culture ratios in practical production. At the end of the experiment, when the ratio of shellfish to algae was 1:1.6, the pCO_2 and the inorganic carbon system components were not significantly different from those in group 2 where the algae were cultured alone. Therefore, it seems possible to assume that the most significant effect on the inorganic carbon cycle was observed in summer when the ratio of Japanese scallop to seaweed was 1:1.6 under experimental conditions. However, in nature there is water exchange in areas occupied by aquaculture, and the real level of water quality parameters may differ significantly from those obtained in a closed experimental system. The optimal ratio of scallop to algae may also differ significantly from that which is experimentally determined in a closed system. Further large-scale field studies are needed to assess the optimal scallop and algae ratio.

Although shellfish–algae farming influences the inorganic carbon system in the water, the "inorganic carbon pump" only enables the transport of CO_2 from the atmospheric carbon reservoir to the oceanic carbon reservoir. For the long-term storage of CO_2 , the CO_2 entering the ocean must be entrained by the "biological carbon pump" [38]. Therefore, the cycling of organic carbon by the aquaculture process is important and should also be considered. Although CO_2 emissions from shellfish respiration do not affect the TA (i.e., the ability to neutralize hydrogen ions) in seawater [20], calcification by shellfish decreases the TA, which limits the ability of seawater to absorb CO_2 and further affects the carbonate chemistry equilibrium [39]. Therefore, if we only look at the direct evidence of inorganic carbon system parameters in the water, shellfish farming should be a source of carbon. However, it has been established that shellfish aquaculture can be a carbon sink, not only via absorbing the DIC and synthesizing the shells, but also through its efficient water filtration and biological activities, which can effectively filter out particulate carbon and transport it to the sediment [6,8]. In our study, it was found that the POC and the DOC had decreased significantly by the end of the experiment in group 1, which had only shellfish cultures. Studies on the shellfish carbon balance have found that 30% of the carbon used by shellfish during a growth cycle was retained by the shellfish itself, 30% sunk to the bottom of the sea as waste, and 30–40% was released by the respiration and calcification processes [40,41]. Therefore, the controversy around whether shellfish aquaculture is a carbon source or sink should not only be based only on the inorganic carbon cycle but should include more detailed studies that track inorganic and organic carbon as well as the physiological processes of the shellfish themselves. Comprehensive studies such as this one may provide insight into the controversial issue of carbon sequestration in shellfish. Additionally, in the groups containing algae, the DOC content significantly increased. The DOC makes up a portion of organic carbon that is effectively sequestered as it enters food webs via the action of microfood loops or forms inert organic carbon and stays in the sea for a long time [42,43]. According to Jiao [44], microbial carbon sequestration as a carbon sink is often overlooked, and the core mechanism of microbial carbon sequestration is its

conversion of DOC into refractory DOC (RDOC) that is stored in seawater for extended periods. Therefore, the organic carbon cycle also plays an important role as a carbon sink in aquaculture systems. The shellfish–algae IMTA mode not only changes the inorganic carbon system, but also effectively changes the organic carbon system, thus increasing the function and role of aquaculture as a carbon sink.

5. Conclusions

In summer the respiratory efficiency of shellfish increases due to high temperatures, and so large-scale shellfish cultures can easily harm environments in semienclosed areas or in areas with poor hydrodynamics. Therefore, it is necessary to utilize macroalgae rotation cultivation strategies in these areas in summer so to alleviate the effects of shellfish monocultures. This study has shown that the summertime rotational aquaculture with macroalgae benefited both the environment and the growth of shellfish. The shellfish-algae IMTA mode not only mutually benefitted the shellfish and algae, but also positively affected carbon sequestration. Moreover, the shellfish-algae IMTA mode not only altered the inorganic carbon system, but also effectively changed the organic carbon system. However, only the appropriate ratio of shellfish to algae can achieve an environmentally friendly aquaculture system. Since this experiment was conducted in an enclosed environment, it is necessary to further investigate and verify these results on a larger scale in the aquaculture area in order to extend these findings to the production level. Such experiments will provide supportive information for making governmental policies that guide the rotational aquaculture of macroalgae in summer, as well as provide data that may help resolve the controversy around aquaculture as a carbon sink.

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References

- 1. Zhang, Y. Comparison of Culture Effect, Discharge of Nitrogen and Phosphorus and Environmental Influence for Three Kinds of Cages. Master's Thesis, Huazhong Agriculture University, Wuhan, China, 2012. (In Chinese with English abstract).
- Fang, J.; Fang, J.; Chen, Q.; Mao, Y.; Jiang, Z.; Du, M.; Gao, Y.; Lin, F. Assessing the effects of oyster/kelp weight ratio on water column properties: An experimental IMTA study at Sanggou Bay, China. J. Oceanol. Limnol. 2019, 38, 3–4. [CrossRef]
- 3. Reid, G.K.; Lefebvre, S.; Filgueira, R.; Robinson, S.M.C.; Broch, O.J.; Dumas, A.; Chopin, T.B.R. Performance measures and models for open-water integrated multi-trophic aqua-culture. *Rev. Aquac.* **2018**, *12*, 47–75. [CrossRef]
- Troell, M.; Halling, C.; Neori, A.; Chopin, T.; Buschmann, A.; Kautsky, N.; Yarish, C. Integrated mariculture: Asking the right questions. *Aquaculture* 2003, 226, 69–90. [CrossRef]
- 5. Tang, Q.S.; Fang, J.G.; Zhang, J.H.; Jiang, Z.J.; Liu, H.M. Impacts of multiple stressors on coastal ocean ecosystems and integrated multi-trophic aquaculture. *Prog. Fish. Sci.* 2013, 34, 1–11, (In Chinese with English abstract).

- 6. Tang, Q.; Zhang, J.; Fang, J. Shellfish and seaweed mariculture increase atmospheric CO2 absorption by coastal ecosystems. *Mar. Ecol. Prog. Ser.* 2011, 424, 97–104. [CrossRef]
- Jiang, W.; Fang, J. Effects of mussel-kelp ratios in integrated mariculture on the carbon dioxide system in Sanggou Bay. J. Sea Res. 2021, 167, 101983. [CrossRef]
- 8. Jiang, Z.; Wang, G.; Fang, J.; Mao, Y. Growth and food sources of Pacific oyster Crassostrea gigas integrated culture with Sea bass Lateolabrax japonicus in Ailian Bay, China. *Aquac. Int.* **2012**, *21*, 45–52. [CrossRef]
- 9. Chopin, T.; Robinson, S.; Troell, M.; Neori, A.; Buschmann, A.; Fang, J. Multitrophic Integration for Sustainable Marine Aquaculture. *Encycl. Ecol.* 2008, 2463–2475. [CrossRef]
- 10. Ning, Z.; Liu, S.; Zhang, G.; Ning, X.; Li, R.; Jiang, Z.; Fang, J.; Zhang, J. Impacts of an integrated multi-trophic aquaculture system on benthic nutrient fluxes: A case study in Sanggou Bay, China. *Aquac. Environ. Interact.* **2016**, *8*, 221–232. [CrossRef]
- 11. Chauvaud, L.; Thompson, J.K.; Cloern, J.; Thouzeau, G. Clams as CO₂ generators: The Potamocorbula amurensis example in San Francisco Bay. *Limnol. Oceanogr.* 2003, *48*, 2086–2092. [CrossRef]
- 12. Suikkanen, S.; Silvia, P.; Engström-Öst, J.; Lehtiniemi, M.; Lehtinen, S.; Brutemark, A. Climate Change and Eutrophication Induced Shifts in Northern Summer Plankton Communities. *PLoS ONE* **2013**, *8*, e66475. [CrossRef]
- 13. Joseph, S.S.; Winston, R.J.; Tirpak, R.A.; Wituszynski, D.M.; Boening, K.M.; Martin, J.F. The seasonality of nutrients and sediment in residential stormwater runoff: Impli-cations for nutrient-sensitive waters. *J. Environ. Manag.* 2020, 276, 111248.
- 14. Su, J.; Cai, W.-J.; Brodeur, J.; Chen, B.; Hussain, N.; Yao, Y.; Ni, C.; Testa, J.M.; Li, M.; Xie, X.; et al. Chesapeake Bay acidification buffered by spatially decoupled carbonate mineral cycling. *Nat. Geosci.* **2020**, *13*, 441–447. [CrossRef]
- 15. Nikinmaa, M. Climate change and ocean acidification—Interactions with aquatic toxicology. *Aquat. Toxicol.* **2013**, *126*, 365–372. [CrossRef] [PubMed]
- 16. Kanerva, M.; Kiljunen, M.; Torniainen, J.; Nikinmaa, M.; Dutz, J.; Vuori, K.A. Environmentally driven changes in Baltic salmon oxidative status during marine migration. *Sci. Total Environ.* **2020**, 742, 140259. [CrossRef] [PubMed]
- Zhou, Y.; Yang, H.; Hu, H.; Liu, Y.; Mao, Y.; Zhou, H.; Xu, X.; Zhang, F. Bioremediation potential of the macroalga Gracilaria lemaneiformis (Rhodophyta) integrated into fed fish culture in coastal waters of north China. *Aquaculture* 2006, 252, 264–276. [CrossRef]
- 18. Fei, X.G.; Lu, S.; Bao, Y.; Wilkes, R.; Yarish, C. Seaweed cultivation in China. World Aquac. 1998, 29, 22–24.
- 19. Fei, X.G.; Bao, Y.; Lu, S. Seaweed cultivation—Traditional way and its reformation. Aquac. Oceanol. Limnol. Sin. 1999, 17, 193–199.
- Zhang, M.; Fang, J.; Zhang, J.; Bin, L.; Ren, S.; Mao, Y.; Gao, Y. Effect of Marine Acidification on Calcification and Respiration of Chlamys farreri. J. Shellfish Res. 2011, 30, 267–271.
- 21. Sugden, A.M. Threats of coastal hypoxia. Science 2017, 356, 38. [CrossRef] [PubMed]
- Levin, L.; Breitburg, D.L. Linking coasts and seas to address ocean deoxygenation. *Nat. Clim. Chang.* 2015, *5*, 401–403. [CrossRef]
 Vaguer-Sunver, R.; Duarte, C.M. Thresholds of hypoxia for marine biodiversity. *Proc. Natl. Acad. Sci. USA* 2008, *105*, 15452–15457.
- Vaquer-Sunyer, R.; Duarte, C.M. Thresholds of hypoxia for marine biodiversity. *Proc. Natl. Acad. Sci. USA* 2008, 105, 15452–15457. [CrossRef]
- 24. Fang, J.H.; Zhang, J.H.; Wu, W.G.; Mao, Y.Z.; Gao, Y.P.; Jiang, Z.J.; Fang, J.G. Carbon and nitrogen budget and environmental optimization in an integrated cage culture model of Japanese flounder with Perinereis aibuhitensis. *J. Fish. Sci.* **2014**, *21*, 390–397, (In Chinese with English abstract).
- 25. Mao, Y.; Yang, H.; Zhou, Y.; Ye, N.; Fang, J. Potential of the seaweed Gracilaria lemaneiformis for integrated multi-trophic aquaculture with scallop Chlamys farreri in North China. *J. Appl. Phycol.* **2009**, *21*, 649–656. [CrossRef]
- 26. Phillips, J.; Hurd, C. Nitrogen ecophysiology of intertidal seaweeds from New Zealand: N uptake, storage and utilisation in relation to shore position and season. *Mar. Ecol. Prog. Ser.* **2003**, 264, 31–48. [CrossRef]
- 27. Pedersen, A.; Kraemer, G.; Yarish, C. The effects of temperature and nutrient concentrations on nitrate and phosphate uptake in different species of Porphyra from Long Island Sound (USA). *J. Exp. Mar. Biol. Ecol.* **2004**, *312*, 235–252. [CrossRef]
- 28. Lartigue, J.; Sherman, T.D. Response of *Enteromorpha* sp. (Chlorophyceae) to a nitrate pulse: Nitrate uptake, inorganic nitrogen storage and nitrate reductase activity. *Mar. Ecol. Prog. Ser.* **2005**, 292, 147–157. [CrossRef]
- 29. Zou, D.H.; Xia, J.R.; Yang, Y.F. Photosynthetic use of exogenous inorganic carbon in the agarophyte Gracilaria lemaneiformis (Rhodophyta). *Aquaculture* **2004**, 237, 421–431. [CrossRef]
- Fisher, T.; Peele, E.; Ammerman, J.; Harding, L. Nutrient limitation of phytoplankton in Chesapeake Bay. *Mar. Ecol. Prog. Ser.* 1992, 82, 51–63. [CrossRef]
- 31. Justić, D.; Rabalais, N.N.; Turner, R.E.; Dortch, Q. Changes in nutrient structure of river-dominated coastal waters: Stoichiometric nutrient balance and its consequences. *Estuar. Coast. Shelf Sci.* **1995**, *40*, 339–356. [CrossRef]
- 32. Redfield, A.C.; Ketchum, B.H.; Richard, F.A. The influence of organism on the composition of seawater. Sea 1963, 2, 26–77.
- 33. Prins, T.C.; Escaravage, V.; Smaal, A.C.; Peeters, J.C.H. Nutrient cycling and phytoplankton dynamics in relation to mussel grazing in a mesocosm experiment. *Ophelia* **1995**, *41*, 289–315. [CrossRef]
- 34. Souchu, P.; Vaquer, A.; Collos, Y.; Landrein, S.; Deslous-Paoli, J.; Bibent, B. Influence of shellfish farming activities on the biogeochemical composition of the water column in Thau lagoon. *Mar. Ecol. Prog. Ser.* **2001**, *218*, 141–152. [CrossRef]
- Dupuy, C.; Vaquer, A.; Lam-Höai, T.; Rougier, C.; Mazouni, N.; Lautier, J.; Collos, Y.; Le Gall, S. Feeding rate of the oyster Crassostrea gigas in a natural planktonic community of the Mediterranean Thau Lagoon. *Mar. Ecol. Prog. Ser.* 2000, 205, 171–184. [CrossRef]

- Lu, J.C.; Huang, L.F.; Xiao, T.; Jiang, Z.; Zhang, W. The effects of Zhikong scallop (Chlamys farreri) on the microbial food web in a phospho-rus-deficient mariculture system in Sanggou Bay, China. *Aquaculture* 2015, 448, 341–349. [CrossRef]
- Han, T.; Jiang, Z.; Fang, J.; Zhang, J.; Mao, Y.; Zou, J.; Huang, Y.; Wang, D. Carbon dioxide fixation by the seaweed Gracilaria lemaneiformis in integrated multi-trophic aquaculture with the scallop Chlamys farreri in Sanggou Bay, China. *Aquac. Int.* 2013, 21, 1035–1043. [CrossRef]
- 38. Schlitzer, R. Export and sequestration of particulate organic carbon in the north pacific from inverse modeling. In Proceedings of the Workshop of the JGOFS North Pacific Process Study Synthesis Group, Sapporo, Japan, 2 October 2002.
- 39. Han, T.; Shi, R.; Qi, Z.; Huang, H.; Wu, F.; Gong, X. Biogenic acidification of Portuguese oyster Magallana angulata mariculture can be mediated through introducing brown seaweed Sargassum hemiphyllum. *Aquaculture* **2020**, *520*, 734972. [CrossRef]
- 40. Ren, L.H.; Zhang, J.H.; Fang, J.G.; Tang, Q.H.; Liu, Y.; Du, M.R. The diurnal rhythm of respiration, excretion and calcification in oyster Crassostre gigas. *Prog. Fish. Sci.* 2013, 1, 75–81, (In Chinese with English abstract).
- 41. Zhang, J.H.; Fang, J.G.; Tang, Q.S.; Ren, L.H. Carbon sequestration rate of the scallop Chlamys farreri cultivated in different areas of Sanggou Bay. *Prog. Fish. Sci.* 2013, 34, 12–16, (In Chinese with English abstract).
- 42. Zhang, Y.; Zhang, J.; Liang, Y.; Li, H.; Li, G.; Chen, X.; Zhao, P.; Jiang, Z.; Zou, D.; Liu, X.; et al. Carbon sequestration processes and mechanisms in coastal mariculture environments in China. *Sci. China Earth Sci.* **2017**, *60*, 2097–2107. [CrossRef]
- Li, H.; Zhang, Y.; Liang, Y.; Chen, J.; Zhu, Y.; Zhao, Y.; Jiao, N. Impacts of maricultural activities on characteristics of dissolved organic carbon and nutrients in a typical raft-culture area of the Yellow Sea, North China. *Mar. Pollut. Bull.* 2018, 137, 456–464. [CrossRef] [PubMed]
- 44. Jiao, N.; Cai, R.; Zheng, Q.; Tang, K.; Liu, J.; Jiao, F.; Wallace, D.; Chen, F.; Li, C.; Amann, R.; et al. Unveiling the enigma of refractory carbon in the ocean. *Natl. Sci. Rev.* 2018, *5*, 459–463. [CrossRef]