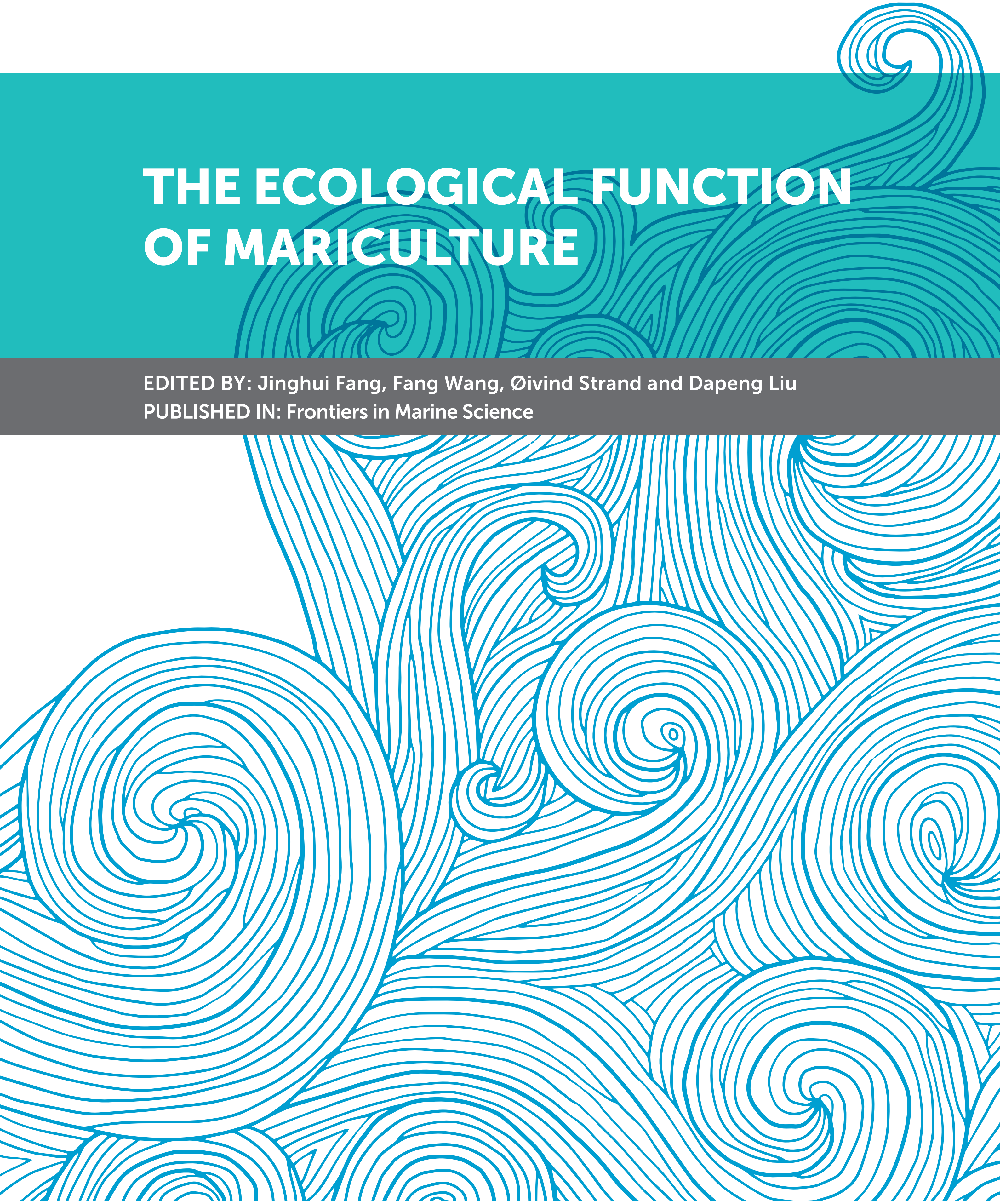
THE ECOLOGICAL FUNCTION OF MARICULTURE



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THE ECOLOGICAL FUNCTION OF MARICULTURE

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Editorial: The ecological function of mariculture

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Editorial on the Research Topic
The ecological function of mariculture

FAO reported that about 90% of the world's commercial marine fishes have been overfished or are on the collapsed verge (maximum production) (FAO, 2020). The continuous increase in consumption of seafood is in contradiction with the seafood supply, especially capture fisheries. The aquaculture production is steadily increasing and surpassed the production of capture fisheries from 2014 (FAO, 2016). It was 57% (122.6 million tons) of the world aquatic production (214 million tons) in 2020 (FAO, 2022). The aquaculture production in the marine environment (mariculture) was 33 million tons in 2020 (FAO, 2022). Mariculture is increasingly seen as an alternative to capture fisheries to provide a growing human population with high-quality protein (Costello et al., 2020).

Aquaculture of fed species (e.g., fish in cages) relies on external food supplies, causing possible associated impact on environment (Tacon and Forster, 2003). Shellfish aquaculture can accelerate the turnover of phytoplankton to reduce eutrophication and play a role in carbon sink processes (Bricker et al., 2018). Seaweeds aquaculture, which can reduce nutrient loadings to the environment from fed species aquaculture, has not yet been attractive in many countries as algal products typically have a low value (Stedt et al., 2022). It is well known that combining different species in aquaculture systems or applying proper aquaculture methods and managements could provide more profit and have concomitant ecological benefits (Fang et al., 2016; Strand et al., 2019).

Sustainable aquaculture will be an eternal theme of the development of aquaculture. The ecosystem approach has emerged as concept in assessments of sustainability of aquaculture development, also through analysis of ecosystem goods and services (Smaal et al., 2019). This can provide methods to integrate environmental, economic and social aspects. Zhou et al. performed a systematic literature review of studies with the aim of understanding the ecosystem goods and services of mariculture in China. The ecosystem services of mariculture in China, not only in China, includes five main ecosystem functions: food supply, nutrient extraction, carbon sequestration, biodiversity conservation, and stock enhancement. Mariculture is closely relative integrated with coastal ecosystems. The inter-connections among mariculture production systems, the surrounding environment and local organisms must be thoroughly understood. As a result, interest in exploring the potential for ecological function of aquaculture in marine ecosystems is growing (Fang et al., 2016; ICES, 2020; Khanjani et al., 2022). The innovations of sustainable mariculture theories, methods, systems, technologies etc., are developing fast. Mariculture provides high quality seafood, and at the same time plays important ecological functions.

The ecological function of seaweed aquaculture

Macroalgae play an important role in nutrient extraction in coastal environments, and their role in carbon sequestration resisting global warming are promoted. At the same time they are affected by the environmental factors impacted by climate change. Zhang et al. found *Sargassum fusiforme* cultivation could increase DO and pH levels and decrease nitrogen and phosphorus levels in addition to enhancing phytoplankton community biodiversity, which was an effective approach for mitigating environmental problems in marine ecosystems. Broch et al. used a high resolution coastal and ocean hydrodynamic model system to investigate the transport and deposition patterns of Particulate Organic Matter (POM) from kelp farm. They underscore the dispersal and deposition of detritus from kelp cultivation associated environmental risks posed by organic loading, and the potential for seafloor carbon sequestration by kelp farming as a nature based climate solution. Endo and Gao reviewed the interactive effects of warming and nutrient enrichment on macroalgae production. They predicted that global warming would enhance bottom-up effects on primary production in cold seasons and areas, and there would be a negative warming effect on production in hot seasons and areas, but it might be possible to mitigate this effect by appropriate levels of nutrient enrichment.

The ecological function of shellfish aquaculture

Shellfish aquaculture interacts with marine ecosystems through processes like nutrient cycling, trophic recourses, substrate engineering etc. Sean et al. proved that the mussel farm enriched benthic communities at line- and bay-scales Îles de la Madeleine, eastern Canada. Spatial structure in the distribution of macrofauna was evident within the aquaculture lease as most species were more abundant directly below and close to mussel lines and anchor blocks. At the same time, oyster farm can provide habitat for marine organisms and increase the biodiversity of microbenthic animals. Chan et al. assessed the biodiversity associated with an abandoned benthic oyster farm. The oyster farm provided habitat and trophic support for associated benthic macrofauna. It showed that oyster aquaculture could improve the restoration potential of oyster reefs. There is also other impacts of aquaculture on the wild animals. Drolet et al. found the aquaculture-related diets from shellfish farm and salmonid farm had different effects on the long-term performance and condition of the rock crab (*Cancer irroratus*). The mussel-only diet impact little on the crabs, whereas the salmonid feed diet resulted in negative impacts on condition. However, the impacts should be investigated in wild environment in the future.

Bivalve aquaculture plays a role in nitrogen extraction. Guyondet et al. used coupled hydro-biogeochemical modeling to integrate all relevant interactions in the assessment of bivalve culture as a nitrogen extraction solution, which provided a new method to assess the eutrophication mitigation. Bivalve culture was shown to provide a net nitrogen removal in the majority of the tested scenarios. Mussel rather than oyster farming provided the strongest potential for nutrient loading mitigation.

The buried bivalve could increase sediment mineralization process. However, they were adversely affected by decreased dissolved oxygen and increased hydrogen sulfide in the sediment induced by marine heat wave, which was proved by Liu et al. from the behavior and physiological metabolism aspects of Manila clam (*Ruditapes philippinarum*).

The ecological function of sea ranching

Artificial reefs are popularly used in the enhancement of sea cucumber (*Apostichopus japonicus*). Xu et al. found that the sea cucumber farm provided spawning and first-year nursery grounds for wild black rockfish (*Sebastes schlegelii*) in Bohai sea, China. The same time, the increased black rockfish could affect other organisms, including crustacea, mollusk and *Hexagrammos otakii*, in both positive and negative way. So, the interaction effects between sea ranching and wild organisms need further research.

The ecological function of polyculture

Polyculture is an important concept in acheiving sustainable development of aquaculture. The well known and successful polyculture example is the shellfish-macroalgae integrated multi-trophic aquaculture (IMTA) (Fang et al., 2016). Liu et al. found the macroalgae and shellfish-macroalgae aquaculture waters absorbed CO_2 from atmosphere by determined the carbonate system and pCO_2 in Sanggou Bay, China. Jiang et al. found that the presence of kelp in integrated aquaculture may help shield oyster from the negative effects of elevated seawater pCO_2, which was due to the kelp improving pHNBS in the enriched CO_2 experimental system, and then the oyster could keep a relevant normal clearance rate and scope for growth. In the polyculture pond, Feng et al. constructed three Ecopath models representing the early, middle, and late culture periods of a *Portunus trituberculatus* polyculture ecosystem, respectively. The results demonstrated that detritus was the main energy source in this polyculture ecosystem. *R. philippinarum* had a dominant influence on phytoplankton community dynamics which changed from nano- to pico-phytoplankton predominance, from the middle to the late period. The ecosystem stability was decreasing during the culturing period, but it can be optimized by stocking more *R. philippinarum* and involving macro-algae in the pond.

Author contributions

JF and FW wrote the editorial. ØS and DL contributed to review and correct the editorial. All authors approved the submitted manuscript for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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A New Classification Tool and a Systematic Review of Macroalgal Studies Disentangle the Complex Interactive Effects of Warming and Nutrient Enrichment on Primary Production

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In order to understand how global warming effects on ecosystem primary production may change depending on nutrient enrichment, a new classification is proposed to disentangle and recognize the combination of interactions among several factors, based on the effect direction (positive, negative, or neutral) and its changes induced in it by the other factor (synergizing, antagonizing, inducing no change, or changing it in some other way). Marine macroalgae were chosen (as primary producers for which there is the most experimental information available) to review the relevant studies on which this new classification can be tested. It was observed the positive effects of elevated temperature and nutrient enrichment often synergized each other within the temperature range between relatively low and optimal growth levels. However, the negative effect of further temperature elevation from optimal to higher levels was antagonized by nutrient enrichment in some studies but was synergized in others, depending on the range of temperature elevation. The positive effect of nutrient enrichment was antagonized (but still positive) by temperature increase above the optimum in many cases, although the effect sometimes switched to a negative effect depending on the magnitude of nutrient enrichment. These results predict that global warming will enhance bottom-up effects on primary production in cold seasons and areas, and there will be a negative warming effect on production in hot seasons and areas, but it may be possible to mitigate this effect by appropriate levels of nutrient enrichment.

Keywords: climate change, cumulative effect, bottom-up control, ecosystem primary producer, interactions between global and local stressors, multiple stressors, non-additive effect

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INTRODUCTION

The combined effects of two different factors can be classified into additive or non-additive (interactive) effects, based on whether interaction of the factors is significant or not, as a result of analysis of variance (ANOVA) (Folt et al., 1999; Crain et al., 2008; Brown et al., 2014; Piggott et al., 2015; Côté et al., 2016; Gunderson et al., 2016). An additive effect is calculated as the sum of the individual effects of the two factors, whereas non-additive effects are not simply summed because the effect of one factor varies depending on changes in the other factor (Folt et al., 1999; Crain et al., 2008; Brown et al., 2014; Piggott et al., 2015; Côté et al., 2016; Gunderson et al., 2016). Historically, this non-additive effect has been classified as either synergism or antagonism, depending on whether the cumulative effect of the factors is greater or less than the additive sum of the individual effects, respectively (Folt et al., 1999; Crain et al., 2008; Brown et al., 2014; Gunderson et al., 2016). Synergism and antagonism can be distinguished easily when both factors produce their effect in the same direction (double positive or double negative). However, this classification is problematic when factors have opposing effects (i.e., one positive, and the other negative), because a synergism of one factor and an antagonism of another can occur at the same time (Piggott et al., 2015). To resolve this problem, Piggott et al. (2015) classified these interactions into 4 types: positive synergism (+S, where the cumulative effect is more positive than the additive sum); negative antagonism (–A, where the cumulative effect is less negative than the additive sum); positive antagonism (+A, where the cumulative effect is less positive than the additive sum); and negative synergism (–S, where the cumulative effect is more negative than the additive sum). This seemed to succeed in classifying all possible combinations of cumulative effects of two factors.

In further considering the study of combined effects of two factors, it is of interest to understand how the effect of one factor varies due to changes in the other factor. However, this is difficult based on the classification proposed by Piggott et al. (2015) because the meanings of the four types can vary depending on whether the two factors combine their effects in the same or opposing direction. For example, positive antagonism (+A) means that the effects of both factors are antagonized when these factors are double positive, but when their effects work in opposing directions, one factor may be antagonized while the other is synergized. Therefore, a deeper classification scheme is needed to directly describe the changes of one factor with respect to the changes in the other.

Global warming and climatic associated changes are considered to be caused by increasing concentration of greenhouse gasses such as CO_2 in the atmosphere (Zandalinas et al., 2021). To mitigate climate change, the conservation and restoration of ecosystem primary producers, such as terrestrial plants, seagrass, and marine macroalgae, are necessary because they contribute to fix and sequester atmospheric CO_2 (Nunes et al., 2020; Watanabe et al., 2020). The biomass and productivity of primary producers are generally regulated by bottom-up (nutrient supply) and top-down (herbivory) effects (Gruner et al., 2008; Sellers et al., 2021), but have also been negatively affected by global warming in recent years (Lobell et al., 2012; Bita and Gerats, 2013; Smale, 2020). Meanwhile, recent studies have shown that the effect of increased temperature on the growth of primary producers can be synergized or antagonized by nutrient enrichment (Strain et al., 2014; Ordóñez et al., 2015; Thomas et al., 2017; Egea et al., 2018; Ostrowski et al., 2021), implying that the management of local nutrient environments might help to mitigate the negative effects of warming. Although the number of such studies is still limited for plant species (Ordóñez et al., 2015; Egea et al., 2018), data have accumulated for marine macroalgae, which can be easily cultured (i.e., they do not have below-ground system) on a small scale (i.e., few incubators) and for shorter experimental periods (a few weeks or months). Hence, a literature review of macroalgal studies to date has the potential to provide data to understand how warming effects vary with nutrient enrichment and inform the management of local nutrient environments to mitigate the negative effects of warming.

Here, a new classification is proposed to disentangle specific effects among two interacting factors, based on the direction of one factor (positive, negative, or neutral) and its change due to the other factor (synergized, antagonized, not changed, or changed in some other way). Based on this classification, a literature review was used to investigate retrospectively variations in the effects of increased temperature on the growth of marine macroalgae due to the influence of nutrient enrichment, and vice versa.

A NEW CLASSIFICATION OF INTERACTION TYPE

A new classification to describe how the effect of a certain factor A, varies with some other factor B is proposed, yielding in 11 types of different interactions (**Figure 1**). In this classification, the initial direction of the effect of factor A was classified as positive (P), neutral (O), or negative (N). The changes of the effect of factor A by factor B were divided into five categories: synergism (S), neutral (O), antagonism (A), inversion to negative (N), and inversion to positive (P). As a result, each of these types of interaction is given a two-letter code, as follows. PS: the positive effect of A is synergized by B. PO: the positive effect of A is not changed by B. PA: the positive effect of A is antagonized by B. PN: the positive effect of A is reversed to a negative effect by B. OP: the effect of A is neutral but becomes positive through the effect of B. OO: the effect of A is neutral and this is not changed by B. ON: the effect of A is neutral but becomes negative through the effect of B. NP: the negative effect of A is reversed to positive by B. NA: the negative effect of A is antagonized by B. NO: the negative effect of A is not changed by B. NS: the negative effect of A is synergized by B.

The directions and changes of two factors can be described by pairing the interaction types, such as PS-PS or PA-PA, because the changes of effect of factor B are basically accompanied by changes of factor A (**Figure 2**). For example, when a positive effect of A is synergized by B (PS), the positive effect of B must be synergized by A (PS), resulting in PS-PS. When a positive effect of A is antagonized by B (PA), a positive effect of B can

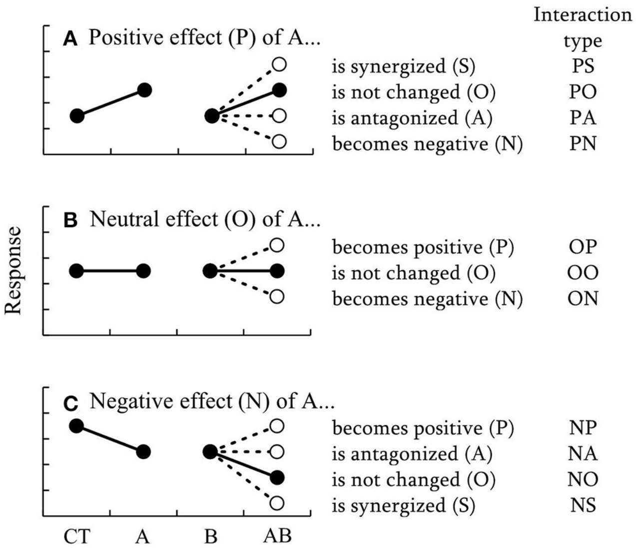

FIGURE 1 | Schematic diagrams of 11 types of interactions proposed as a new classification to describe how the effect of a certain factor, A, varies with some other factor, B. The changes of the **(A)** positive, **(B)** neutral, **(C)** negative effects of A by B are shown separately. CT, A, and B indicate control, and treatments with elevated level of factors A and B, respectively. Black and white circles indicate the results of additive and non-additive effects, respectively.

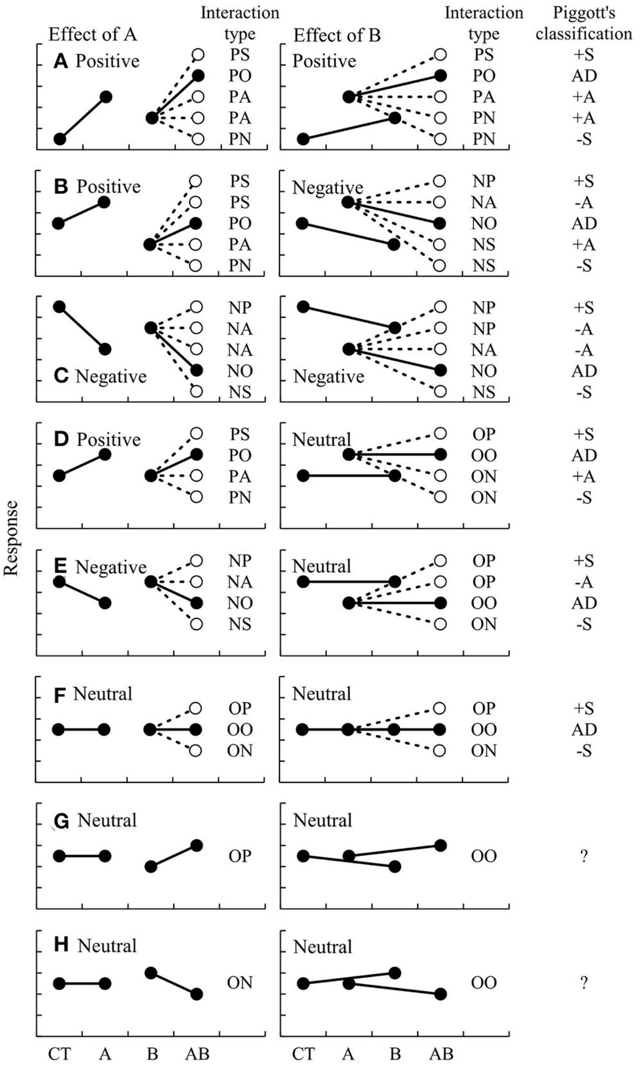

FIGURE 2 | Schematic diagrams of potential types of interactions between two factors, A and B, and comparison with the classification by Piggott et al. (2015). The changes of A and B are separately shown at left and right, respectively, when the effects of A and B are **(A)** double positive, **(B)** opposing, **(C)** double negative, **(D)** positive and neutral, **(E)** negative and neutral, **(F–H)** double neutral, respectively.

be antagonized (PA) or reversed by A (PN), resulting PA-PA or PA-PN, respectively.

This classification of interaction types is compared with that proposed by Piggott et al. (2015) in **Figure 2**. There are several similarities between the two schemes. For example, when the factors have opposing effects (e.g., one is positive, but the other is negative), both schemes classify the possible interactions into five types, including PS-NP (positive synergism, +S), PS-NA (negative antagonism, –A), PO-NO (additive effect, AD), PA-NS (positive antagonism, +A), and PN-NS (negative synergism, –S). Similarly, the interactions are classified into PS-OP (+S), PO-OO (AD), PA-ON (+A), and PN-ON (–S) when the two factors have positive and neutral effects, respectively; and into NP-OP (+S), NA-OP (–A), NO-OO (AD), and NS-ON (–S) when the two factors have negative and neutral effects, respectively. When both factors have neutral effects, they can be classified as OP-OP (+S), OO-OO (AD), or ON-ON (–S).

However, there are critical differences in some cases. When both factors have positive effects, the interactions can be classified only into 4 types by Piggott's classification (+S, AD, +A, and –S); but into 5 types according to the new classification: PS-PS (+S), PO-PO (AD), PA-PA (+A), PA-PN (+A), and PN-PN (–S). Note that type +A is here subdivided into PA-PA and PA-PN. Similarly, when both factors have negative effects, the interactions are classified as NP-NP (+S), NA-NP (–A), NA-NA (–A), NO-NO (AD), and NS-NS (–S). Moreover, there are two new possible types of interaction, OP-OO and ON-OO, which are not recognized by the scheme of Piggott et al. (2015).

Overall, there are few differences between the two schemes but the new classification, including the information about the directions and changes of both factors, provide more comprehensive coverage of the possible combination of interactions than that of Piggott et al. (2015). For example, +S of the Piggott's scheme can be subdivided according to the new scheme into PS-PS, PS-NP, NP-NP, PS-OP, NP-OP, and OP-OP.

REVIEW OF THE COMBINED EFFECTS OF TEMPERATURE AND NUTRIENTS ON MACROALGAE

To perform a retrospective study to evaluate the new scheme, academic publications reporting the combined effects of temperature and nutrients on the growth of marine macroalgae were searched with Google Scholar by combining four categories of keywords: category 1, combined and interactive effects; category 2, temperature and warming; category 3, nutrient, nitrate, and dissolved inorganic nitrogen (DIN); and category 4, macroalga, alga, and seaweed. This search identified 31 articles where the combined effects on growth (i.e., relative growth rate and size) or recruitment density (the result of growth after settlement) of several factors including, at least, temperature and nutrients, were reported using ANOVA. The direction of changes in temperature and nutrients were unified to "elevated temperature" (i.e., warming) and "nutrient enrichment," respectively, because a negative effect of decreased temperature results in changes in the same direction as a positive effect of elevated temperature (keeping them separate complicates understanding of the trends of the interactions).

The effect of each factor on growth was classified into 11 types of interaction based on the results of ANOVA and/or multiple comparison test, even when a significant probability of interaction was not described in the article. When the study cultured macroalgae at 3–5 levels of temperature (e.g., 5, 10, and 15°C) or nutrients, the classification was conducted for each consecutive level separately (e.g., 5–10 and 10–15°C). When multiple comparison tests were not conducted in a study, the potential type of interaction was speculated from figures (indicated by a question mark in **Supplementary Table 1**; such as PS?) but this was excluded from subsequent analysis.

MODIFICATION OF NUTRIENT EFFECTS BY ELEVATED TEMPERATURE

A total of 78 cases (including all species, variables, temperature range, and nutrient range) involving temperature-nutrient interactions were identified and analyzed using the new scheme (**Supplementary Table 1**). The results are presented below in two parts: modification of nutrient effects by elevated temperature; and modification of temperature effects by nutrient enrichment.

The effects of nutrient enrichment on macroalgal growth were positive in 35 cases, neutral in 38 cases, and negative in 5 cases (**Table 1**). These nutrient effects varied due to elevated temperature in 27 cases but not in 51 cases.

The positive effect of nutrients was synergized (PS) by elevated temperature in 7 cases (Lotze and Worm, 2002; Steen, 2003, 2004; Gao G. et al., 2017; Gao X. et al., 2017). For example, the effect of nutrient enrichment on recruit density of the green alga *Ulva intestinalis* was weak at the relatively low temperature of 5°C but was strong at 12 and 17°C; both the latter seemingly optimal temperatures for recruitment (Lotze and Worm, 2002). Also in the other cases, the ranges of temperature elevation were between relatively low and optimal levels for their growth: 7–17°C for *Ulva compressa* (Steen, 2004); 14–18°C for *Ulva rigida* (Gao G. et al., 2017); and 5–15°C for juveniles and 5–10°C for larger size classes of the kelp *Saccharina japonica* (Gao X. et al., 2017).

Additionally, neutral nutrient effect became a positive effect (OP) as a result of elevated temperature from relatively low to around optimal levels in other 3 cases: 7–17°C for the fucoid brown algae *Fucus vesiculosus* and *Sargassum muticum* (Steen and Rueness, 2004); and 10–15°C for the red alga *Porphyra amplissima* (Kim et al., 2007). These results can be explained by a lowered nutrient uptake rate (Pedersen et al., 2004 and references therein) and down-regulation of nitrogen transporter genes (Takahashi et al., 2020) under relatively low temperatures.

In contrast, a positive nutrient effect was antagonized (PA) by elevated temperature in 10 cases (Kim et al., 2007; Gao et al., 2013; de Faveri et al., 2015; Kay et al., 2016; Gao X. et al., 2017; Gouvêa et al., 2017; Piñeiro-Corbeira et al., 2019). For example, the nutrient effect on relative growth rate of the brown alga *Saccharina japonica* was synergized by temperature elevated from 5°C to the optimal level of 10°C; but was antagonized by further temperature elevation from 10 to 15°C (Gao X. et al., 2017). Also in other cases, the antagonized effects of nutrients were found between around optimal growth and relatively high temperatures: 10–15°C for *Porphyra linearis* and 15–20°C for *P. amplissima* (Kim et al., 2007); 15–24°C for the kelp *Undaria pinnatifida* (Gao et al., 2013); 15–25°C for the red alga *Hypnea musciformis* (de Faveri et al., 2015); 16–20 and 20–24°C for the fucoid brown alga *Ascophyllum nodosum* (Kay et al., 2016); 24–28°C for the red alga *Laurencia catarinensis* (Gouvêa et al., 2017); and 14–26°C for *F. vesiculosus* (Piñeiro-Corbeira et al., 2019). Additionally, the negative effect of a high level of nutrient enrichment (from 20 to 100 μM DIN) was also antagonized (NA) by elevated temperature from optimal growth to higher levels (16–20°C for *A. nodosum*; Kay et al., 2016). Although the physiological mechanism of these types of interactions is unclear, it may be related to changes in nutrient demands with elevated temperature, because increased phosphorus uptake rates with temperature elevation have been demonstrated in a brown macroalgae species (Ohtake et al., 2020) and corals (Ezzat et al., 2016), whereas nitrogen uptake rate was not affected (Ohtake et al., 2020) or was decreased by warming (Ezzat et al., 2016).

Thus, the effect of nutrient enrichment on macroalgal growth is often synergized by elevated temperature (PS) from relatively low to optimal levels but was antagonized by further temperature elevation (PA). However, there were few cases where neutral nutrient effect became positive (OP) in response to temperature elevated to relatively high levels: 10–15°C for *P. amplissima* and *Porphyra umbilicalis*, and 15–20°C for *P. linearis* (Kim et al., 2007); and 25–35°C for *H. musciformis* (de Faveri et al., 2015). Because elevated temperature can decrease the nitrogen content of plants and macroalgae (Hay et al., 2010; Wu et al., 2019), nutrient enrichment may have a positive effect when the macroalgal thallus is nitrogen-starved under warm conditions.

Further, there was just one case where a positive effect of relatively high levels of nutrient enrichment (DIN 50 to 120 μM) on relative growth rate (of *L. catarinensis*) became negative (PN) when temperature was elevated from 24 to 28°C, whereas the effect of relatively low levels of nutrient enrichment (DIN 1.3–50 μM) was still positive at the higher temperature (Gouvêa et al., 2017). This indicates that the magnitude of nutrient enrichment can affect whether or not a positive nutrient effect can become negative.

TABLE 1 | Distribution among different groups of macroalgae of interaction types involving a nutrient effect (from published experiments that included species, variables, temperature range, and nutrient range).

Interaction type		Piggott's class	Number of cases*						
Nutr.	**Temp.**		**Green**	**Red**	**Kelp**	**Fucoid**	**Total**		
PS	PS	+S	3		2	1	6	7	35
	NP	+S		1			1		
PO	PO	AD	2	1	1	3	7	17	
	OO	AD	1	1		3	5		
	NO	AD			2	3	5		
PA	PA	+A		2			2	10	
	ON	+A		2	1	2	5		
	NS	+A			1	2	3		
PN	NS	–S		1			1	1	
OP	PS	+S				2	2	6	38
	OO	?**		1			1		
	NA	–A		3			3		
OO	PO	AD			3	7	10	32	
	OO	AD		1	3	5	9		
	ON	?**			1	1	2		
	NO	AD		2	3	6	11		
NA	OP	–A		1			1	3	5
	NA	–A				2	2		
NO	PO	AD			1		1	2	
	OO	AD				1	1		

*"Green", "red", "kelp", and "fucoid" indicate the major groups of macroalgal taxa. **This type of interaction is not recognized by the scheme of Piggott et al. (2015).

MODIFICATION OF TEMPERATURE EFFECTS BY NUTRIENT ENRICHMENT

The effects of elevated temperature on macroalgal growth were positive in 28 cases, neutral in 26 cases, and negative in 24 cases (**Table 2**). These temperature effects varied due to nutrient enrichment in 28 cases but not in 50 cases.

The positive effect of elevated temperature was synergized (PS) by nutrient enrichment in 8 cases. This type of interaction simultaneously occurred with the synergism of nutrient effect by elevated temperature (PS-PS) in 6 cases (Lotze and Worm, 2002; Steen, 2003, 2004; Gao G. et al., 2017; Gao X. et al., 2017) and turning a neutral nutrient effect into positive (PS-OP) in 2 cases (Steen and Rueness, 2004). These results indicate that this interaction occurred when the elevated temperature weakened the negative effect of low temperature on nutrient uptake rates, as discussed above.

The negative effect of elevated temperature was synergized (NS) by nutrient enrichment in 4 cases (Kay et al., 2016; Gao X. et al., 2017; Gouvêa et al., 2017; Piñeiro-Corbeira et al., 2019). A neutral effect of elevated temperature became negative under the influence of nutrient enrichment (ON) in 7 cases. Most of these cases occurred with the antagonism of the nutrient effect by elevated temperature (NS-PA and ON-PA). Therefore, this interaction can occur when the strong effect of nutrient enrichment around the optimal growth temperature is suppressed by further temperature elevation. Note that the nutrient effect is still positive even when it synergizes the negative effect of elevated temperature in this type of interaction.

In contrast, the negative effect of elevated temperature was antagonized (NA) by nutrient enrichment in 5 cases. Many of these cases occurred when a neutral nutrient effect became positive because of elevated temperature (NA-OP) in 3 cases: 15–20°C for *P. linearis* and 10–15°C for *P. umbilicalis* (Kim et al., 2007); and 25–35°C for *H. musciformis* (de Faveri et al., 2015). In other cases, NA occurred when the negative effect of a high level of nutrient enrichment (DIN 20–100 μM) was antagonized by elevated temperature (NA-NA) in 2 cases: 16–20°C for changes in weight and length of *A. nodosum* (Kay et al., 2016). Hence, this interaction can occur when temperature is elevated between optimal and higher levels. Even in both cases, macroalgal growth was enhanced by nutrient enrichment.

There was only one report showing that a negative effect of elevated temperature can become positive and can be synergized by nutrient enrichment: Gouvêa et al. (2017) showed that the negative effect of temperature elevated from 20 to 24°C became positive because of nutrient enrichment (between 1.3 and 50–120 μM DIN) on relative growth rate of *L. catarinensis*. In addition, they showed that neutral effect of further temperature elevation from 24 to 28°C (at 1.3 μM DIN) became negative under low levels of nutrient enrichment (between 1.3 and 50 μM DIN), whereas the negative effect of temperature elevated from 24 to 28°C (at 50 μM DIN) was synergized by further nutrient enrichment (between 50 and 120 μM DIN). Thus, the

TABLE 2 | Distribution among different groups of macroalgae of interaction types involving a temperature effect (from published experiments that included species, variables, temperature range and nutrient range).

Interaction type		Piggott's class	Number of cases*						
Temp.	**Nutr.**		**Green**	**Red**	**Kelp**	**Fucoid**	**Total**		
PS	PS	+S	3		2	1	6	8	28
	OP	+S				2	2		
PO	PO	AD	2	1	1	3	7	18	
	OO	AD			3	7	10		
	NO	AD			1		1		
PA	PA	+A		2			2	2	
OP	NA	–A		1			1	1	24
OO	PO	AD	1	1		3	5	16	
	OP	?**		1			1		
	OO	AD		1	3	5	9		
	NO	AD				1	1		
ON	PA	+A		2	1	2	5	7	
	OO	?**			1	1	2		
NP	PS	+S		1			1	1	26
NA	OP	–A		3			3	5	
	NA	–A				2	2		
NO	PO	AD			2	3	5	16	
	OO	AD		2	3	6	11		
NS	PA	+A			1	2	3	4	
	PN	–S		1			1		

*Abbreviations as in **Table 1**. **This type of interaction is not recognized by the scheme of Piggott et al. (2015).*

effect of elevated temperature strongly depends on the range of temperature elevation and nutrient concentrations.

ECOLOGICAL IMPLICATIONS

Marine macroalgal forests dominated by kelp and fucoid brown algae are highly productive and play an important role in coastal ecosystems through the provision of habitat and spawning grounds for a wide range of marine organisms (Steneck et al., 2002). Recent ocean warming has resulted in the increased abundance and/or expansion of range of marine forests in arctic reefs (Smale, 2020). The elevated temperature from relatively low to higher levels in these areas may enhance a bottom-up effects on macroalgae, as shown in this review. In contrast, ocean warming has caused range contraction, decreased abundance, and local extinction of marine forests in temperate reefs worldwide (Smale, 2020). Previous studies have shown that above-average temperatures combined with low nutrient availability during summer causes mass mortality and failed recruitment in kelp species (Dayton and Tegner, 1984; Dean and Jacobsen, 1984, 1986; Gerard, 1997; Tegner et al., 1997), but nutrient enrichment can enhance their recruitment, growth, and survival (North and Zimmerman, 1984; Dean and Jacobsen, 1986; Hernández-Carmona et al., 2001).

From the viewpoint of the interaction between temperature and nutrients, the present review has demonstrated that negative warming effects were antagonized by nutrient enrichment according to some studies but was synergized in others. However, even when the negative warming effect was synergized by nutrient enrichment, the positive effect of nutrient enrichment was still positive in many cases. Therefore, negative warming effects on macroalgal forests may be mitigated by *in situ* nutrient enrichment in coastal waters. Specifically, nutrient enrichment is predicted to be effective on macroalgae growing in regions, where nutrient concentrations in the surface water has been declining because vertical mixing of nutrient-poor surface water and nutrient-rich deep water has been suppressed by ocean warming or long-term natural climate change (Watanabe et al., 2005; D'Alelio et al., 2020).

The aquaculture production of commercially important macroalgae (genera *Undaria*, *Saccharina*, *Sargassum, Neopyropia*, *Gracilaria*, *Eucheuma*, and *Kappaphycus*), is increasing worldwide due to the rising demand for their utilization as human food, bait, fertilizer and raw industrial materials (Hurd et al., 2014; Chung et al., 2017). Specifically, the kelp *Undaria pinnatifida* has been extensively cultivated in China, Korea, and Japan, and has recently been introduced to Europe for commercial culture (Peteiro et al., 2016). Although the negative warming effect on this species is reported to be offset by nutrient enrichment (Gao et al., 2013), both the elevated temperature and nutrient enrichment are predicted to increase the risk of herbivory by isopods during autumn, when the outdoor cultivation of this species is started (Endo et al., 2021). Hence, warming may force the beginning of cultivation to be delayed by 1 month or more until the temperature drops into optimal levels. In contrast, elevated temperatures during winter may enhance macroalgal growth by the synergistic positive effect of increased nutrient concentration during this season, as shown in the present review, although there is also a possibility that warming might decrease nitrogen concentration in the surface water *via* suppression of vertical water mixing, as mentioned above (Watanabe et al., 2005; D'Alelio et al., 2020).

Green algae belonging to the genus *Ulva* are known to cause severe algal bloom phenomena (so-called green tides) in eutrophied coastal waters and have negative ecological impacts such as decreased oxygen levels in coastal environments due to their decomposition (Gao G. et al., 2017; Lee and Kang, 2020). The present review has shown that the positive effects of nutrient enrichment on *Ulva* species was often synergized by temperatures elevated from 5–7 to 11–17°C (Lotze and Worm, 2002; Steen, 2004) but there was no change with further temperature elevation from 15 to 30°C (Lee and Kang, 2020), whereas the effect was often antagonized by similar temperature elevations in red and brown algae. Hence, blooms of *Ulva* under eutrophication are predicted to be enhanced by warming in cold seasons and areas, but might not strongly be affected under hot conditions (i.e., around 30°C).

The combined effects of temperature and nutrient availability have also been examined in a number of other groups of primary producers, including terrestrial plants (Ordóñez et al., 2015 and references therein), seagrasses (Egea et al., 2018; Ontoria et al., 2019; and references therein), and phytoplankton (Thomas et al., 2017 and references therein), although the number of reports showing the results of ANOVA and multiple comparison tests is quite limited. For example, Ordóñez et al. (2015) reported that the negative effect of elevated temperature on the yield of maize (*Zea mays*) was synergized by nutrient enrichment. Although a similar result was obtained from macroalgal studies, the present review showed that whether the negative warming effect was synergized or antagonized depended on the magnitude of nutrient enrichment. Based on this information, there is still a possibility that reducing the amount of fertilizer might mitigate the negative warming effect on the crop yield, although this hypothesis needs to be tested.

Thomas et al. (2017) revealed that the optimal growth temperature of the diatom *Thalassiosira pseudonana* increased as a result of nutrient enrichment. This result is consistent with a macroalgal study conducted by Gouvêa et al. (2017), who showed that the optimal growth temperature of the red macroalga *L. catarinensis* was around 20°C in low nutrient treatments but increased to 24°C in nutrient enriched treatments. Further evaluation of optimal growth temperature under both nutrient-deficient and nutrient-rich conditions, using thermal performance curve (e.g., Fernández et al., 2020) or response surface methodology (e.g., Mendes et al., 2012; Sato et al., 2020), will contribute to understand the relationships between nutrient availability and optimal growth temperature of primary producers.

CONCLUSIONS

Using a more detailed scheme to classify the interactions of different factors on the growth of plants, this review was able to show that the effects of elevated temperature and nutrient enrichment on the growth of marine macroalgae are often synergized within the temperature range between relatively low to optimal growth levels. Thus, global warming is predicted to enhance a bottom-up effect on macroalgal productivity in cold-temperate and subarctic zones. In contrast, a negative effect of further temperature elevation on macroalgal growth can be both antagonized and synergized by nutrient enrichment, depending on the range of temperature elevation. Although the positive effect of nutrient enrichment was often antagonized by this temperature elevation, the effect was still positive in most cases even when it was antagonized. Therefore, the negative effect of warming on primary production in warming hotspots may be mitigated by nutrient enrichment. However, caution is advised in determining the amount of fertilizer, because there is evidence that a positive effect of relatively high levels of nutrient enrichment can become negative as a result of increased temperature. Additionally, nutrient enrichment under warm conditions was predicted to strengthen the top-down (herbivory) effects on primary producers (e.g., Endo et al., 2021). Further studies on the effect of elevated temperature on the nutrient requirement and interactive effects of temperature, nutrients, and herbivory on the productivity of marine macroalgae and terrestrial plants are needed in order to understand how to use fertilizer to mitigate negative warming effect on these primary producers.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

HE: idea and conceptualization. HE and XG: literature review, writing, and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.774801/full#supplementary-material

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Dispersal and Deposition of Detritus From Kelp Cultivation

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A high resolution coastal and ocean hydrodynamic model system was used to investigate the transport and deposition patterns of Particulate Organic Matter (POM) from kelp farmed at three locations of different properties: a sheltered location, an exposed location, and an offshore location. Published values on the sinking speeds of organic particles from kelp were used, spanning several orders of magnitude. Recent work on quantifying the release of particulate organic matter from farmed kelp was used to link the release of carbon to possible cultivation volumes and scenarios, and finally to link this to the potential for carbon loading on the ocean floor. The results are presented in terms of loading and distribution per unit harvested kelp, and the loading estimates are compared with estimates of natural (background) primary production. According to the simulation results, organic matter may be transported anything from a few (hundred) meters up to a hundred km away from the release site, depending on the sinking rates, time of release, and the location. The depth at which the matter settles on the sea floor likewise depends on the properties of the matter and the sites. The time until settlement varied from minutes to several hundred hours. The results underscore the importance of constraining the dispersal and deposition of detritus from kelp cultivation in order to better understand and quantify associated environmental risks posed by organic loading, and the potential for seafloor carbon sequestration by kelp farming as a nature based climate solution.

Keywords: seaweed aquaculture, ocean model, organic loading, carbon export, sedimentation—dispersion model, carbon sequestration, kelp

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1. INTRODUCTION

Recent work suggests that the potential for macroalgae aquaculture globally is great, also outside of Asia, that currently produces more than 99% of the 32 million tons wet weight cultivated per annum (Lehahn et al., 2016; Hadley et al., 2018; van der Molen et al., 2018; Broch et al., 2019; FAO, 2020; Forbord et al., 2020; Aldridge et al., 2021; Duarte et al., 2021). During the grow out phase in culture, as in natural populations, macroalgal tissue fragments are shredded and entire plants dislodged (Parke, 1948; Sjtun, 1993; Krumhansl and Scheibling, 2012; Zhang et al., 2012; Pedersen et al., 2020; Fieler et al., 2021). Both these processes contribute to the pool and export of Particulate Organic Matter (POM). In particular kelps, large brown macroalgae of the order *Laminariales*, display meristematic growth with ensuing erosion of the distal end(s) of the frond(s). In natural kelp forests, 50% of the Net Primary Production (NPP) may be released as POM (Pedersen et al., 2020). The erosion of cultivated *S. japonica* in Sungo Bay in China has been reported at up to 61% of the cultivated kelp NPP (Zhang et al., 2012). Recent results from Norwegian kelp cultivation indicate a POM export of 8 to 13% of the NPP if the kelp is harvested early during spring, and up to 49% if harvested later in the growth season during summer (Fieler et al., 2021). The export includes a wide range of kelp fragment sizes (Fieler et al., 2021). POM released from natural kelp forests is an important source of food and habitat for bacteria, macro- and meiofauna (Duggins et al., 1989; Renaud et al., 2015; Queirs et al., 2019; de Bettignies et al., 2020a; Brunet et al., 2021; Harbour et al., 2021a). It has been suggested that organic matter from intense kelp cultures may have negative environmental impacts (Walls et al., 2017; Campbell et al., 2019), as in fin fish farming (Carroll et al., 2003). However, investigations indicate that the impacts of organic loads even from large-scale seaweed farming are modest (Zhang et al., 2009; Walls et al., 2017; Visch et al., 2020).

Regardless of the source, kelp POM contributes to carbon sequestration through export and subsequent deposition and permanent burial of carbon rich organic detritus in coastal or deep sea soft sediments (Krause-Jensen and Duarte, 2016; Duarte et al., 2017, 2021; Smale et al., 2022). Macroalgal tissue may be transported distances at the order of 1000 km and to depths of several thousands of meters (Harrold et al., 1998; Ortega et al., 2019). Simulation studies and *in situ* observations have also shown that POM from natural kelp populations can be transported beyond the natural habitats, both horizontally and vertically (Filbee-Dexter et al., 2018, 2020), and that it may degrade over time periods of months (Frontier et al., 2021; Smale et al., 2022). Despite this, it is yet unclear how exported biomass of kelp detritus is dispersed (Pedersen et al., 2021), how far it is transported, and how the matter is distributed between the near and far field. Neither have there been any studies focusing on cultivated kelp detritus, and in particular the importance of kelp farming location and physical characteristics.

Here, we investigate how kelp farming contributes to the export of POM, how far it is transported and how it is subsequently distributed on the seafloor. These questions are approached by using a high resolution 3 dimensional hydrodynamic model (SINMOD) with a detritus transport module. We then relate the results to cultivation scenarios for the commercially important kelp *Saccharina latissima* (sugar kelp) and present the results in terms of fractions of production volumes so that they are scalable and not directly related to production technologies, species, or cultivation practices at any one particular farm.

2. MATERIALS AND METHODS

2.1. Ocean Model SINMOD

The 3 dimensional model framework SINMOD (Slagstad and McClimans, 2005) was used for the simulations. The hydrodynamic component solves the primitive Navier-Stokes equations using a finite difference scheme on an Arakawa C-grid (Aarakawa and Lamb, 1977). In the present simulations, *z*-layers were used (i.e., each vertical layer had a fixed thickness except the surface and bottom layers) and a hydrostatic assumption was applied (Slagstad and McClimans, 2005).

A model domain of 160 m horizontal resolution was used (**Figure 1**). Boundary conditions were produced in a 3 step nesting procedure, running models of successively finer grids from 20,000 m, to 4,000, to 800 m and finally to 160 m resolution, e.g., Broch et al. (2019, 2020). The model setup in 160 m horizontal resolution used here had depth layer thicknesses ranging from 1 to 5 m for the upper 25 m, followed by 25 m thick layers down to 650 m depth. The simulation time step for the 160 m model was 30 s.

Atmospheric forcing was applied using ECWMF's ERA-Interim data (Dee et al., 2011). Forcing by freshwater from rivers and land was implemented by using data from the Norwegian Water Resources and Energy Directorate (www.nve.no) generated by a version of the HBV-model (Beldring et al., 2003).

Previous studies have shown that the model system is able to approximate the local current system at and around aquaculture sites in a realistic manner (Broch et al., 2020). On a larger scale, the model has been shown to reproduce the circulation dynamics at the Norwegian Shelf outside Northern Norway (Skardhamar and Svendsen, 2005).

2.2. Transport and Deposition Modeling

A conceptual diagram of the deposition model is presented in **Figure 2**. An Eulerian approach was taken in the transport simulations. This entails the calculation of concentration fields of suspended or sedimented POM. A summary of all the parameters and variables of the transport model, including choices of numerical values where applicable, is given in **Table 1**.

Four model compartments (D_1, D_2, D_3, D_4) representing the concentrations of kelp POM (unit: gC m^{-3}) of different sinking speeds were used (**Table 1**). The concentration D_j of component number j is calculated according to the following equation (Wassmann et al., 2006):

$$\frac{\partial D_j}{\partial t} + \mathrm{Adv}(D_j) + \mathrm{Diff}(D_j) = G_j,\ j = 1, 2, 3, 4. \tag{1}$$

Here, Adv and Diff are the 3 dimensional advection and diffusion operators, respectively, while the source term G_j represents release of POM from the cultivation sites (Slagstad and McClimans, 2005). It is tacitly assumed that all expressions and equations are valuated in spatial position (x, y, z). A Richardson scheme for vertical mixing is used (Sundfjord et al., 2008).

The sea floor concentration of deposited POM from compartment j (unit: gC m^{-2}) is denoted by S_j, and the concentration of kelp POM in compartment j in the model's bottom layer by $D_{j,\mathrm{bot}}$. The fluxes and inter-actions between $D_{j,\mathrm{bot}}$ and S_j are given by the equations:

$$\frac{\partial S_j}{\partial t} = s - r, \tag{2}$$

and

$$\frac{\partial D_{j,\mathrm{bot}}}{\partial t} = \frac{r}{\Delta z_{\mathrm{bot}}} - s + \mathrm{Adv}(D_{j,\mathrm{bot}}) + \mathrm{Diff}(D_{j,\mathrm{bot}}), \tag{3}$$

for $j = 1, 2, 3, 4$. Here $r = r(x, y)$ is the amount of POM resuspended and $s = s(x, y)$ is the fraction deposited in position (x, y); Δz_{bot} is the thickness of the bottom layer (**Figure 2**).

The resuspension and advection of POM from the bottom is calculated as follows. Let $u = (u_x, u_y)$ denote the current velocity

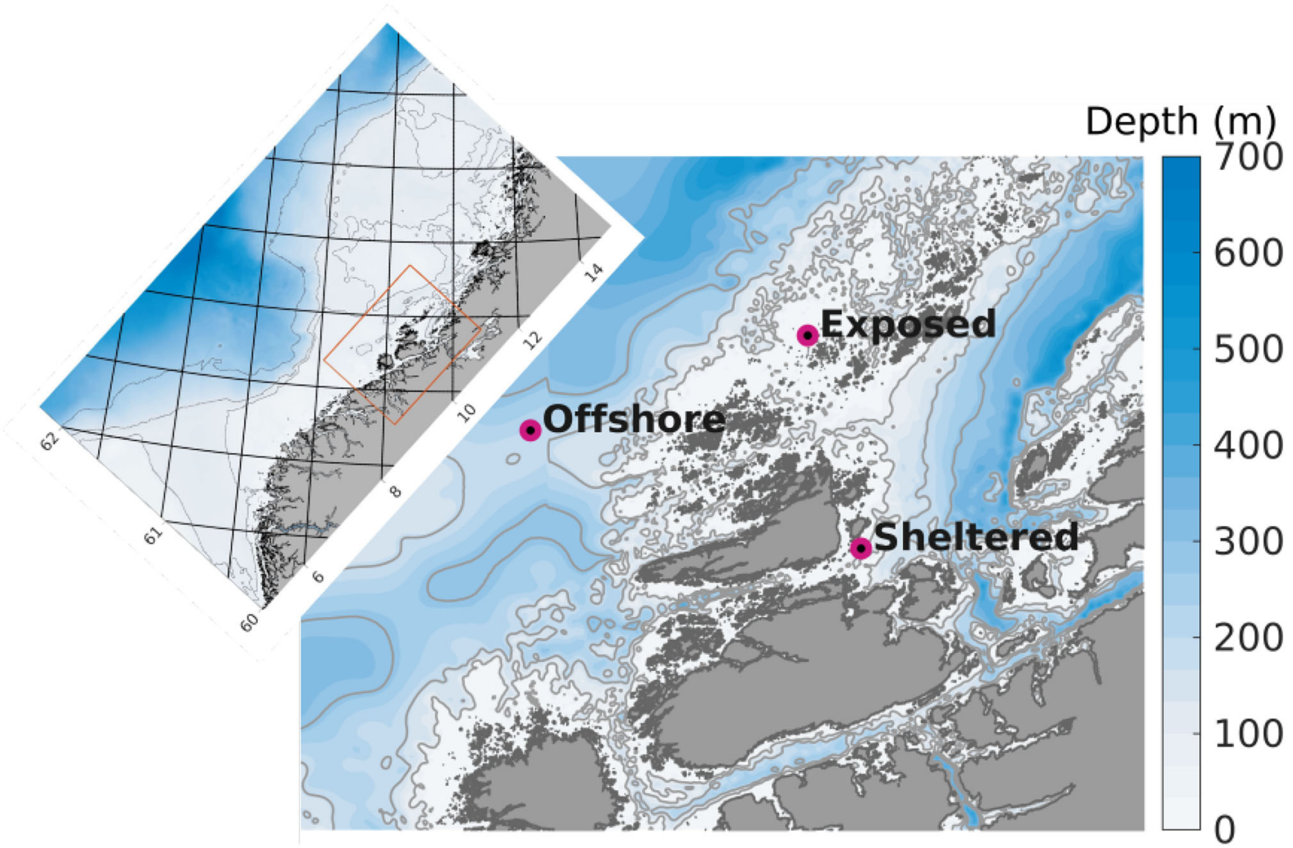

FIGURE 1 | Detail of the model domain in 160 m horizontal resolution used for the dispersal simulations. The purple dots indicate the positions of the release sites. The colors indicate bottom depth (m), and the thin gray curves represent the 100, 200, and 300 m isobaths. The inset map displays the 800 m model domain that was used to generate boundary conditions for the 160 model (orange rectangle in inset map). The numbers along the axes denote latitude and longitude.

in the middle of the bottom grid cell. The *shear velocity* u_* is calculated using a logarithmic law (Kuhrts et al., 2004; Warner et al., 2008),

$$u_* = \frac{\kappa\sqrt{(|u_x| + |u_y|)|u|}}{\ln(z/z_0)} \tag{4}$$

where $\kappa = 0.41$ is the universal von Kármán constant, z is the distance from the bottom to the middle of the $D_{j,\mathrm{bot}}$ grid cell, and z_0 is the *roughness height*: the height above the bottom at which the current speed $|u|$ tends to 0 (**Figure 2**, **Table 1**). Whether the POM is sedimented, remains neutral, or is resuspended from the sediment, i.e., the values of s and r in Equations (2) and (3), depends on the value of u_* relative to the critical shear velocity for reuspension $u_{*,R}$, thus:

$$s = \begin{cases} v_j D_{j,\mathrm{bot}}, & u_* \leq u_{*,\mathrm{R}}, \\ 0, & \text{otherwise} \end{cases} \tag{5}$$

where v_j denote the sinking speed of compartment j and

$$r = \begin{cases} \rho g M u_*^2, & u_* \geq u_{*,\mathrm{R}} \\ 0, & \text{otherwise,} \end{cases} \tag{6}$$

where ρ is the density of sea water and M is a parameter depending on the properties of the matter (Kuhrts et al., 2004). The interactions of sinking and resuspension with horizontal and vertical advection leads to the possibility of aggregation of POM, in contrast to passive tracers.

2.3. Detritus Sinking Speeds and Release

The sinking speeds v_j used for POM compartments j are recorded in **Table 1**. The sinking speed of D_2 to D_4 are based on the lower end of the range of values published in Wernberg and Filbee-Dexter (2018). They are sinking speeds for whole fronds, frond fragments, and of sea urchin fecal particles (shredded kelp) for *Laminaria hyperborea*. The sinking speed of D_1 is about an order of magnitude lower than the lowest ones recorded in Wernberg and Filbee-Dexter (2018). Consequently, a wide range of sinking speeds are covered.

The transport of POM from the beginning of April until the end of June was considered, assuming deployment of the kelp cultures in January-February (Boreal winter). Before this period, the absolute biomass, and biomass export of a farm is low, even if the size and biomass specific growth rates usually are high (Forbord et al., 2020; Fieler et al., 2021).

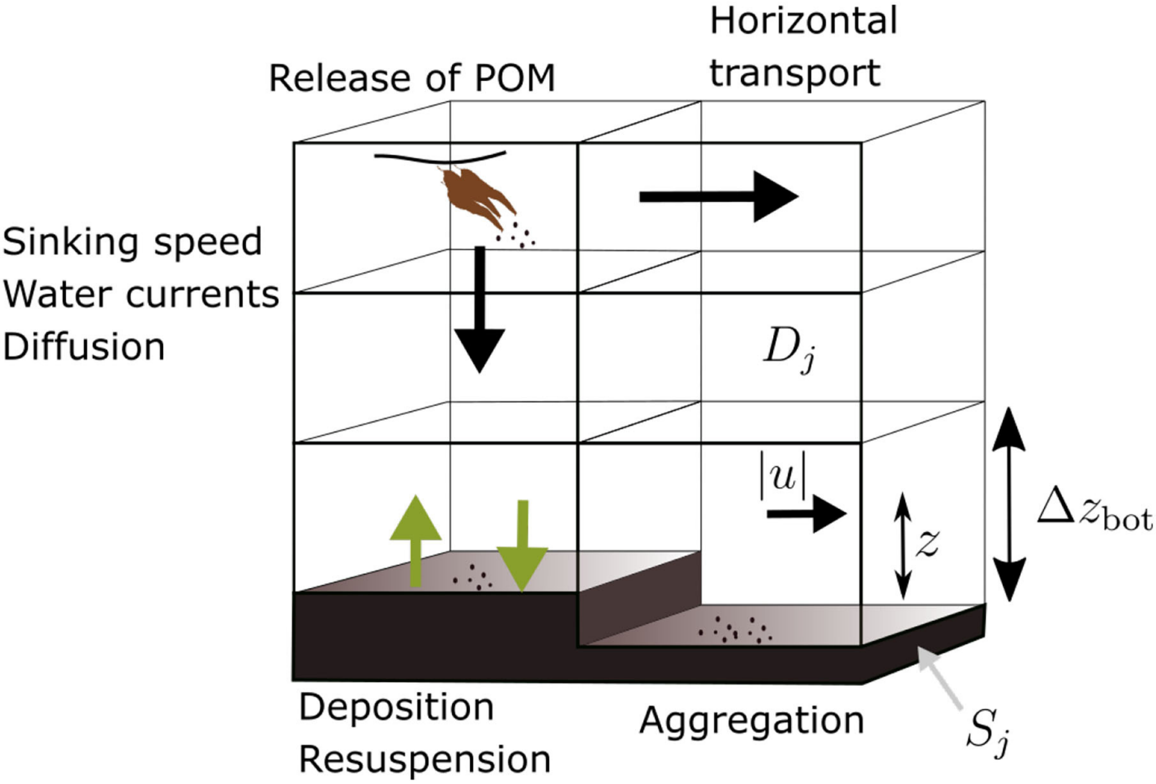

FIGURE 2 | Diagram of the POM transport model, illustrating the main processes: horizontal transport, vertical sinking, sedimentation, resuspension, and aggregation. The source is indicated (brown "kelp," upper left), as well as the quantities $|u|$, z, and Δz_{bot} involved in calculation of the shear velocity (Equation 4).

It was assumed that 10% of the total amount of POM was released in April, 20% in May, and 70% in June (Fieler et al., 2021). The release was assumed to be constant over each month, i.e., the same amount of matter was released for every model time step within each month.

One simulation was run for each of three types of farm locations (**Figure 1**):

- Sheltered: a nearshore farm in a coastal environment with a mean water depth of 18 m influenced by tidal mixing and coastal water currents.
- Exposed: a nearshore farm in an exposed environment with a mean water depth of 40 m influenced by tidal mixing and the Norwegian Coastal Current.
- Offshore: a farm in a fully open ocean environment with a mean water depth 232 m influenced by the North Atlantic and Norwegian Coastal currents.

For more information in the prevailing currents along the Norwegian coast cf. (Sætre, 2007). The sheltered farm site is an actual kelp cultivation location with a permit for kelp cultivation of 30 ha. The other two (hypothetical) farms are assumed to cover an area of 125 ha (7 × 7 model grid cells). Although the concepts of "sheltered," "exposed," and "offshore" may sometimes be used differently, the words serve to distinguish easily between the sites in the present case.

2.4. Organic Loading Calculations

The generic dispersal simulation results were translated into estimates for organic loading from sugar kelp (*Saccharina latissima*) aquaculture. While the conditions are suitable for *S. latissima* cultivation along most parts the Norwegian coast (Forbord et al., 2020), the full cultivation potential is not yet known. Simulation model results indicate an average potential for *S. latissima* cultivation in Norwegian coastal waters inside the maritime baseline (Harsson and Preiss, 2012) of around 75 t ha^{-1} year^{-1} (February-June), though with substantial spatial variability and a maximum potential close to 200 t ha^{-1} near shore and even higher offshore (Broch et al., 2019).

According to Fieler et al. (2021), 8 to 13% of the *S. latissima* NPP in culture from January/February until June in Central Norway is lost as POM through frond erosion and plant dislodgement. This translates into the losses by harvest time recorded in **Table 2**. The results form the dispersal simulations were up-scaled accordingly.

The sensitivity of POM loading to variations in the loss fraction and production intensity was calculated as follows. We consider only the carbon (C) fraction released. Assuming a harvested WW biomass of B_{harvest} t WW ha^{-1} and a loss of a fraction p of the NPP B_{NPP} t WW ha^{-1} (that is, the biomass had

TABLE 1 | Parameters and variables used in the transport and sedimentation model.

Symbol	Value	Unit	Definition, references
$D_j, j = 1, \ldots, 4$	Variable	$\mathrm{gCm^{-3}}$	Concentration of suspended kelp detritus
$S_j, j = 1, \ldots, 4$	Variable	$\mathrm{gCm^{-2}}$	Concentration of sedimented kelp detritus
r	Variable		Resuspension rate
s	Variable		Sedimentation rate
v_1	10^{-4}	$\mathrm{ms^{-1}}$	Sinking speed of detritus compartment D_1
v_2	10^{-3}	$\mathrm{ms^{-1}}$	Sinking speed of detritus compartment D_2
v_3	10^{-2}	$\mathrm{ms^{-1}}$	Sinking speed of detritus compartment D_3
v_4	2×10^{-2}	$\mathrm{ms^{-1}}$	Sinking speed of detritus compartment D_4
u_*	Variable	$\mathrm{ms^{-1}}$	Shear velocity
$u_{*,\mathrm{R}}$	6×10^{-3}	$\mathrm{ms^{-1}}$	Critical shear velocity for resuspension. Value corresponding with Kuhrts et al. (2004)
κ	4.1×10^{-1}		von Kármán's constant
M	2×10^{-7}	$\mathrm{sm^{-1}}$	Resuspension parameter (Kuhrts et al., 2004)
z_0	1.67×10^{-5}	m	Bottom roughness height (Warner et al., 2008)

TABLE 2 | Relations/assumptions between production and release of organic matter/release of matter per 1 t harvested biomass.

Form of biomass	Amount	Remarks
Harvested WW biomass	1 t	Assumed to be cultivated from Jan/Feb until June
Net Primary Production (harvested + lost biomass)	1.088 t	Assuming a loss of 8.1% of NPP (Fieler et al., 2021)
Released WW biomass	8.8×10^{-2} t	Assuming a loss of 8.1% of NPP (Fieler et al., 2021)
Released DW biomass	1.06×10^{-2} t	A dry matter content of 12% (Handå et al., 2013)
Released carbon (C)	3.18×10^{-3} t	Assuming a carbon content of 30% of the dry matter (Handå et al., 2013)

no POM been lost), we have that

$$C_{\mathrm{loss}}(p, C_{\mathrm{harvest}}) = k_{\mathrm{C}}(B_{\mathrm{NPP}} - B_{\mathrm{harvest}}) = k_{\mathrm{C}} \frac{p}{1-p} B_{\mathrm{harvest}}, \quad (7)$$

with the unit g C $\mathrm{m^{-2}}$, that is: g C per unit of the farmed area. The factor k_{C} converts from t WW to g C (**Table 2**). The maximum loadings (i.e., the loading in the model grid cell/location with the highest carbon loading) from each of the simulations described above for a loss fraction of $q = 0.08$ (equivalent to 8%), normalized to the unit g C $\mathrm{m^{-2}}$ $(\mathrm{t\,ha^{-1}})^{-1}$ is denoted by $S_{\max}(q)$. This was scaled from the results for $q = 0.08$ to a production scenario of C_{harvest} t $\mathrm{ha^{-1}}$ and a loss fraction of p as

$$C_{\mathrm{load,\,max}}(p, B_{\mathrm{harvest}}) = \frac{(1-q)}{q} S_{\max}(q) \frac{p}{(1-p)} B_{\mathrm{harvest}}, \quad (8)$$

again of the unit g C $\mathrm{m^{-2}}$. In summary

- Equation (7) denotes the average carbon loss from a farm harvesting B_{harvest} t WW $\mathrm{ha^{-1}}$ assuming a loss fraction of p of the NPP (the biomass had nothing been lost) as POM. This may also be interpreted as the average amount of carbon deposited per unit area assuming direct deposition without any horizontal advection.
- Equation (8) represents the *maximum* organic loading (g C $\mathrm{m^{-2}}$) to the sea floor from a farm harvesting B_{harvest} t WW $\mathrm{ha^{-1}}$ assuming a loss fraction of p of the gross production. Here, sinking rates, horizontal transport and diffusion, sedimentation, and resuspension processes have been accounted for, and are calculated by the ocean model dispersal simulations.

We consider two main dispersal and organic loading scenarios with different distribution of the organic matter between different sinking speeds:

- Scenario A, with 50% slowly sinking matter and the mass distributed evenly between the four detritus compartments D_1 to D_4;
- Scenario B, with 90% fast sinking matter: 5% of the matter allocated to each of the D_1 and D_2 compartments and 45% to each of the D_3 and D_4 compartments.

3. RESULTS

3.1. Dispersal of POM

The average (standard deviation, maximum) simulated current speeds (hourly data April, May, and June) at the middle of the sheltered, exposed and offshore sites were $0.078\,(0.048, 0.443), 0.166\,(0.091, 0.8530)$, and $0.219\,(0.125, 0.681)\ \mathrm{ms^{-1}}$, respectively.

The dispersal distances and patterns of POM differed between the three release sites (**Figure 3**). The least dispersive site was the sheltered one, while the offshore location was the most dispersive one in the sense that the 90% of the POM deposited within a much grater region at the offshore site than at the sheltered one (**Figures 3**, **4**). A higher proportion of faster sinking POM lead to less dispersal, but did not change the ranking between the sites. Almost 80% of the sedimented POM released from the sheltered site was transported less than 1 km away from the center of the release site, and thus sedimented within the farm itself in Scenario A. By contrast, around 60% of the POM released from the offshore site was transported at least 2 km from the center of the release site in Scenario A, and more than 20% of the POM was transported more than 16 km away from the offshore site.

Matter in the slowest sinking POM compartment D_1 was transported relatively far for all release sites. In particular at the offshore location, there was a potential for transport of around 40% of the POM more than 60 km away (**Figure 4**). In contrast, the fastest sinking POM (compartment D_4) released from the

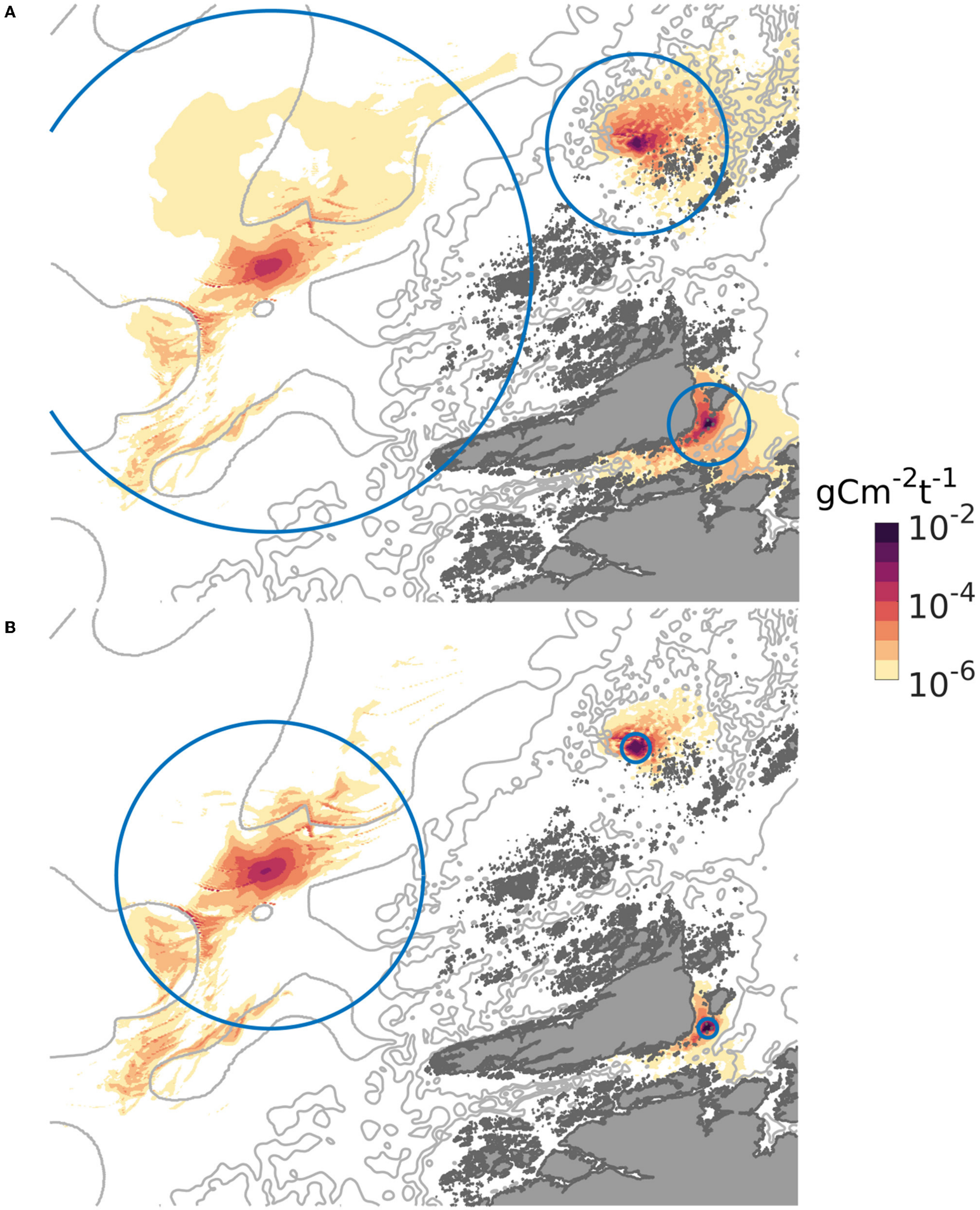

FIGURE 3 | Spatial distribution of POM from the three locations on the sea floor, expressed as g C m^{-2} (t WW harvested biomass) $^{-1}$, June. The gray curves are 100, 200, and 300 m isobaths. The circles indicate the region within which 90 per cent of the released POM deposited. In **(A)**, representing scenario A, the radii of the circles were 4, 10, and 28 km, for the sheltered, exposed, and offshore scenarios, respectively. In **(B)**, representing scenario B, the radii of the circles were 1, 1.5, and 16 km for the sheltered, exposed, and offshore scenarios, respectively. The colormap used is described in Thyng et al. (2016).

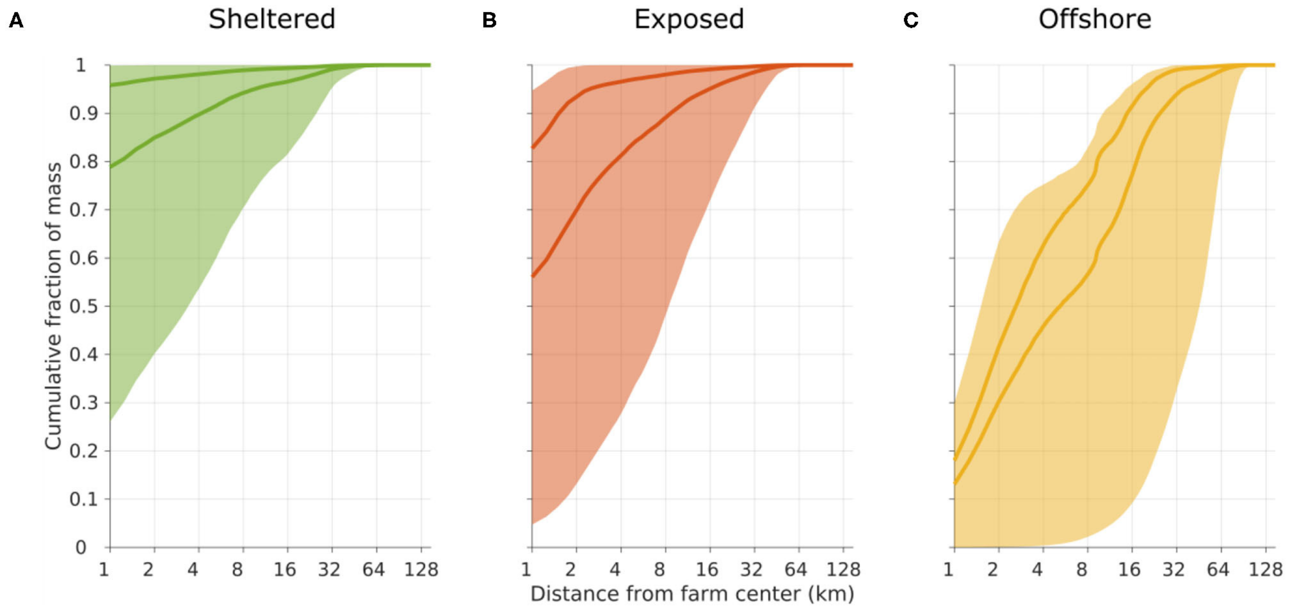

FIGURE 4 | Cumulative frequency plot of the transport distance of POM before sedimentation on the seafloor, released from the sheltered [**(A)**, green], exposed [**(B)**, red], and offshore [**(C)**, yellow] sites. The shaded regions indicate the span from considering only the slowest sinking D_1 (lower limit) to considering only the fastest sinking D_4 (upper limit) compartments. The darker curves represent scenarios A (lower curve) and B (upper curve); cf. **Figure 3**.

sheltered and exposed sites, never moved more than 2 km, thus in practice remaining within the near zone. At the offshore site, around 30% the D_4 POM was transported more than 4 km away before depositing.

3.2. Deposition Depth

The average deposition depths of the POM varied according to the location, reflecting the bottom depth in the region around the release sites (**Figures 1**, **5**). POM released from the offshore location deposited significantly deeper than that released from both the sheltered and exposed sites for all POM compartments D_1 to D_4 (see confidence intervals in **Figure 5**). In this case, the deposition depth varied significantly between POM compartments as well, except between D_3 and D_4. The D_1 POM deposited shallower than the other compartments probably because the settling velocity allowed for transport out into deeper waters, and then toward the shallow watered archipelago to the East of the offshore site and directly South West and East of the exposed site (**Figure 3**). For each POM component D_j the median deposition depth was shallower when released from the sheltered than the exposed site. Between POM compartments, there were significant differences in the median deposition depths between D_1 and D_4 only.

3.3. Time to Settlement of Released POM

The time to settlement of the released POM was estimated based on the median deposition depths δ_j (**Figure 5**) and the sinking velocities (**Table 1**) of POM compartments $j = 1, 2, 3, 4$:

$$T_{\text{settlement},j} = \frac{\delta_j}{\nu_j}. \tag{9}$$

The fastest sinking POM compartments (D_3, D_4) deposited within a few hours at all the locations (**Table 3**). The slower sinking compartments spent, on average (median) up to 3(D_2) and 20 days (D_1) in suspension.

3.4. Organic Loading

The dispersal results were translated into maximum organic loading through (8) and visualized as a function of harvested kelp biomass (t ha^{-1}) and biomass loss [in percentages of the NPP, Equation (7); **Figure 6**]. The carbon loading at the seafloor was greatest at the sheltered site, in line with the distribution pattern (**Figure 3**). The partitioning of the biomass between the different POM compartments impacted significantly on the magnitude of the organic carbon loading. Thus, the increase in the maximum carbon loading from scenario A (slow sinking) to B (fast sinking) was 65% for the sheltered location, 58% for the exposed location, and 56% for the offshore location, as POM accumulated over a smaller area.

The maximum carbon loading relative to the average (= max) carbon loading in a situation where the POM sinks straight down (no horizontal advection) is expressed by the quotient of Equation (8) to (7):

$$Q = \frac{C_{\text{load, max}}}{C_{\text{loss}}} = \frac{q}{k_C(1-q)} S_{\text{max}}^{-1}, \quad \text{(present case: } q = 0.08). \tag{10}$$

This quotient is independent of the cultivation volume and the fraction of matter deposited. A value below 1 means that the matter is dispersed more than in the passive (no horizontal advection) scenario, while a value above 1 indicates that aggregation of matter contributes to the maximum carbon

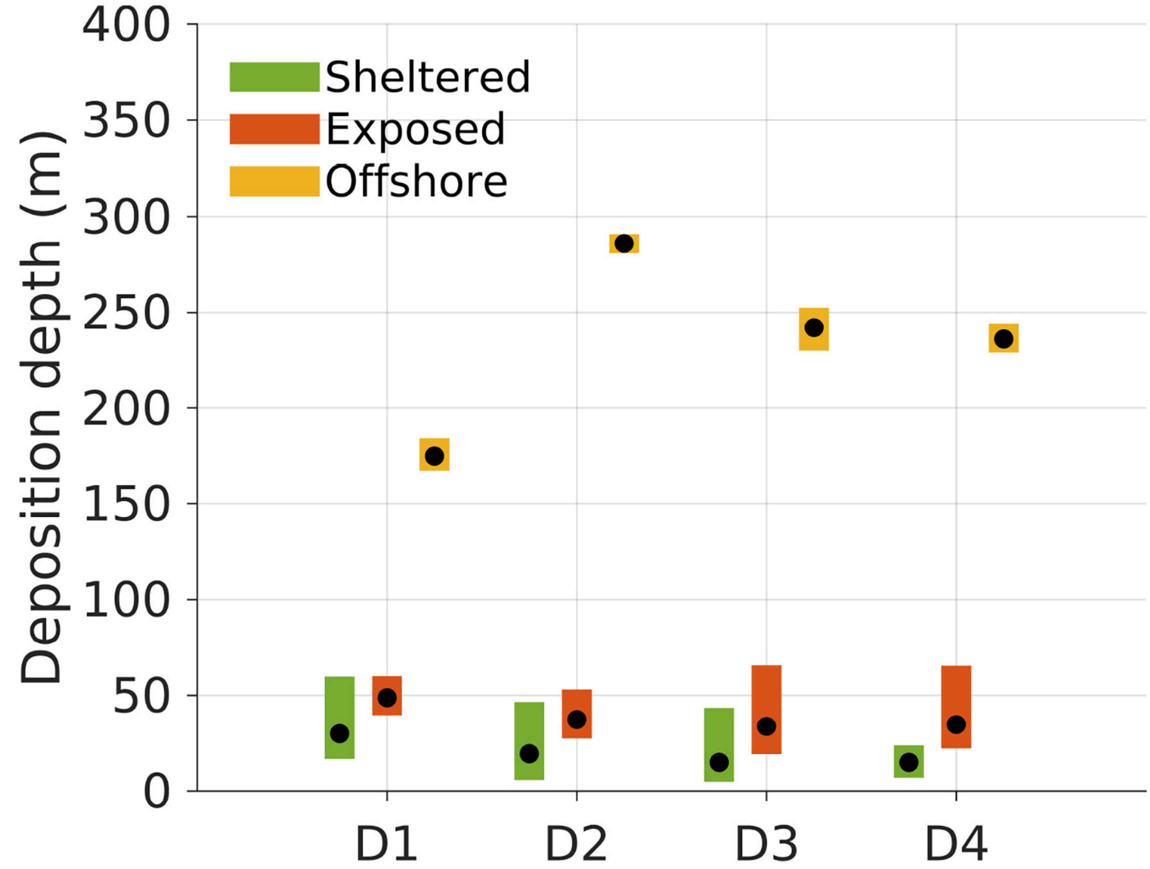

FIGURE 5 | Deposition depths of POM compartments D_1 to D_4 released from the surface at the three locations (sheltered, 18 m depth; exposed, 40 m depth; offshore, 232 m depth; **Figure 1**). The black dots indicate the median deposition depth of the POM for each release site and compartment. The colored bars represent the 95% confidence intervals for the median deposition depths based on the simulation results. The confidence intervals were computed by bootstrapping, extracting 10,000 subsamples from the original deposition field 100,000 times.

loading (**Figure 2**). The average loading rates should be the same, assuming no degradation or consumption. The fractions (rounded to one decimal place) for the Sheltered location were 0.8 and 1.2 for Scenarios A and B, respectively. For the exposed location the fractions were 0.7 and 1.2. For the offshore location, the fractions were 0.1 and 0.2.

4. DISCUSSION

POM is inevitably released from kelp farms during the growth season (Zhang et al., 2012; Fieler et al., 2021) and enters the surrounding environment. How this POM is dispersed, transported and deposited, depends on a number of factors, including the position and exposure level of the farm site. On the seafloor, kelp POM is a food source for the benthic community (Renaud et al., 2015; Queirs et al., 2019) and/or potentially impacts the fauna community negatively (Campbell et al., 2019; Harbour et al., 2021b). A fraction of the carbon in the POM escapes faunal digestion and microbial degradation and is consequently buried in the seafloor. This leads to sequestration of the organic carbon and thus forms a pathway for climate mitigation through removal of carbon initially fixed from atmospheric CO_2 during kelp growth (Krause-Jensen and Duarte, 2016; Duarte et al., 2017).

In this article, we have approached the problem of how kelp POM released from different kelp cultivation sites is distributed in time and space by applying a hydrodynamic model. The purpose has been to investigate how some important properties (detritus fragment size and sinking speeds) and site characteristics (water depth, current speeds) impact the foot print of kelp farms and their contribution of organic loading on the seafloor and downstream potential for carbon sequestration. The dispersal results were used to provide a general formulation of the distribution and traveling distance for three scenarios of coastal, exposed and offshore kelp farming, respectively. This enabled predictions of the deposition and maximum organic loading potential (g C m^{-2} per t WW harvested biomass) for future cultivation scenarios (Broch et al., 2019) as a function of the total biomass harvested and the loss fraction. Previous studies have considered dispersal of kelp detritus from natural populations (Filbee-Dexter et al., 2018).

TABLE 3 | Estimated time until settlement (h) of the four POM compartments released from the three sites.

Location type	D_1	D_2	D_3	D_4
Sheltered	69	6	0.5	0.25
Exposed	139	10	1	0.5
Offshore	486	78	7	3.5

While a Norwegian region was the setting for this study, similar kelp communities exist in other European temperate regions (Smale et al., 2022), and cultivation of *S. latissima* presently takes place from Portugal to sub-arctic regions of Norway (Azevedo et al., 2019; Forbord et al., 2020). The results described here are therefore relevant for other regions as well.

4.1. Sinking Speeds and Transport

The deposition area and transport distance from the release point(s) depended substantially on the sinking rates used in the simulations.

The export transport distance and thus the ecological impact area is closely linked to fraction size and particulate sinking rates. Smaller detritus fractions have lower sinking rates and potentially travel further (Wernberg and Filbee-Dexter, 2018). This leads to larger impact areas of kelp farming but with potentially a lower organic loading and following ecological impact (Sweetman et al., 2014). Conversely, larger POM fractions (with greater sinking speeds),will deposit over a smaller area, with potentially greater local effects.

Even the fast sinking POM components (D_3, D_4) were transported far relative to fecal and feed particles from fin fish farming (Broch et al., 2017). This can be explained by two factors. Firstly, the sinking rates even of D_3 and D_4 were low relative to those used in deposition studies for fin fish farming. Settling velocities used in Chang et al. (2014) were a mean of 3.2×10^{-2} ms^{-1} for fecal and 11.0×10^{-2} ms^{-1} for feed particles). Secondly, the waters around the exposed and offshore release locations were deep relative to typical coastal fin fish farming locations (Chang et al., 2014; Broch et al., 2017).

Sinking speeds ranging 3 orders of magnitude were considered, allowing for interpretation of the results in the context of a wide range of possible distributions of the biomass between different sinking speeds. In particular, sinking speeds for blade fragments of *S. latissima* have not been properly established. In Wernberg and Filbee-Dexter (2018), the seaweed POM was correlated to the size and mass of *L. hyperborea* particles. The weight per unit area of frond tissue in cultivated *S. latissima* seems to be a lot lower than in naturally growing *L. hyperborea* (Foldal, 2018; Wernberg and Filbee-Dexter, 2018). This indicates that the sinking speeds of *S. latissima* POM may be lower than for, e.g., *L. hyperborea*. On the other hand the average density of *L. hyperborea* blade tissue fragments reported by Wernberg and Filbee-Dexter (2018) was $1064 \pm 96 \mathrm{kg\ m}^{-3}$. Corresponding values for *S. latissima* blade fragments are 1092 ± 91 (Vettori and Nikora, 2017) and 1120 ± 130 (Norvik, 2017).

Many deposition and transport models employ Lagrangian (i.e., particle based) rather than Eulerian (i.e., concentration fields) approaches (Cromey et al., 2002). One advantage of Eulerian models, used in the present study, is that there is no need to convert from particles representing various parts of the released organic mass to concentration fields. On the other hand, it is possible to track the history of single particles in Lagrangian approaches, and to include many properties of the particles (size, sinking rates, composition) without increasing the computational costs unduly.

There is evidence that the bottom type may impact on the resuspension (Carvajalino-Fernández et al., 2020), though we have not explicitly taken this into consideration here. The effects of resuspension are probably not underestimated since we have used a critical shear velocity for resuspension very close to the parameter used by Kuhrts et al. (2004) for the "fluff" layer of fine matter.

Degradation of kelp POM may take several weeks or even months (de Bettignies et al., 2020b; Smale et al., 2022), with the photosynthetic capacity still partially intact (Frontier et al., 2021). This impacts on the potential for large-scale and long-term transport, and for how long the POM is left on the sea floor has bearing on the degradation rates and the associated bacterial community (Brunet et al., 2021). Therefore, a number of coupled inter-actions between physical transport and biological degradation determine where and in what state kelp POM settles on the sea floor. This should be addressed in more detail in future studies.

4.2. Organic Loading and CO_2 Sequestration

The organic loading rates were presented in terms of g C m^{-2} per t WW harvested biomass. While a somewhat unorthodox unit, it allows for interpretation of the results both in terms of cultivation intensities and for farm-size independent assessments and generalized appraisals. Thus, the maximum organic loading on the seafloor can be visualized as a function of the production density and the biomass loss (**Figure 6**). For instance, the organic loading from a farm at a sheltered site with an export of fast sinking ("large") POM producing 150 t WW ha^{-1} and having a loss rate of 11% of the gross production [defined in this context by Equation (7)] will result in an added organic loading of 60–80 gC m^{-2} y^{-1}, equivalent to the average net pelagic primary production in the Norwegian sea, estimated at 65–79 gC m^{-2} y^{-1} (Skogen et al., 2007; Hansen and Samuelsen, 2009). Thus, the potential for organic input to the sea floor per unit area doubles in this case, assuming that neither kelp nor phytoplankton POM is grazed of remineralized in the water column before depositing on the sea floor. This leads to a conservative estimate for the relative contribution of kelp organic loading, as phytoplankton is largely consumed by secondary producers (e.g., zooplankton) or remineralized in the water column. The present results may be used as a starting point to estimate loading for used in risk management (upper estimates, max loading) until further, more detailed data from large scale operations become available.

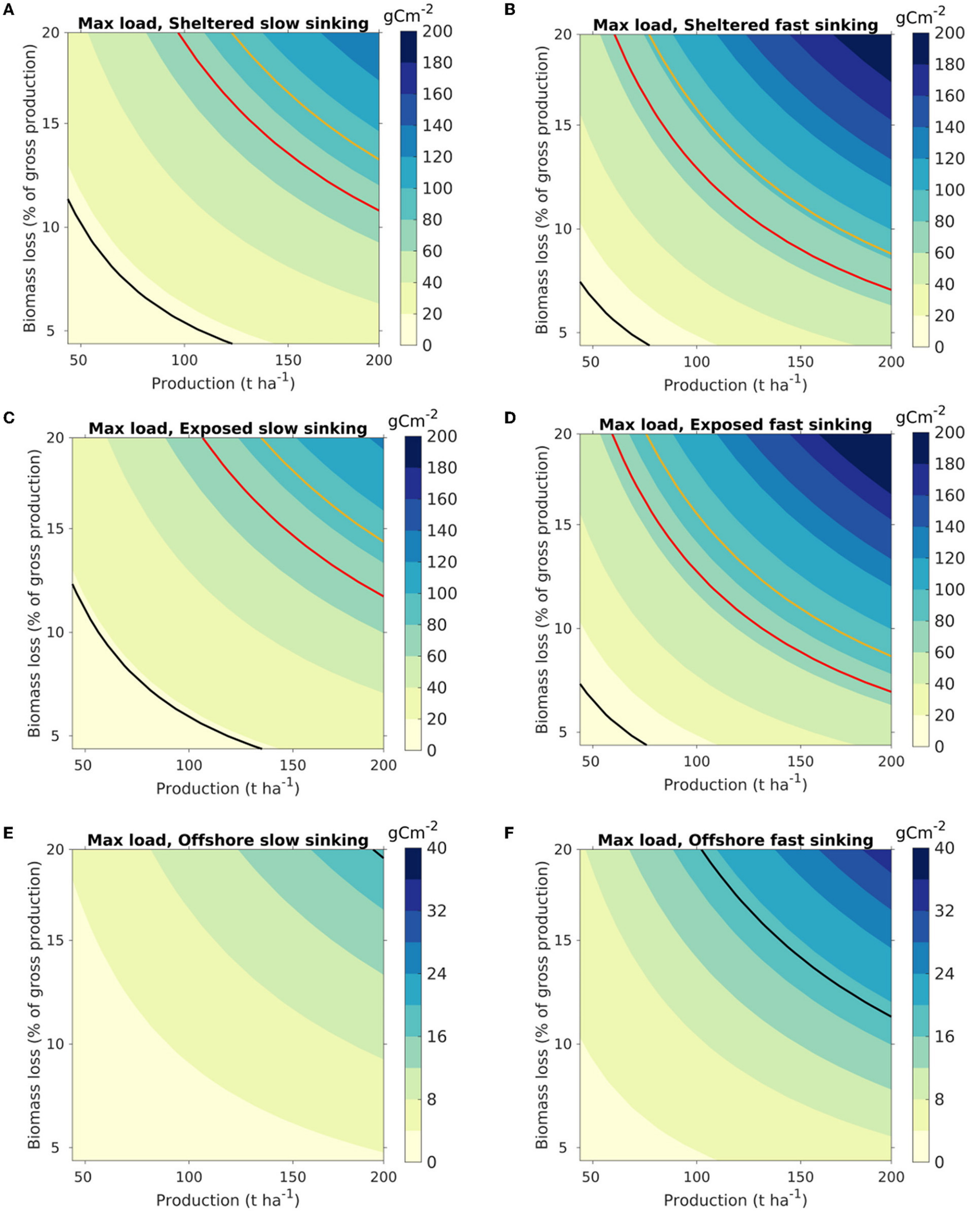

FIGURE 6 | Maximum organic loading on the seafloor from kelp cultivation, visualized as a function of the production density (t WW harvested biomass ha^{-1}) of *S. latissima* (abscissa) and the biomass loss (ordinate) (Equation 8). The colors indicate the highest loading within the model domain, with release from each of the three locations [sheltered: **(A,B)**; exposed: **(C,D)**; offshore: **(E,F)**]. The color scaling varies between the release locations. Results are shown for scenarios A [slow sinking POM, **(A,C,E)**] and B [fast sinking POM; **(B,D,F)**]. The isocurve in red represents 72 g C m^{-2}, corresponding to estimated net pelagic primary production in the Norwegian Sea (Skogen et al., 2007; Hansen and Samuelsen, 2009). The black and yellow isocurves represent loadings of 19 and 90 g C m^{-2}, equivalent to the loading from an *S. latissima* production of 100 t WW ha^{-1} with, respectively, 5 and 20% loss calculated from (Equation 7), assuming direct deposition without horizontal advection of the POM (cf. Equation 7).

Kelp POM deposition on the seafloor also potentially contributes to carbon sequestration through sedimentation and long-term storage of carbon in seafloor sediments or deep water layers (Duarte et al., 2017, 2021). This implies that kelp organic matter is escaping faunal digestion and microbial degradation on the sea floor and is locked away by sedimentation at climatically significant time scales of decades to centuries (IPCC, 2019).

Our simulation results indicate that none of the matter released from any of the 3 locations considered here would reach far and deep enough to move into the North Atlantic thermohaline circulation. A large fraction of the D_1 compartment had not deposited by the end of the simulation, and was even transported out of the model domain, leaving deposition in deep waters outside the continental shelf an open question.

The carbon sequestration potential is, ultimately, dependent on how POM released from kelp farming degrades. This, in turn, depends not only on the composition of the matter, but on the size fraction and sinking rates, as well as oxygen, temperature, and the bacterial community (Wernberg and Filbee-Dexter, 2018; Brunet et al., 2021; Filbee-Dexter et al., 2021). As the time in suspension is strongly affected by sinking rates, we see that the degradation of kelp POM in the water column thus depends on the size of the particles; the average suspension time for the slowest sinking POM (model compartment D_1) was more than 100 times as long as that of the fastest sinking POM (model compartment D_4). This relates well to the difference in sinking speeds applied. However, the model has not included a compartment for burial of POM, so that, in principle, all the deposited matter was available for resuspension. This may have contributed to increasing the average suspension time.

4.3. Conclusions

The issue of transport and deposition is basically simple: The horizontal dispersal distance increases with water depth and decreases with increasing sinking rates. Thus, while a specific model setup has been applied in the present study, the simulation scenarios link general features of the released matter (sinking speeds) to water currents and depth, and the results can thus be applied generally. The results are scalable to any farm size and actual release rates from seaweed farming.

This study underscores the importance of constraining the dispersal and deposition of detritus from kelp cultivation in order to better understand and quantify the associated environmental risks (the effects of organic loading), and to explore the potential for seaweed farming as a climate mitigation solution through sediment carbon sequestration. This is becoming increasingly important with the global increase of the seaweed farming industry, and the urgent needs for decreasing the atmospheric and marine CO_2 concentrations.

We suggest that further research includes focus on the size distribution and sinking velocity of POM from seaweed farms, spanning the range of cultivated species and their physical properties and physiological conditions when detached from the farm. Also, the distribution of detritus between size fractions should be further investigated. Finally, the degradation dynamics, and how this interacts with the size spectrum and transport, should be given attention.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

OB, KH, and IE conceived and planned the article, contributed to the analysis of the results, and the writing of the article. OB ran the dispersal simulations. All authors contributed to the article and approved the submitted version.

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Temperature-Induced Environmental Chain Reaction in Marine Sedimentation and Its Impact on Manila Clam *Ruditapes philippinarum*

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Marine Heatwaves (MHWs) events have been increasing, causing severe impacts on marine ecosystems and aquaculture. In this study, the effects of temperature on changes in sediment dissolved oxygen (DO) and hydrogen sulfide (H_2S) concentrations and their effects on the behavioral and physiological responses of a bivalve were investigated by simulating different sudden summer temperature change conditions. The results showed that temperature was an important factor affecting DO consumption and H_2S release in sediments, and sediment type also played a key role. At higher temperatures, DO was consumed more rapidly and H_2S release increased in sediments, and the DO was consumed and H_2S released in sandy sediments was less than in silty sand sediments. The response of Manila clam's exercise behavior under environmental pressure was also rapid. The excavation index of the Manila clam decreased with the DO consumption, indicating that the Manila clam could alter its burial depth and move toward the sediment-water interface when stressed. Compared with phenol oxidases (PO) activity being activated under experimental conditions, succinate concentrations only increased slightly at 32°C, and did not surpass the threshold indicative of anaerobic metabolism. However, when toxic substances such as H_2S start to accumulate, they may damage the immune system and tissues of the Manila clam, thereby affecting its future survival. Therefore, when temperatures are high for long periods in summer, it is necessary to take timely action to prevent and guard against harm caused by DO and H_2S to aquaculture organisms.

Keywords: *Ruditapes philippinarum*, temperature, dissolved oxygen, hydrogen sulfide, behavioral characteristic, physiological response

INTRODUCTION

Global climate change is responsible for increased frequency, intensity, and duration of extreme events, such as marine heatwaves (MHWs) are having far-reaching impacts on marine ecosystems (Gall et al., 2021; Mcpherson et al., 2021). MHWs are driving temperature increases at rates and levels well above projected warming scenarios (Babcock et al., 2019; Holbrook et al., 2019). The coupled impacts of acute temperature change and species thermal tolerance are causing havoc across the aquaculture industry as seen in the mass mortality of bivalves and seaweeds (Smale et al., 2019; He et al., 2021; Plecha et al., 2021; Xu et al., 2022). Increasing temperatures appear to be the more important contemporary stressor for many marine organisms (Hughes et al., 2017; Smale et al., 2019; Scanes et al., 2020a,b).

Increasing temperatures are not the only environmental source of stress to marine organisms. Increases in temperature are naturally accompanied by decreases in dissolved oxygen (DO) concentration. Globally, the prevalence of hypoxic areas caused by high temperatures in summer continues to increase, especially in offshore eutrophic areas (Diaz and Rosenberg, 2008; Suikkanen et al., 2013; Smith et al., 2020). Following the excessive reproduction of phytoplankton, massive amounts of organic matter sink to the seafloor where they are degraded *via* processes that consume oxygen and produce hypoxia or anoxia regions (Li et al., 2018). Especially in summer, haloclines and thermoclines weaken the vertical exchange of water, hindering the vertical mixing of DO, and aggravating the hypoxic or anoxic conditions of bottom waters (Wei et al., 2007). Additionally, biological sedimentation resulting from benthic shellfish culture has also led to increased sediment oxygen consumption, which exacerbated the risk of hypoxia or anoxia in the sediment environment of culture areas (Gilbert et al., 1997; Yang et al., 2007). In hypoxic or anoxic environments, the sedimentary environment shifts from an oxidative state to a reductive state (Nagasoe et al., 2011), which accelerates the process of acid volatile sulfur (AVS) reduction to produce hydrogen sulfide (H_2S) (Kodama et al., 2018). H_2S can be harmful, and even lethal, to benthic organisms (Smith et al., 1977; Joyner-Matos et al., 2010; Soldatov et al., 2018). To date, many studies regarding the environmental impact on shellfish have focused on the variation of DO concentration and the content of AVS in the water column, little attention has been paid to sedimental DO concentration and the H_2S content which is confirmed to be the only sulfide that causes harm to aquatic animals (Kodama et al., 2018; Liang, 2019). Therefore, the variation of DO and H_2S concentrations in the sedimental environment under high summertime water temperature are currently unknown.

Changes in environmental factors significantly affect the behavioral and physiological responses of marine organisms (Long et al., 2008; Li et al., 2019). When benthic shellfish experience hypoxia, they often seek access to additional oxygen by altering their digging behavior to reduce their burial depth (Lee et al., 2011). Bivalves mainly rely on glycolysis to meet their energy requirements when exposed to hypoxic conditions, and when the DO concentration continues to decrease, the dissimilation of glycolysis and the accumulation of succinate or propionate will be observed (Lee et al., 2011). Therefore, succinate concentrations can be used as an indicator of hypoxia stress in bivalve molluscs (Lee et al., 2011). Additionally, phenol oxidases (PO) and hydrolases, which are usually involved in detoxification, inflammation, and digestion, are considered to be the immune functions of bivalve molluscs that are most susceptible to environmental stressors (Hellio et al., 2006). Meanwhile, PO also plays an important role in the melanization of soft tissues and shells of bivalves (Cerenius et al., 2008). Even though the multiple environmental pressures often overlap in practical cases, most studies focus on individual pressures and few have considered the relationships between multiple pressure sources and how they affect marine organisms (Parisi et al., 2017).

The Manila clam *Ruditapes philippinarum* aquacultured in America and Europe was originated from Asia, and is now one of the most popular clams worldwide. However, Manila clam is threatened with mass death every summer (Jonsson and Andé, 1992; Wootton et al., 2003). Although there is evidence that environmental factors play a decisive role in the survival of Manila clam (Gestal et al., 2008; Matozzo and Marin, 2011), it does not seem possible to pin the cause of a large-scale Manila clam die-offs in summer on a single environmental pressure (Gajbhiye and Khandeparker, 2019). This is because any change in an environmental factor will trigger a series of environmental chain reactions. Therefore, a single change can drive changes in other environmental factors that may jointly affect the survival of marine organisms in an environment, resulting in large-scale mortality in conditions that would normally be considered tolerable (Parisi et al., 2017; Kim et al., 2018). In this study, temperature-controlled experiments were conducted to simulate sudden temperature changes in summer, to identify the characteristics of variation of DO and H_2S in different substrate types of sediments under different temperature conditions, and to clarify the environmental chain reactions brought about by temperature, as well as the effects on the behavioral and physiological responses of Manila clam. This study will serve as a reference for future research on artificial breeding, resource conservation, behavior, and early warning mechanisms of the Manila clam and related molluscs.

MATERIALS AND METHODS

Method

In June 2020, following previous studies, box-corer and water sampler were used to collect sediments and bottom seawater from high-density and low-density breeding areas in the Weifang Longwei group breeding area in the Xiaoqing River Estuary (37°16.290′N, 119°04.248′E) in Shandong Province, China. Sediment samples were firstly filtered to remove macrobenthic organisms. After the samples were collected, kept separate according to different sites and put into a refrigerator, and the experiment was initiated immediately after returning to the laboratory. The environmental characteristics of the sampling sites are shown in **Table 1**.

TABLE 1 | Environmental characteristics of the sampling site.

	High-density area	Low-density area
Depth at high tide (m)	4.3	8.7
Water temperature (°C)	25.50	24.86
Sediment temperature (°C)	26.17	25.84
Salinity	28.43	28.51
Mediangrain size of surface sediment (μm)	67.35	110.33
Silty and Mud (%)	46.62	11.23
Substrate types	Silty sand	Sandy

Manila clams were provided by the Shandong Longwei Group. To avoid any transportation/transition effect on the shellfish in this experiment, the clams were acclimatized to the laboratory for 1 week before the experiment. During acclimation, the water temperature was 24°C, salinity was 30, the clams were fed chlorella every day, and oxygen was maintained and water changed daily.

The sediments of different types were spread evenly in experimental tanks [20 cm (L) × 15 cm (W) × 25 cm (H)] to a depth of approximately 10 cm. The bottom seawater collected *in situ* was added (water depth of about 10 cm), and the experimental tanks were placed in the thermostatic water baths at 24 28, and 32°C. The water level in the water bath exceeded the height of the overlying water in the experiment tanks. There were two different sediment types in each temperature water bath, making 6 experimental groups, and each experimental group had three replicate tanks. The experiment lasted for 12 h (one tidal cycle).

A particularly designed cylindrical collector (5 cm in diameter and 20 cm in depth) was inserted in each experimental tank to avoid the interference of experimental organisms. The sedimental DO and H_2S content at different depths were measured in the collector using a microelectrode system (Unisense, Aarhus, Denmark). The experimental monitoring parameters were measured every 3 h. The measurements were conducted to 10 cm below the surface sediment with microelectrode steps of 5 mm.

At the beginning of the experiment, the Manila clams in the holding tank were directly placed into the experimental tanks to simulate sudden temperature change in natural conditions. 10 clams (5.3 ± 0.27 g, 30.38 ± 1.23 mm shell length) were placed in each experimental tank and 30 Manila clams with the same specifications as the experimental clams were selected from the temporary culture flume to serve as the control (3 groups). The experiment lasted for 12 h. The succinate concentration in the muscle and PO activity in the hepatopancreas in the Manila clams were measured before and after the experiment, and the excavation index of Manila clam was measured after the experiment.

Sample Measurement

H_2S at different depths were measured using the manual propeller paired with the microelectrode system. The overlying water-sediment interface was recorded as the depth of 0 cm. The change of DO was quantified based on the overlying water DO concentration, penetration depth, and average DO concentration of the penetration layer. Specific operational parameters: the DO penetration depth referred to the depth from the sediment water interface to the depth where the DO concentration was lower than 0.1 mg/L, and the average concentration of DO in the penetration layer was recorded (Liang, 2019).

After the experiment, the digging scores of all Manila clams in each experimental group were recorded. The specific scores were graded as follows: 1, shells were completely buried in the sediment; 0.5, shells were partially exposed; 0, shells were entirely exposed on the sediment surface (Lee et al., 2007, 2011). The digging scores of all shellfish were added together and divided by the number of shellfish to obtain the digging index for the Manila clam. The higher the index was, the deeper the Manila clam was. After that, the Manila clams from each tank were collected and the hepatopancreas and soft tissues were dissected, sampled, and stored in liquid nitrogen for later use.

Succinate was measured in clams exposed to different temperatures and compared to the control clams measured before exposure. After freeze-drying the muscle tissue, the samples were ground into a powder with a freeze grinder. 100 mg dried sample powder was weighed and 1 mL of 50% methanol/water solution was added, this solution was vortexed for 30 min, centrifugated at 12000 rpm for 5 min, and 50 μL of supernatant was collected. 50 μL of propionic acid isotope standard (5 μg/ml), 50 μL of 3-nitrophenylhydrazine (3-NPH) (250 mM, prepared with 50% methanol/water solution), and 50 μL of EDC [150 mM, prepared with 75% methanol/water solution (containing 7.5% pyridine), i.e., methanol: water: pyridine = 69.375: 23.125: 7.5] were added to the supernatant and it was placed in a shaking mixer for derivatization at 30°C for 30 min. After that, 50 μL of 2,6-di-tert-butyl-p-cresol (BHT) methanol solution (2 mg/mL) and 250 μL of 75% methanol aqueous solution were added. The sample was centrifuged at 12000 rpm for 5 min at 4°C. 200 μl of the supernatant was extracted and analyzed by mass spectrometry.

The separation was performed using a Waters UPLC BEH C8 column (2.1 × 100 mm, 1.7 μm) with the mobile phases of A-phase (water, 0.01% formic acid) and B-phase (methanol: isopropanol = 8: 2) at a flow rate of 0.3 ml/min. The column temperature was 45°C. Mass spectrometry was performed using a Waters XEVO TQ-S Micro tandem quadrupole mass spectrometer system. The ion source voltage was 3.0 kV, the temperature was 150°C, the desolvation temperature was 350°C, the desolvation gas flow rate was 1000 L/h, and the cone well gas flow rate was 10 L/h.

The PO activity was measured in the hepatopancreas of each clam. The frozen samples were ground into powder using a cold grinder (SPEX, United States) then 500 μL of lysate was added and the solution was homogenized before centrifugation at 1000 r/min at 4°C for 45 min. The supernatant was collected and the PO activity was determined by a previously reported method. Specifically, 50 μL of supernatant and 50 μL of Tris-HCl buffer were incubated at 25°C for 10 min, then 100 μL of 0.04 M L-DOPA was added and the absorbance value at 492 nm was read 30 min later with a microplate reader at 25°C. Meanwhile, the spontaneous oxidation of L-dopa was measured using the

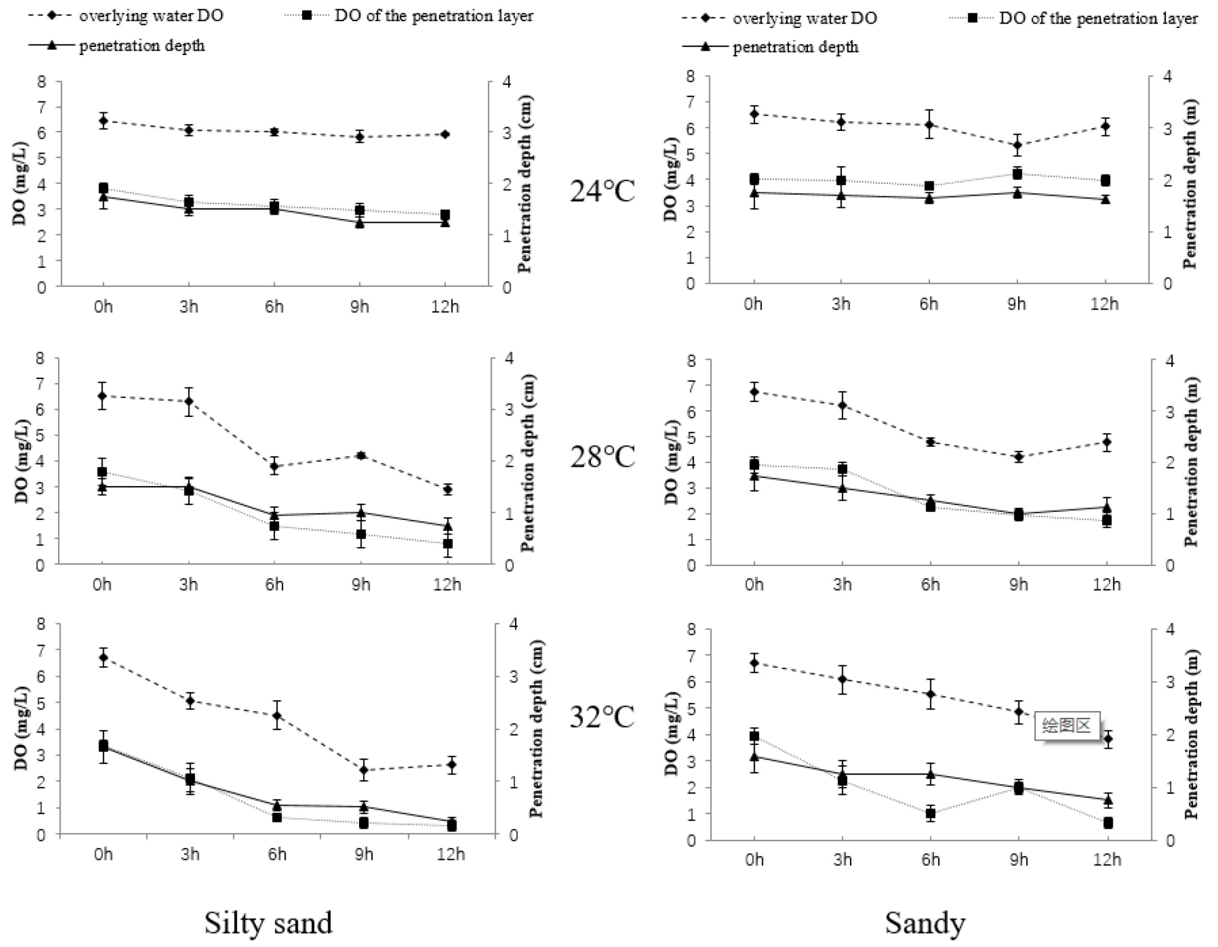

FIGURE 1 | Changes in the overlying water DO concentration, penetration depth of the penetration layer, and average DO concentration of the penetration layer in different sediment substrate types over time.

TABLE 2 | Two-way ANOVA results of DO-related parameters.

Variables	Source	*F*	*P*	Source	*F*	*P*
Overlying water DO concentration	Temperature	27.003	0.00	Temperature × Substrate	5.793	0.02
	Substrate	8.792	0.01	Temperature × Time	19.564	0.00
	Time	79.119	0.00	Substrate × Time	2.698	0.11
Average DO concentration of the penetration layer	Temperature	76.140	0.00	Temperature × Substrate	0.257	0.62
	Substrate	15.501	0.00	Temperature × Time	16.254	0.00
	Time	62.272	0.00	Substrate × Time	1.461	0.24
Penetration depth of the penetration layer	Temperature	101.842	0.00	Temperature × Substrate	1.240	0.28
	Substrate	31.694	0.00	Temperature × Time	20.091	0.00
	Time	104.801	0.00	Substrate × Time	6.758	0.02

same method, but pure water was used as the reactant instead of supernatant and any spontaneous oxidation results were removed from the measured values during calculation.

The PO specific activities were calculated as:

PO specific activities (U/mg prot) = (ΔA/min × dilution factor)/total protein concentration

where, ΔA/min is the value of the increase in absorbance per minute; dilution factor is the sample's dilution factor; and total protein concentration is the sample's protein concentration assayed with Bradford's method.

Data Analysis

Three-factor analysis of variance was used to explore the influences of temperature, time, and geological type on the variables. One-way analysis of variance was used to explore the influences of temperature, DO, and H_2S on behavior and

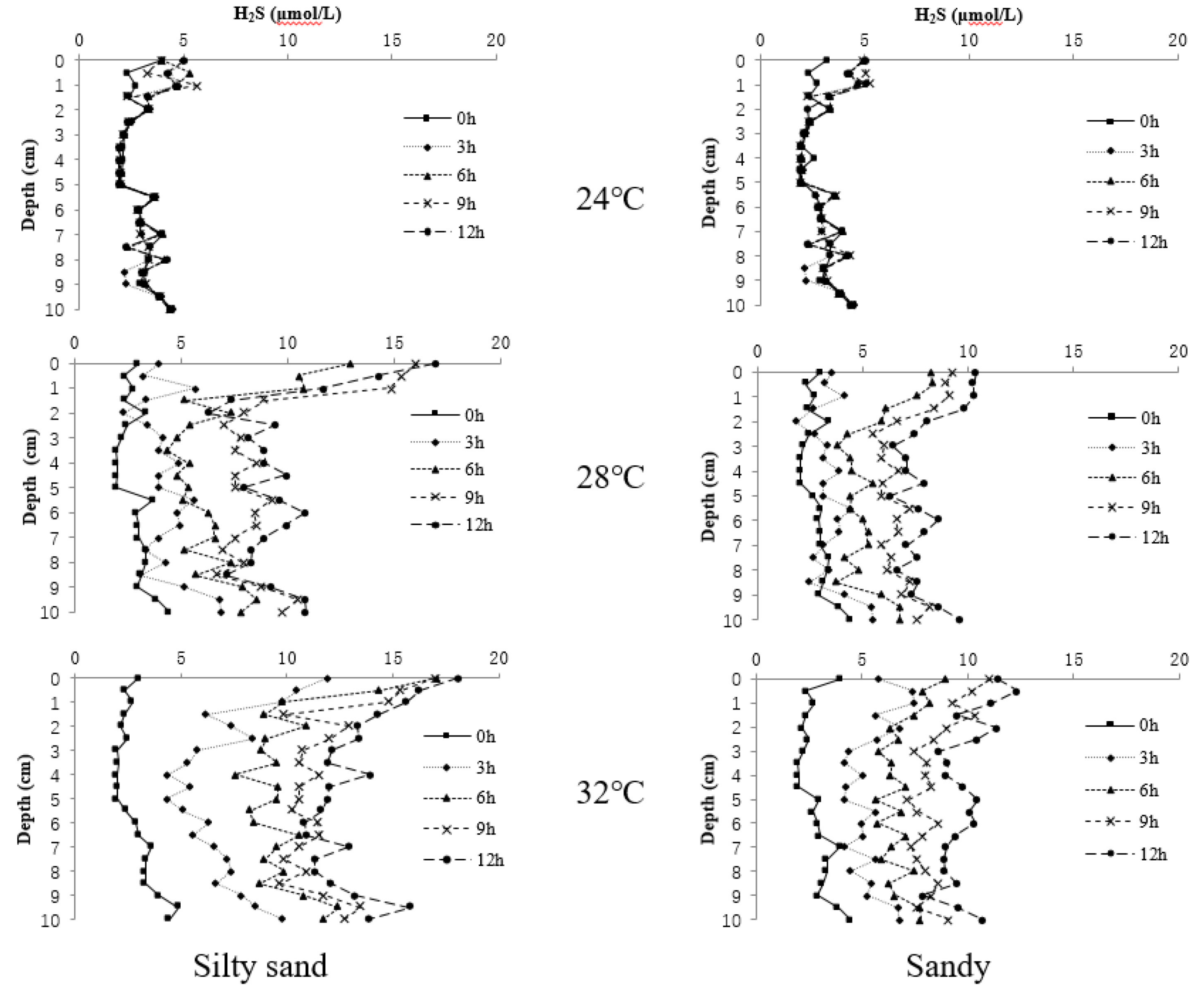

FIGURE 2 | H_2S concentrations at different depths over time in two sediment substrate types.

physiological indicators. The above analysis is completed in the R language program using the "vegan" package.

RESULTS

Characteristics of Environmental Factor Changes in Sediments

Dissolved Oxygen Content in Sediment

At 24°C, the average DO content of the penetration layer, penetration depth of the penetration layer, and DO content of overlying water in silty sand sediments and sandy sediments difference was not significant ($p > 0.05$, **Figure 1**). At 28°C, DO-related parameters began to decline as the experiment progressed. The average DO content of the penetration layer, penetration depth of the penetration layer, and DO content of overlying water in silty sand and sandy sediments decreased from 3.58 mg/L, 1.50 cm, and 6.51 mg/L and 3.91 mg/L, 1.75 cm, and 6.75 mg/L at the beginning of the experiment to 0.79 mg/L, 0.75 cm, and 2.88 mg/L and 1.75 mg/L, 1.14 cm, and 4.78 mg/L at the end of the experiment, respectively. Likewise, at 32°C, all parameters of both sediment substrate types showed significantly decreases ($p < 0.05$) after 3 h. The average DO content of the penetration layer, penetration depth of the penetration layer, and DO content of the overlying water in the silty sand and sandy sediments at 32°C decreased from 3.37 mg/L, 1.65 cm, and 6.71 mg/L and 3.92 mg/L, 1.59 cm, and 6.71 mg/L at the beginning of the experiment to 0.32 mg/L, 0.25 cm, and 2.62 mg/L and 0.63 mg/L, 0.75 cm, and 3.82 mg/L at the end, respectively. In comparison, the decrease in DO concentration of the penetration layer (55–84%) was much higher than that of the overlying water (29–43%).

The multivariate analysis of variance showed that the DO-related parameters were significantly affected by temperature, substrate, and time. The DO content of the overlying water was significantly affected by the interaction between temperature and substrate, as well as temperature and time individually. The average DO content of the penetration layer was significantly affected by the interaction between temperature and time, and the penetration depth was significantly affected by the interaction between temperature and time as well as substrate and time individually (**Table 2**).

H_2S Content in Sediment

As shown in **Figure 2**, at the beginning of the experiment, there were no significant differences in H_2S concentrations among the experimental groups ($p > 0.05$). After the experiment started, there were still no significant differences in H_2S concentration at different measurement time points in silty sand or sandy sediment substrates at 24°C ($p > 0.05$), but there was a significant change with depth ($p < 0.05$). The change characteristics were small in the middle and high in the surface and bottom layers. In the other temperature groups, the H_2S concentrations gradually increased as time progressed, and the H_2S concentration changed significantly with depth ($p < 0.05$), but there was no obvious change law. At 28°C, the H_2S concentrations in both sediment types increased drastically after 6 h ($p < 0.05$), and the maximum H_2S concentrations in silty sand and sandy sediments were 16.90 and 10.41 μmol/L, respectively. At 32 °C, H_2S concentrations in both sediments showed a significant increase ($p < 0.05$) after 3 h and the maximum H_2S concentrations in silty sand and sandy sediments were 18.10 μmol/L and 12.32 μmol/L, at the end of the experiment, respectively.

The multivariate analysis of variance showed that the H_2S concentrations in sediments were significantly affected by temperature, substrate, and time (temperature: $F = 358.458$, $p < 0.01$; substrate: $F = 86.124$, $p < 0.01$; time: $F = 352.653$, $p < 0.01$). There were significant interactions between temperature, time, and substrate (temperature × time: $F = 89.948$, $p < 0.01$; temperature × substrate: $F = 44.648$, $p < 0.01$; time × substrate: $F = 13.553$, $p < 0.01$). At high temperatures, the H_2S concentrations in silty sand sediments were significantly higher than those in sandy sediments ($p < 0.05$).

Behavioral and Physiological Responses of the Manila Clam

While none of the Manila clams died during the experiment, their digging behavior was affected. With increased temperature, the digging index of Manila clam decreased, and the number of Manila clam that appeared on the sediment surface began to increase. Temperature and the concentrations of DO and H_2S at the sediment-water interface at the end of the experiment all exhibited significant effects on the digging index of Manila clams (temperature: $F = 6.104$, $p < 0.05$; DO: $F = 5.54$, $p < 0.05$; H_2S: $F = 6.275$, $p < 0.05$) (**Figure 3**).

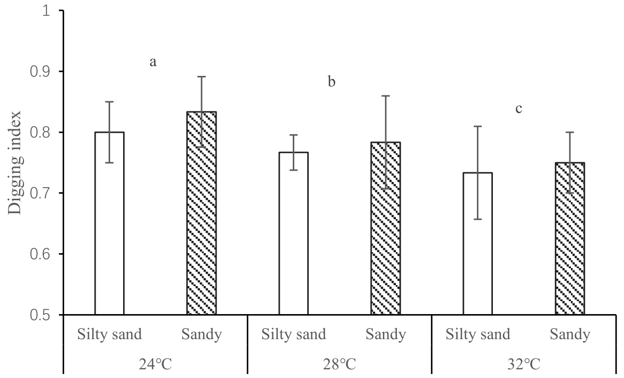

FIGURE 3 | Digging index of Manila clam at different temperatures and in different sediment types. The different letters indicate significant difference of digging index at different temperatures.

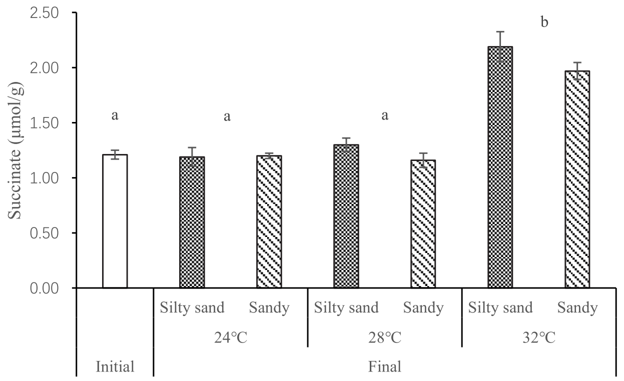

FIGURE 4 | Concentrations of succinate in Manila clam soft tissue at different temperatures and in different sediment types. The different letters indicate significant difference of succinate at different temperatures.

After the experiment, the concentrations of succinate in the soft tissues of the Manila clams exhibited significantly accumulation, but only at 32°C ($p < 0.01$), with a concentration of about 2 μmol/g, which was 60% higher than that at the beginning of the experiment (**Figure 4**). However, the concentration was far lower than the anoxic stress index of bivalves reported in the literature (4.4 μmol/g; Lee et al., 2011), therefore, the Manila clams were not utilizing anaerobic metabolism under the experimental conditions of this study.

The largest difference was seen in the activity of PO in Manila clam hepatopancreas, which was activated as the temperature rose (**Figure 5**). The temperature and concentrations of DO and H_2S at the sediment-water interface at the end of the experiment had a significant impact on the PO activity of the Manila clam (temperature: $F = 145.9$, $p < 0.001$; DO: $F = 54.23$, $p < 0.001$; H_2S: $F = 123.6$, $p < 0.001$), and the interaction between H_2S and DO also significantly affected PO activity (DO × H_2S: $F = 6.381$, $p < 0.05$).

DISCUSSION

Typically, global pressure sources (e.g., global warming and ocean acidification) tend to vary at large time scales. Indeed, while climatic events are playing an important in marine organisms and the ecosystems they support, extreme environmental changes (e.g., MHWs) on a short time scale, in some cases, may generate even more profound impacts. This impact is more apparent for

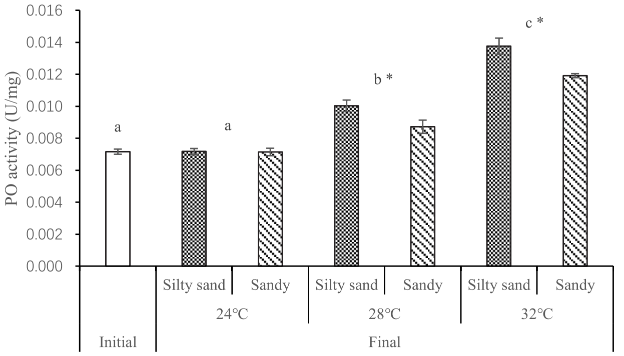

FIGURE 5 | The activity of PO in Manila clam hepatopancreas at different temperatures and in different sediment types. The different letters indicate significant difference of PO at different temperatures. * indicates significant difference of the activity of PO in manila clam in different sediment types under the same temperature conditions.

mudflat bivalves living in estuarine which are more likely to be exposed to drastic temperature change. These rapid temperature change (e.g., within a single tidal cycle) over small spatial scales may trigger unexpected environmental chain reactions (Helmuth et al., 2014). In this study, increased temperature and more prolonged duration resulted in a significant decrease in the DO of the sediment. The higher the temperature, the more dramatic the effect. Therefore, hypoxia or anoxic conditions are more likely to occur in summer with higher temperatures (Diaz, 2001). Furthermore, the effect of temperature on sedimental DO is much higher than that on the overlying water. Likely, the DO concentration of overlying water has not been reduced to hypoxic conditions (DO < 4 mg/L) when the sediment is already in anoxic condition (DO < 2 mg/L). So it may not be accurate to focus only on the DO concentration in the bottom water in benthic shellfish culture area.

When high temperatures and low DO conditions co-occur, the increase in H_2S release also becomes obvious. At the end of this study, the H_2S concentrations in the sediments under normal conditions (24°C) were about 3∼5 μmol/L, while at higher temperatures, the maximum concentration of H_2S in sediment samples was 18.10 μmol/L. However, even this high concentration was still far lower than the concentration of AVS in sampling area sediments (45∼180 mg/kg) (Liu et al., 2009; Yang et al., 2018), but it still approached or exceeded the half lethal concentration for many polychaetes with strong tolerances to H_2S (Wang and Chapman, 1999). So, it is important to accurately measure H_2S concentrations (Liang, 2019). In addition, H_2S is very easy to oxidize and hardly exists in oxygen enriched water, so increases of H_2S concentration have usually been associated with hypoxic or anoxic conditions (Nagasoe et al., 2011). H_2S concentration would increase under the condition of decreasing DO concentration in the sediment. The increased H_2S will also be oxidized by DO, which will accelerate the consumption of the remaining DO and lead to more serious hypoxia phenomenon. In summary, temperature changes may bring about a correlated environmental change of DO and H_2S anomalies in the sediment, resulting in benthic organisms that can be exposed to three stresses simultaneously.

In addition, we note that the effects of DO depletion and H_2S release vary significantly among substrate types in this study, and the environmental indicators of sediments with larger grain diameters are relatively better. Because the water is more easily exchanged, which prevents the accumulation of pollutants (Ichimi et al., 2019) and can effectively increase the oxidation intensity in sandy sediments (Yang et al., 1999). The finer the sediment particles are, the more quickly they accumulate organic matter (Hatcher et al., 1994; Mayer, 1994), which drives sediments to shift to a reductive state. Indeed, the phenomenon where DO concentrations decrease and H_2S concentrations increase does not occur every summer (Kodama et al., 2018). Since the increase of H_2S was also related to the consumption of DO in the bottom waters. Therefore, in a natural environment, under the influence of tidal and other hydrodynamic effects, the exchange of water might disassociate the effect of temperature from the increase in H_2S concentration. Because of this, it's likely that sediment DO and H_2S concentrations in summer were related substrate in addition to magnitude and duration of elevated temperatures. It also reminds us that in summer, when there is a continuous high temperature, it is necessary to monitor H_2S concentrations in addition to changes in the sediment DO.

In this study, increasing temperature and decreasing DO levels did not affect the survival on Manila clams. While there is evidence that increasing temperature and decreasing DO did appear to influence Manila clam mortality additively because temperature increases narrow the tolerable DO range (Kim et al., 2018). However, this conclusion was based on prolonged stress conditions in the laboratory. In a natural environment, under the influence of tides and other hydrodynamic effects, benthic organisms rarely have the opportunity to be exposed to prolonged environmental stress. Manila clam is highly tolerant of temperature or DO variations. The results of succinate proved that Manila clam did not utilize anaerobic metabolism during the experiment. Therefore, the increasing temperature and decreasing DO within a specific range is not the direct cause of the massive mortality of Manila clam. Nevertheless, even increasing temperature and decreasing DO level within know tolerable ranges affected on the behavior of Manila clam. When faced with environmental pressure, marine organisms have various coping strategies, including behavioral and physiological responses (Wu, 2002). This study found that when the sedimental DO decreased, Manila clam will reduce their burial depth to be closer to the sediment-water interface. It also seemed that the Manila clam's locomotory response to environmental pressure was relatively rapid. Firstly, Manila clam may emerge from sediment to breathe more efficiently to cope with stress and then through regulating physiological metabolism, energy supply strategy, and the other physiological processes (Kang et al., 2016). However, the shallower burial depth may increase the risk of predation, indirectly increasing the likelihood of high mortality (Lee et al., 2011; Howard et al., 2017).

Dissolved oxygen, rather than other environmental factors, is a significant stressor in the digging behavior of Manila clam (Kim et al., 2018). Due to the release of H_2S along with increased temperature and decreased DO, Manila clam may be harmed by higher H_2S concentrations in the sediment surface while seeking more oxygen. Indeed, changes in behavioral characteristics of Manila clam also increase the concentration of H_2S. Because the lack of movement (excavation) decreased the irrigation and penetration of DO into the sediments and, therefore, increased H_2S production. In this study, PO activity did change significantly. Our results showed that the release of H_2S might activate PO activity and damage the immune system of the Manila clam. In mollusks, PO is an important substance involved in immune defense that can be activated by external factors and is a key enzyme for melanin formation in the soft tissues and shells of bivalves (Asokan et al., 1997). Changes in the activity of this key component of the immune system may directly affect the survival of mollusks under environmental stress (Cajaraville et al., 1996; Xing et al., 2008). Nagasoe et al. (2011) reported that that exposure to high-H_2S concentrations can cause tissue melanization and damage of the Manila clam, including damage to the siphon, gills, adductor muscle, etc., and further affect physiological processes involved in breathing and feeding. Therefore, even though several environmental factors had no significant effect on the survival of the Manila clam during the initial exposure, as the environmental stress frequency or duration increased, damage to immune system could occur, potentially leading to significantly reduced performance in the future (Kozuki et al., 2013). MHWs induced elevated water temperatures are not the unique source of stress for marine organisms, and the environmental effects on the behavior and physiology of organisms should be considered in an integrated manner.

CONCLUSION

At higher temperatures, DO was consumed more rapidly and H_2S release increased in sediments. Sediment type also affected oxygen consumption and H_2S release rates. When Manila clams face environmental pressure, they respond quickly with locomotory behaviors (e.g., digging). Manila clams shows a strong tolerance to both high temperature and low DO and their physiological metabolism may not be affected by temperature increases or DO reduction within a certain range. However, when toxic substances such as H_2S start to accumulate, they may damage the immune system and tissues of the Manila clam, thereby affecting its future survival. Therefore, when temperatures are high for long periods in summer, and especially when the bottom water DO begins to decrease, it is necessary to take timely action to prevent and guard against harm caused by H_2S to aquaculture organisms. Therefore, areas that experience weak hydrodynamic exchange or that have small sediment particle sizes should be avoided in when selecting benthic bivalves culture locations.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

YL and JZ conceptualized the study. WW and QK were responsible for the experimental operation. XW was in charge of the data curation. YL prepared and wrote the original draft. CL reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Influence of an Offshore Mussel Aquaculture Site on the Distribution of Epibenthic Macrofauna in Îles de la Madeleine, Eastern Canada

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Mussel farming influences benthic environments by organic loading and the addition of physical structure within aquaculture leases. This study evaluated near-field (distance to mussel aquaculture structures, line-scale) and bay-scale (inside vs. outside a blue mussel, *Mytilius edulis*, farm) effects of an offshore mussel farm in Îles de la Madeleine (Canada) on epibenthic macrofaunal communities. Benthic communities were evaluated by underwater visual counts using SCUBA in June and July 2014. The mussel farm influenced benthic communities at line- and bay-scales. Overall, crabs (*Cancer irroratus*, *Pagarus pubecsens, Pagarus acadianus*), sea stars (*Asterias rubens*), Northern moon snails (*Polinices heros*), and American lobsters (*Homarus americanus*) were more abundant in farm sites than outside of farm sites and ocean quahogs (*Arctica islandica)* were more abundant outside. No clear spatial trend was observed for winter flounder (*Pseudopleuronectes americanus)* and the sand dollar (*Echinarachnius parma*) but both species differed (flounder) or showed a trend to differ (sand dollar) between sampling dates. Spatial structure in the distribution of macrofauna was evident within the aquaculture lease as most species were more abundant directly below and close to mussel lines and anchor blocks. There was no spatial structure in non-farm sites. Further investigation is needed to evaluate if mussel farms serve as ecological traps for the species that congregate within them. The long-term effects of the observed spatial effects of offshore mussel culture on macrofauna fitness remains unknown.

Keywords: offshore mussel aquaculture, *Mytilus edulis*, *Homarus americanus*, *Cancer irroratus*, benthic macrofauna, spatial variation

INTRODUCTION

Declining wild stocks and increasing demand for seafood have led to a rapid expansion of the aquaculture industry and concerns of its potential impact on the environment. Bivalve aquaculture has various near- and far-field effects on marine ecosystems. Far-field effects are mostly limited to effects on water column processes, such as alteration of plankton communities

(Prins et al., 1997) and hydrodynamic processes (Plew et al., 2005). Although near-field effects (i.e., within-and immediately surrounding farms) may occur in the water column due to hydrodynamic modifications and farmed bivalves grazing on plankton (see review in Weitzman et al., 2019), most research on near-field effects of bivalve culture has focused on the benthic environment with an emphasis on infaunal and epibenthic macrofaunal communities, sediment structure, and nutrient fluxes and benthic respiration (Souchu et al., 2001; Giles et al., 2006; McKindsey et al., 2011).

The effects of bivalve culture on benthic macrofauna may be evident at several spatial scales. At a bay-scale, culture sites may differ from non-culture sites in the same general area and, at the farm-scale, communities may differ between farm areas at different stages of production (e.g., Toupoint et al., 2008; Drouin et al., 2015). At a smaller spatial scale (line-scale), communities close to or directly below culture gear may differ from those further from such structures (McKindsey et al., 2012; Drouin et al., 2015). In all cases, variations in benthic communities among locations are largely due to two main mechanisms: the addition of physical structure to the environment and organic loading effects.

As suspended bivalve culture is commonly done above unvegetated soft-bottom habitats, culture gear adds considerable physical structure and complexity to an environment that is largely devoid of three-dimensional surfaces (Dumbauld et al., 2009; Forrest et al., 2009; McKindsey et al., 2011). The introduction of aquaculture gear (e.g., ropes and anchor blocks) modifies habitat characteristics and may attract vagile organisms directly (as shelter) or indirectly (as substrate for sessile hard-bottom prey) (Drouin et al., 2015). When fouled, aquaculture structures may increase the abundance and diversity of fauna in farm sites relative to non-farm sites (Saranchova and Flyachinskaya, 2001; Miron et al., 2002; D'Amours et al., 2008) but not necessarily the productivity of these species (Clynick et al., 2008). Bivalve fall-off also alters the habitat of otherwise flat, soft bottoms, adding physical structure through the addition of living hard-bodied organisms and shell debris, although this effect is most likely to occur in mussel farms as other bivalves (e.g., oysters and scallops) are most typically grown in baskets or other structures and do not fall from culture structures to the extent that mussels do.

Many studies of the effects of bivalve culture on the benthic environment have focused on the accumulation of biodeposits (organic material in feces and pseudofeces) from farmed bivalves (Matisson and Lindén, 1983; Callier et al., 2008; Weise et al., 2009). In general, this type of organic loading may alter benthic infaunal and epifaunal communities. This, in turn, may influence the types of larger animals that may feed on these communities (McKindsey et al., 2011; Froehlich et al., 2017). However, as noted by Cranford et al. (2006) and Fréchette (2012), organic loading also occurs in the form of fallen mussels (and associated fauna). Casual observations (authors' pers. obs.) show that there are often notable quantities of fallen mussels on the seabed within mussel farm sites, particularly directly below and close to culture structures, in eastern Canada. This has also been reported elsewhere around the world where mussels are farmed (e.g., Kaspar et al., 1985; Freire and González-Gurriarán, 1995; Inglis and Gust, 2003; Wilding and Nickell, 2013). Fallen mussels represent a direct input of food and may act as a trophic subsidy for the animals that live in and around culture sites with scavengers and opportunistic predators likely benefiting from this novel prey source. Evidence of this trophic link was provided by Freire and González-Gurriarán (1995), who documented a greater proportion of mussels in the diet of crabs located in a Spanish mussel farm area than comparable areas outside of it. More recently, Sardenne et al. (2019) used lab and field studies to determine that large lobsters fed mainly on mussels from a mussel farm.

At the bay-scale, predators and scavengers are commonly more abundant within mussel farms than outside of them because of the greater abundance of physical structure and trophic interactions (i.e., modified benthic communities and the addition of prey through fall-off of farmed and associated species). At the farm-scale, predators and scavengers may be more abundant in some areas than others as the level of fall-off and physical structure in areas is a function of the stage of production (e.g., fall-off is greater and more anchor blocks are deployed to maintain culture structures in place for larger mussels). Farm-related physical structures, such as anchor blocks, ropes, associated farmed animals, and fall-off are not randomly distributed in farm sites – they occur in well-defined rows. Accordingly, vagile macrofauna have been observed to be more abundant in the vicinity of culture structures and fallen mussels than in areas between mussel lines, thus accounting for line-scale variation in species abundances (Inglis and Gust, 2003; D'Amours et al., 2008; Wilding and Nickell, 2013; Drouin et al., 2015).

Numerous studies have documented that the abundance of vagile predators and scavengers (e.g., crustaceans, sea stars, and gastropods) is increased within bivalve culture sites, particularly mussel farms (Romero et al., 1982; Inglis and Gust, 2003; D'Amours et al., 2008; Callier et al., 2018; Barrett et al., 2019). These studies have mostly focused on mussel farms in shallow, protected embayments. However, the expansion of the aquaculture industry is increasingly leading to the development of offshore farm sites as the inshore industry is becoming saturated in some areas and conflicts with other users increase (Jansen et al., 2016; Mascorda Cabre et al., 2021). According to Jansen et al. (2016) and Mascorda Cabre et al. (2021), cultivation of lower trophic species, such as bivalves and specifically mussels, has a high biological and economic potential for offshore production and this type of aquaculture will most likely expand in the future. Offshore sites have a different architecture (e.g., cultured bivalves being suspended higher off the bottom, longlines with greater spacing between them) and the general environment is different from that of enclosed embayments (e.g., commonly stronger currents, colder waters, deeper areas, different species). Such differences may affect interactions between bivalve culture and benthic communities. Few studies have concentrated on the distribution of vagile epibenthic macrofauna in offshore sites, including those for mussel farms (Froehlich et al., 2017). In the context of marine spatial planning, it is important to quantify the effects of offshore aquaculture on capture fisheries (and ecologically important) species to better predict if farms result in net gains or losses for impacted populations (Clavelle et al., 2019). Indeed, Barrett et al. (2022) recently suggested that non-fed aquaculture may increase productivity of associated species, providing a more holistic view of the costs and benefits of aquaculture production in support of decision-making and the development of sustainable aquaculture (Weitzman, 2019).

The general objective of this study was to evaluate the effects of offshore suspended mussel *Mytilus edulis* aquaculture on the spatial distribution of vagile epibenthic macrofauna. Two hypotheses were evaluated: (1) that the abundance of epibenthic macrofauna is greater within farm sites relative to non-farm sites (hereafter, reference sites—bay-scale variation), and (2) that the abundance of epibenthic macrofauna within farm sites increases with proximity to farm infrastructure (e.g., mussel longlines and anchor blocks—line-scale variation). Sampling was done twice to evaluate if the benthic communities display temporal variation.

MATERIALS AND METHODS

Study Sites

The study was done in Baie de Plaisance (N 47°21′, W 61° 44′), îles de la Madeleine, Québec, Canada (**Figure 1**). The mussel lease covers an area of approximately 2.5 km^2, 4 km offshore, where 187 longlines are deployed primarily for mussel aquaculture but the area also supported limited oyster aquaculture. Longlines are 100 m long and are anchored at each end by cement blocks or screw anchors, suspended off-bottom by variable numbers (depending on grow-out stage) of 40 cm spherical buoys and ballasted by ca. 10 concrete anchor blocks (typically about 30 × 30 × 40 cm high) spaced at ca. 10 m. Rows of longlines are spaced 50 m from adjacent ones and individual longlines within rows are separated by 70 m. The site is about 20 m deep and 2 m-long mussel socks are suspended from longlines ca. 10 m above the bottom. The studied area experiences a tidal range of 0.60 m and water temperature can reach 20°C in summer and drop below 0°C during the winter (December to April). The bottom is characterized by a very flat sandy substratum with little physical heterogeneity. In all sites, underwater visibility was typically >5 m. Up to 85% of the mussel lease is used at a time and contains mussels at different stages of production.

Sampling Protocol

Sampling was done at two different periods, June 8–14 and July 19–23, 2014. Two areas were studied, one inside (Farm area) and one outside (Reference area, encompassing all areas surrounding the farm and > 500 m to about 1 km distant from it) the offshore mussel farm site. At each area and each period, 8 stations were chosen haphazardly to cover the surface of the mussel farm (concentrating on longlines with 2-year-old mussels) and non-farm areas, for a total of 32 sampling stations. As described by D'Amours et al. (2008), two SCUBA divers made underwater visual counts along transects. The first diver swam near the bottom holding a 2 m pole equipped with a compass and swam transects along the same bearing that mussel lines were oriented while deploying a measuring tape. The second diver identified, counted, and noted epibenthic macroinvertebrates and fish by swimming directly above the bar guided by the first diver. All taxa were distinct and a clear distinction between hermit crabs was made; large hermit crabs were identified as *Pagarus pubecsens* and small hermit crabs as *Pagarus acadianus*. Inside the farm, benthic macrofauna were noted along 30 m transects, divided into contiguous ($n = 3$) 10 m segments, parallel to mussel lines at each of 4 distances from them: 0 m (directly under mussel lines), and at 5, 10, and 25 m away from them (i.e., directly between two adjacent longlines). Transects evaluated in non-farm stations were done following the same pattern and orientation of four transects, with the exception that there was no mussel line, to evaluate if spatial structure of benthic communities differed between farm and reference areas.

Data Analysis

Temporal, bay- and line-scale variation in abundances and taxonomic richness were evaluated using 4-way mixed effect

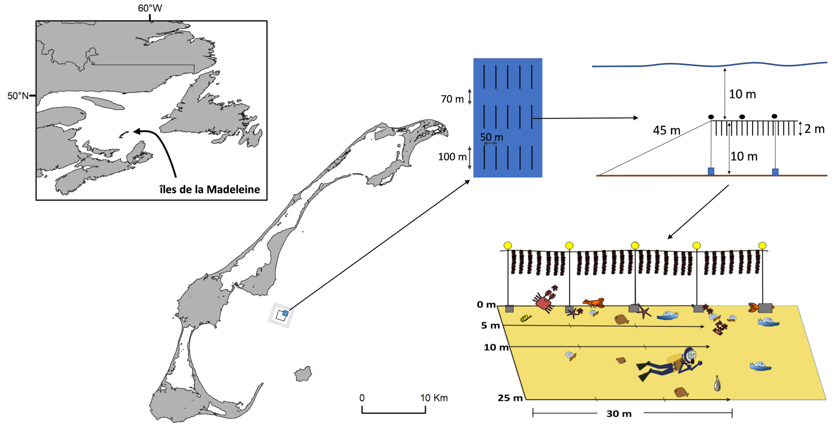

FIGURE 1 | Location of the mussel lease site in Baie de Plaisance, îles de la Madeleine, eastern Canada. The black polygon indicates the mussel lease (Farm area) and the gray area surrounding it the Reference area. Details of farm layout and sampling are also provided.

models with the factors Farm (fixed; farm area or reference area), Period (random; June or July), Distance (fixed; from mussel line: 0, 5, 10, and 25 m) and Site (random; nested in Farm and Period) and the interactions among these factors. Samples from contiguous transect are considered as replicates nested within each Distance in a given Site. The assumptions of normality and homoscedasticity for parametric analyses were verified by visual evaluation of residuals and Cochran's test and were never met, despite trying various data transformations. Thus, all univariate models (i.e., for abundances of each species, total abundance, and richness) were evaluated using PERMANOVA with PRIMER 7 + PERMANOVA based on Euclidean distances (n = 4,999 unrestricted permutations) as suggested by Anderson (2001). All univariate analyses (with the exception of winter flounder and small hermit crabs) showed that the factor Period and its interactions with other factors were not significant ($P >$ 0.2) and thus this factor was removed from the analyses and the reduced model rerun for all species other than winter flounder and small hermit crabs. Significant factors were further examined using appropriate pairwise comparisons. Variation in multivariate community structure was visualized using non-metric multidimensional scaling (MDS) and evaluated using PERMANOVA (fourth-root transformation of abundance data and based on Bray Curtis similarity distances, n = 4,999 unrestricted permutations). PERMDISP was performed on the same Bray–Curtis matrix to evaluate variation in multivariate dispersion among *a priori* groups (i.e., contrasting variation in multivariate community structure between outside and inside of the farm and among distance classes within the farm). Likewise, species accounting for differences between Farm and Reference areas and between distances within the farm were identified using SIMPER.

RESULTS

Epibenthic macrofaunal communities were dominated by sand dollars *Echinarachnius parma* (70.5%), small hermit crabs *Pagarus acadianus* (9.59%), large hermit crabs *Pagarus pubecsens* (5.2%), sea stars *Asterias rubens* (4.3%), Atlantic rock crabs

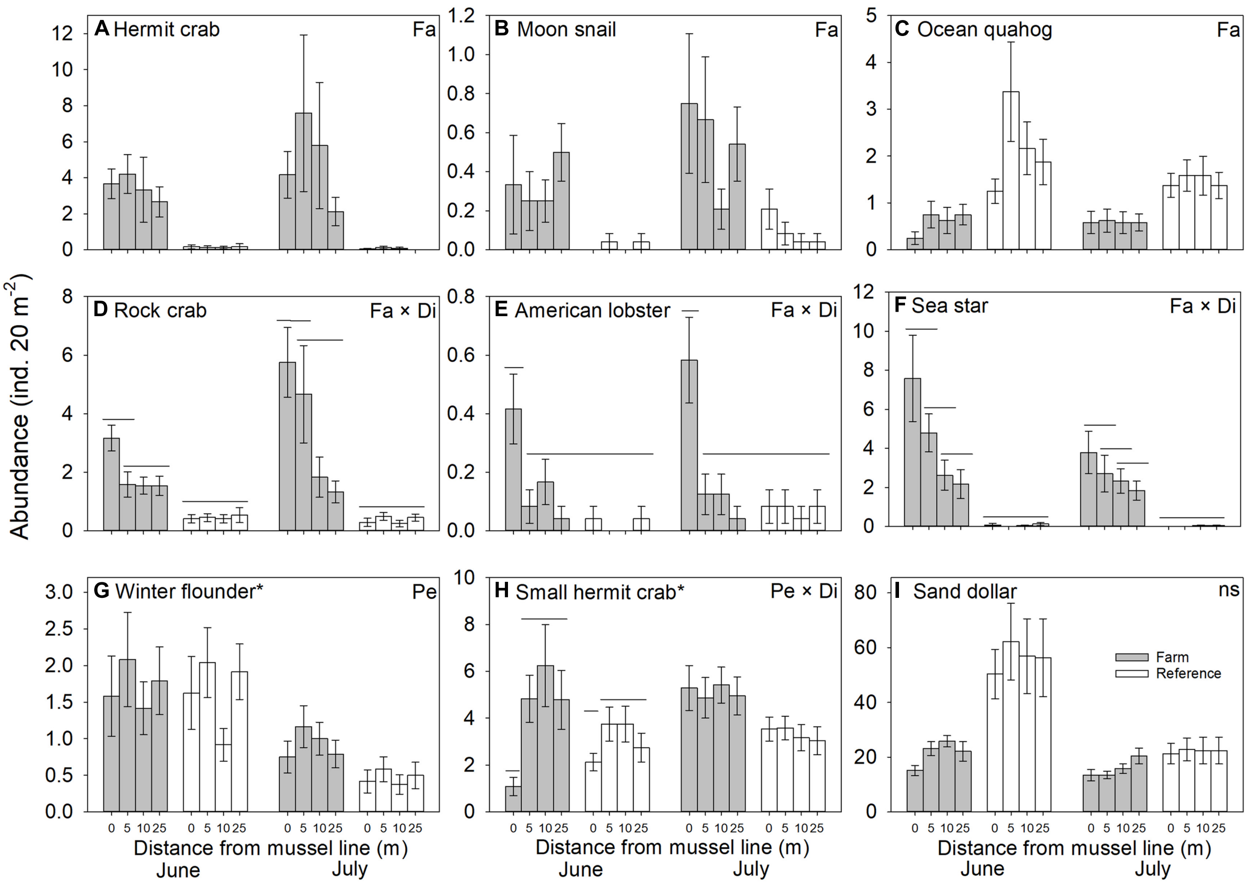

FIGURE 2 | Abundance (mean ± *SE*, n = 24) of macrofauna recorded along transects in Farm (gray) and Reference (white) areas at two sampling Periods (June and July) with Distance to mussel lines (0, 5, 10 and 25 m) in an offshore blue mussel (*Mytilus edulis)* culture site. Significant effects on macrofauna abundance among different treatments are specified in the upper right-hand corner; only significant differences between distances where interactions are significant are indicated (by non-adjoining bars); effects of Farm are evident. All pair-wise comparisons were made with reduced model (i.e., without the factor Period) except for species indicated by an asterisk; *ns* = not significant.

Cancer irroratus (3.8%), ocean quahogs *Arctica islandica* (2.9%), winter flounders *Pseudopleuronectes americanus* (2.9%), Northern moon snails *Polinices heros* (0.6%), and American lobsters *Homarus americanus* (0.3%). Other vagile organisms were occasionally observed, mostly within mussel farms and directly on anchors blocks (data not shown), such as sea ravens *Hemitripteridae* sp. and sculpins *Myoxocephalus* sp., although they were not considered in the present study due to their rarity.

Univariate Analyses

Variation in abundance was observed between farm and reference sites for seven of the nine epibenthic species evaluated. The abundance of large hermit crabs, Northern moon snails, and ocean quahogs varied significantly between farm and reference areas, being more abundant in farm than reference sites, with the exception of ocean quahog, which was more abundant in reference than in farm sites (**Figure 2** and **Table 1**). The abundance of American lobster, Atlantic rock crab and common sea star showed significant 2-way interactions between Farm and Distance. Atlantic rock crab and sea star abundance increased significantly with proximity to longlines, whereas American lobster abundance was greater at 0 m than at other distances from the longlines (**Figure 2** and **Table 1**). Winter flounder was significantly more abundant in June than July but did not show any spatial variation in abundance (**Figure 2** and **Table 2**). The abundance of small hermit crabs varied as a function of the Period × Distance interaction (**Figure 2** and **Table 2**) such that its abundance decreased with proximity to longlines—being least abundant at 0 m in June—but no trend was observed in July. Despite a fairly clear trend in the abundance of sand dollars (**Figure 2** shows that sand dollar abundance in reference sites in June to be about twice that observed in farm sites and of reference sites in July), this effect was not statistically significant (**Table 1**), likely due to very high variability among sites. Overall, taxonomic richness was significantly greater inside the mussel farm than in the reference sites (**Figure 3** and **Table 1**). There were no statistically significant patterns for total abundance, again most likely due to the abundance of sand dollars among sites. When the abundance of this species is removed from the calculation of "total abundance," the abundance of all other species combined is greater in Farm sites than Reference sites (**Figure 3** and **Table 2**), mirroring the overall trends observed for most individual species.

Multivariate Analyses

Macrofaunal community structure varied as a function of the Farm × Distance interaction (**Table 2**), with communities from farm and reference areas differing. Within the farm, communities directly under the mussel lines differed from those at other distances (i.e., 0 m $\neq$ 5 m = 10 m = 25 m) and were most different from those in reference sites. In both periods, MDS plots (**Figure 4**) show clear farm-scale effects where Farm sites differ from Reference sites with little overlap between site points. Multivariate dispersion differed between farm and reference sites, with variation among replicate contiguous transects being greater inside of the farm than outside of it [$\overline{x}$ = 25.026 and 20.642 for dissimilarity outside and inside of the farm, respectively,

TABLE 1 | Results of PERMANOVA analysis (reduced model; untransformed data) for variation in the abundance of American lobster *Homarus americanus,* Atlantic rock crab *Cancer irroratus,* hermit crab *Pagarus pubecsens,* common sea star *Asterias rubens,* northern moon snail *Polinices heros,* sand dollar *Echinarachnis parma* and ocean quahog *Arctica islandica,* total abundance, taxonomic richness, and multivariate community structure to test the blue mussel (*Mytilus edulis)* culture lease effects of Farm (Farm vs. reference), Distance (from mussel line: 0, 5, 10, and 25 m) and Site (1–32) and their interactions.

Source of variation		***Homarus americanus***			***Cancer irroratus***			***Pagarus pubecsens***			***Asterias rubens***		
	df	**MS**	**Pseudo-F**	**p (perm)**	**MS**	**Pseudo-F**	**p (perm)**	**MS**	**Pseudo-F**	**p (perm)**	**MS**	**Pseudo-F**	**p (perm)**
Farm (Fa)	1	2.19	22.093	**0.002**	490.51	10.741	**0.001**	1604.8	5.231	**0.001**	1134.4	13.222	**0.001**
Distance (Di)	3	1.093	9.289	**0.001**	45.622	4.598	**0.001**	51.912	1.486	0.246	63.965	5.229	**0.002**
Si(Fa)	30	0.099	0.928	0.566	45.666	11.621	**0.001**	306.78	8.193	**0.001**	85.797	11.634	**0.001**
Fa × Di	3	0.961	8.168	**0.001**	48.955	4.934	**0.001**	49.579	1.419	0.239	66.299	5.42	**0.002**
Si (Fa) × Di	90	0.118	1.102	0.274	9.922	2.525	**0.001**	34.94	0.933	0.709	12.232	1.659	**0.002**
Res	256	0.107			3.93			37.443			7.375		
Total	383												
		Polinices heros			***Echinarachinus parma***			***Arctica islandica***			**Taxonomic richness**		
	df	**MS**	**Pseudo-F**	**p (perm)**	**MS**	**Pseudo-F**	**p (perm)**	**MS**	**Pseudo-F**	**p (perm)**	**MS**	**Pseudo-F**	**p (perm)**
Farm (Fa)	1	13.878	5.713	**0.004**	41003	3.013	**0.070**	145.04	8.686	**0.005**	217.5	20.275	**0.001**
Distance (Di)	3	0.704	1.212	0.308	670.73	2.431	**0.066**	8.438	1.211	0.332	1.718	1.034	0.375
Si(Fa)	30	2.429	5.585	**0.001**	13608	57.396	**0.001**	16.699	11.853	**0.001**	10.728	7.758	**0.001**
Fa × Di	3	0.343	0.59	0.635	186.78	0.677	0.577	4.021	0.577	0.673	0.926	0.558	0.668
Si (Fa) × Di	90	0.581	1.336	**0.043**	275.94	1.164	0.179	6.97	4.947	**0.001**	1.661	1.201	0.134
Res	256	0.435			237.09			1.409			1.383		
Total	383												

Values in bold indicate statistically significant (α = 0.05) effects. Pseudo-F (F) and permutationaly-derived probability (P) values, respectively, were calculated using PERMANOVA.

TABLE 2 | Results of PERMANOVA analysis (full model; untransformed data for univariate metrics but fourth-root transformed data for multivariate community structure) for variation in the abundance of winter flounder *Pseudopleuronectes americanus* and small hermit crab *Pagarus acadianus,* multivariate community structure, total abundance and total abundance without sand dollar *Echinarachnius parma* to test the effects of Farm, Period, Distance, Site and their interactions.

Source ofvariation		***Pseudopleurnectes americanus***			***Pagarus acadianus***			**Multivariate community structure**			**Total abundance (N)**			**Total abundance (N) (without sand dollars)**		
	df	**MS**	**Pseudo-F**	**p (perm)**	**MS**	**Pseudo-F**	**p (perm)**	**MS**	**Pseudo-F**	**p (perm)**	**MS**	**Pseudo-F**	**p (perm)**	**MS**	**Pseudo-F**	**p (perm)**
Farm (Fa)	1	7.315	2.293	0.274	208.570	20.179	0.243	54948.000	37.952	0.254	9272.800	0.384	0.482	92095.000	58.762	0.234
Period (Pe)	1	91.065	5.534	**0.018**	30.940	0.313	0.551	7528.300	2.315	**0.040**	38420.000	2.617	0.100	11863.000	2.848	**0.024**
Distance (Di)	3	5.107	1.837	0.317	46.961	0.818	0.699	1244.700	4.089	**0.020**	796.460	1.482	0.397	1790.700	4.114	**0.022**
Fa × Pe	1	3.190	0.194	0.687	10.336	0.104	0.768	1447.800	0.445	0.824	24146.000	1.644	0.229	1567.300	0.376	0.827
Fa × Di	3	1.100	6.005	0.094	19.225	1.552	0.325	1255.500	20.974	**0.004**	106.110	1.152	0.473	1917.400	26.927	**0.011**
Pe × Di	3	2.780	1.606	0.208	57.433	3.536	**0.008**	304.410	0.655	0.743	537.300	1.644	0.183	435.270	0.536	0.809
Si (Fa × Pe)	28	16.454	7.998	**0.001**	98.916	11.315	**0.001**	3251.900	11.311	**0.001**	14683.000	41.929	**0.001**	4165.400	8.223	**0.001**
Fa × Pe × Di	3	0.183	0.106	0.961	12.385	0.762	0.507	59.857	0.129	0.986	92.114	0.282	0.844	71.209	0.088	0.987
Si (Fa × Pe) × Di	84	1.731	0.841	0.802	16.245	1.858	**0.001**	465.020	1.618	**0.001**	326.800	0.933	0.666	812.070	1.603	**0.001**
Residual	256	2.057			8.742			287.490			350.190			506.550		
Total	383															

Values in bold highlight statistically significant (α = 0.05) effects. Pseudo-F (F) and permutationaly-derived probability (P) values, respectively, were calculated using PERMANOVA.

$F_{(1,382)} = 21.225$, $p = 0.001$]. In contrast, multivariate dispersion within the farm did not differ among distances [$F_{(3,\ 39)} = 0.128$, $p = 0.961$]. In all contrasts among distance classes within farms, sea stars, small and large hermit crabs, flounders, and rock crabs, together, accounted for between 71 and 77% of the observed dissimilarity in community structure among distances, with the contribution of the various species differing depending on the contrast.

DISCUSSION

This study shows that offshore suspended mussel aquaculture in Îles de la Madeleine influences the distribution and abundance of epibenthic macrofauna at both the bay- and line-scale, although the effects varied among species and, at times, between sampling periods. Community structure analyses highlighted three distinct species assemblages: outside the farm sites, within the farm sites but not below mussel lines, and directly below mussel lines. This was reflected by the distribution of most species, except for winter flounder and sand dollars, whose abundance did not vary between farm and reference sites, most of which (except for ocean quahogs, which is actually an infaunal species) were most abundant in farm sites and many of which were more abundant directly below mussel lines than between them. Taxonomic richness and total abundance (less sand dollars) were also greater in farm areas than non-farm areas. This increased taxonomic richness and total abundance likely homogenized benthic communities within the farm relative to those outside of the farm, as evidenced by the decreased multivariate dispersion of samples from farm sites relative to those from reference sites.

Two main mechanisms likely account for the spatial distribution of the vagile macrobenthic organisms observed in the present study (see review in McKindsey et al., 2011). First, effects on trophic structure (increased input of prey resulting from fallen mussels and associated fouling organisms may attract predators, as may biodeposit-enriched and modified infaunal communities). Second, the addition of physical structure to the benthic environment in the form of anchor blocks and fallen mussels alters the seabed by creating colonization surfaces and increasing possible shelter or refuges for a variety of organisms. While other studies have focused on a few species (e.g., Freire and González-Gurriarán, 1995; Inglis and Gust, 2003; Wilding and Nickell, 2013), we focused on entire local macrofaunal communities to allow more general conclusions to be drawn. Given that the impact of this offshore mussel farm on benthic infauna is quite limited (Lacoste et al., 2018) and that lobster, but not crabs, sea stars, or flounders are impacted by the presence of anchor blocks alone without the presence of mussels (Drouin et al., 2015), we assume that observed impacts on species distributions of most species are likely mostly attributable to the presence of fallen mussels as a food source. That is, a simple trophic effect (i.e., the supply of food in the form of fallen mussels to scavengers or predators; Forget et al., 2020) is likely the main reason for the increased abundance of several taxa in farm sites, particularly close to mussel lines within the farm. Mussels are considered the main prey of rock crabs in coastal

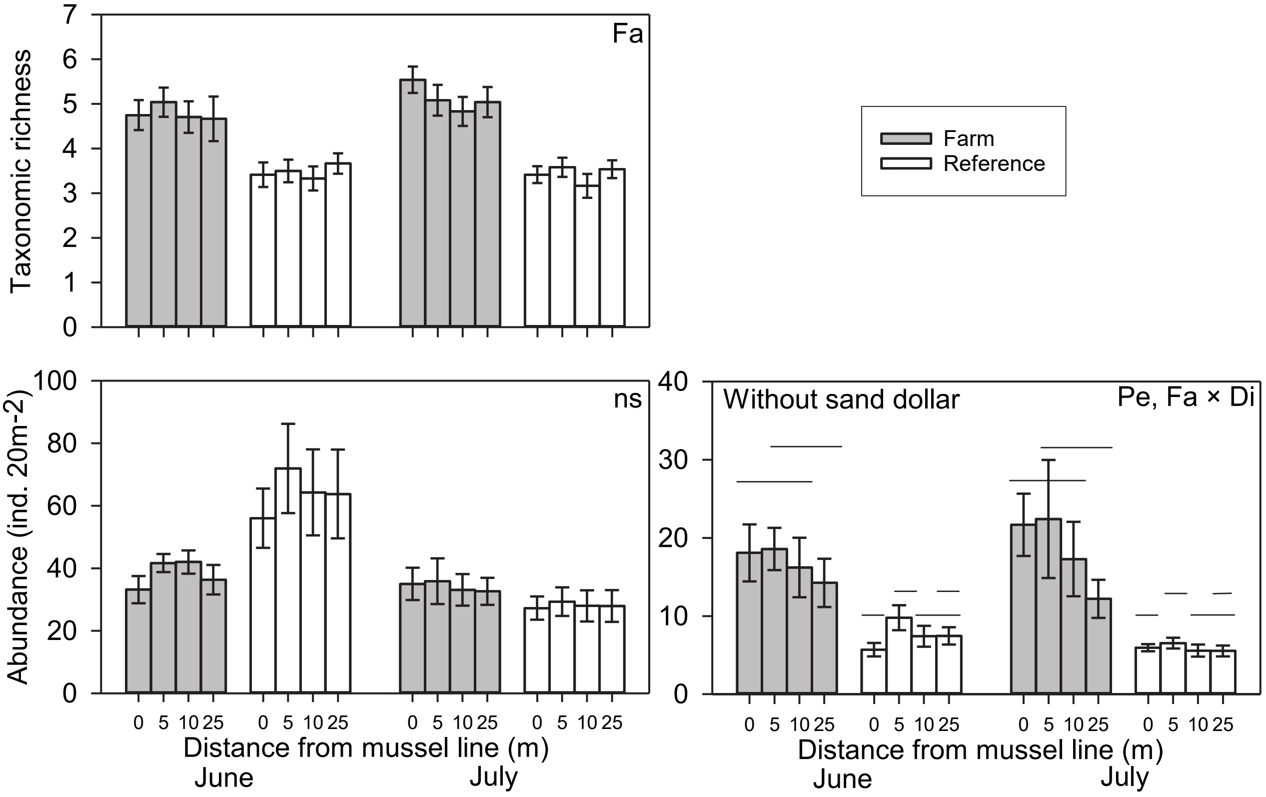

FIGURE 3 | Abundance (with and without sand dollars) and taxonomic richness (mean ± *SE*, *n* = 24) of macrofauna recorded along transects in Farm (gray) and Reference (white) areas at two sampling Periods (June and July) with Distance to mussel lines (0, 5, 10, and 25m) in an offshore blue mussel (*Mytilus edulis)* culture site. Significant differences in macrofauna abundances and taxonomic richness among different treatments are specified in the upper right-hand corner; only significant differences between distances where interactions are significant are indicated (by lines on different planes); effects of Farm are evident. The pair-wise comparison was made with full model (i.e., with the factor Period).

areas (Drummond-Davis et al., 1982). Given that many mussels fall from culture structures during grow-out, particularly more or less directly below mussel longlines (Wilding and Nickell, 2013), this provides a great trophic advantage to crabs in the area, logically concentrating them where the mussels have fallen. Romero et al. (1982) noted a greater abundance of various crabs in areas with mussel rafts relative to areas without them, suggesting that they are attracted to aquaculture sites where they feed on fallen cultured mussels and associated epifauna. As noted by Hudon and Lamarche (1989), mussels may account for an important fraction of American lobster diet due to their high calorific value. Moreover, a great proportion of the diet of large lobsters is mussels in an area with available farmed, but few wild, mussels (Sardenne et al., 2019). However, rock crabs play a key role in the growth, condition and ovary development of American lobsters of all sizes due to their high protein content and ratio of amino acids (Gendron et al., 2001), likely accounting for the finding that smaller lobsters feed mostly on rock crabs associated with mussel farms (Sardenne et al., 2019). In contrast, Sainte-Marie and Chabot (2002) found that larger lobsters preyed on large, vagile, nutritious prey, such as crustaceans, although this latter work was done in an area that lacked nearby mussel aquaculture sites. Together, this suggests that lobsters are, in part, more abundant in farm sites, particularly close to mussel lines, because of both the abundant prey and the addition of physical structure in the form of anchor blocks.

Sea stars were orders of magnitude more abundant in farm sites than in reference sites, showing the great attractiveness of the farm for these animals. Increased abundance of sea stars within mussel sites associated with mussel lines was also reported by Inglis and Gust (2003) and Barrett et al. (2020) for *Coscinasterias muricata* and D'Amours et al. (2008) for *Asterias* sp. Gaymer et al. (2004) showed that mussels (*M. edulis*) are the preferred prey of *Asterias* sp., again suggesting a trophic link. Similarly, Wilding and Nickell (2013) reported a negative correlation between sea star abundance and distance to mussel lines and related this to mussel fall-off. However, sea stars may also recruit in large quantities to mussel lines (Barkhouse et al., 2007) and this too could, in part, account for the greater abundance of these animals in the farm relative outside of it.

Large hermit crabs and Northern moon snails were more abundant in mussel farm sites relative to reference sites. Hermit crabs are omnivorous detritivores (Hazlett, 1981) and thus likely able to take advantage of the general increase of detritus in farm areas due to incomplete consumption of fallen mussels and associated organisms by other macropredators in the site.

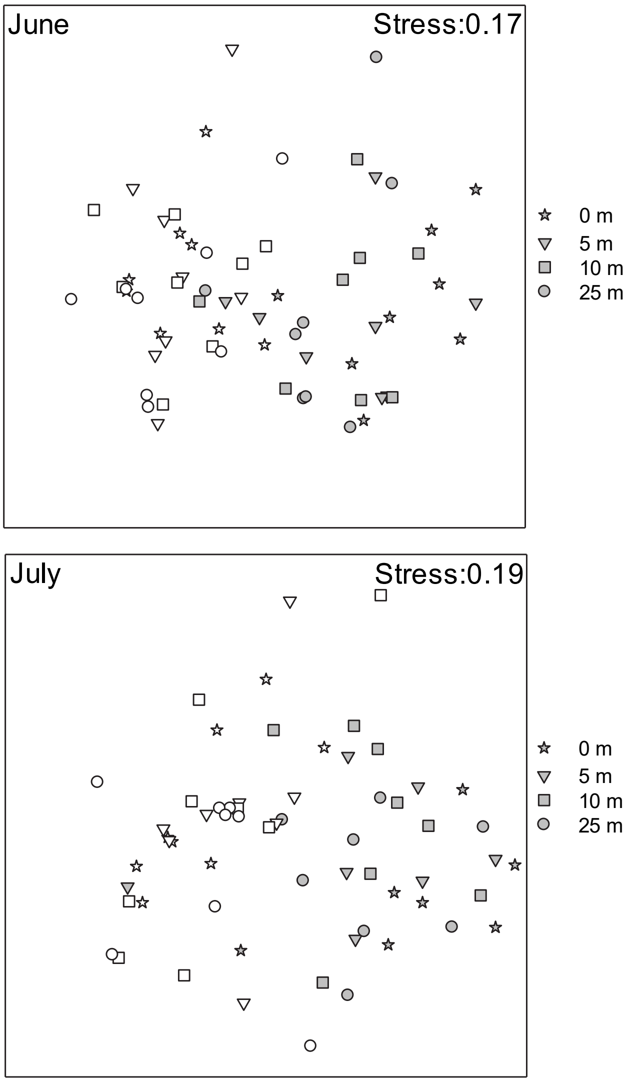

FIGURE 4 | MDS plots of macrofauna (√√) community structure at two sampling Periods (June and July 2014) in mussel farms (gray) with distance to mussel lines (0 m through 25 m) and reference areas (open circles).

In contrast, Northern moon snails are active foraging predators of molluscs (Commito, 1982) that readily feed on mussels if found in their environment (Kenchington et al., 1998) and thus were likely more abundant in the farm site because of the great concentration of fallen mussels in the area. Other authors reported similar findings of increased abundance of predatory gastropods in Eastern Canada (Grant et al., 1995; D'Amours et al., 2008).

Although small hermit crabs were, overall, more abundant in mussel farm sites than reference sites, they displayed line-scale variation in abundance in June, when they were less abundant directly below mussel lines than at other distances and locations. Small hermit crabs are vulnerable to dexterous predators, including larger hermit crabs, brachyuran crabs (e.g., rock crabs) and lobsters (Angel, 2000) and these predators were particularly more abundant in farm sites close to mussel longlines, potentially explaining their line-scale variation in abundance. Similarly, Drummond-Davis et al. (1982) found that *P. acadianus* is the most frequently consumed prey by rock crabs in kelp beds in Nova Scotia. Although more food is available in farm sites (e.g., fallen mussels, associated organisms), small hermit crabs may avoid such areas since predation risks are elevated, illustrating a behavior defined by Kerfoot and Sih (1987) whereby aquatic prey adjust to spatial and temporal variation in predation risk.

In contrast to the positive association of animals to the mussel culture site, both ocean quahogs and sand dollars tended to be less abundant in the farm than outside of it. In both cases, this may be due to the increased abundance of predators in the farm, which may also prey on these two species. Predators of ocean quahogs include invertebrates such as brachyuran crabs (e.g., *C. irroratus*) (Stehlik, 1993), crustaceans (Kraus et al., 1992), seas stars (Kennish et al., 1994), and teleost predators such as sculpins (*Myxocephaus* sp.) (Langton and Bowman, 1980), all which were most abundant in farm sites. Likewise, predators of sand dollars include sea stars, common rock crabs, and benthic fish (i.e., winter flounder) (Jalbert et al., 1989; Himmelman and Dutil, 1991; Gaymer et al., 2004). Most of these predators were more abundant in farm sites than reference sites. In addition to predation, mortality of ocean quahogs may also occur due to increased sedimentation (Anger et al., 1977). Thus, organic loading from biodeposition below mussel lines may, in part, explain the decreased abundance of these bivalves in farm sites. Sand dollars are more mobile than ocean quahogs and commonly burrow in sediments (Cabanac and Himmelman, 1996), suggesting that increased sedimentation may not account for the observed trends in sand dollar abundance.

The presence of physical structure in marine habitats allows for a greater abundance of organisms and species richness compared to less complex habitats (Gratwicke and Speight, 2005). Anchor blocks, ropes and other aquaculture items, including fallen mussels and shell debris, may create a more heterogeneous benthic environment and modify community richness and biomass (Chesney and Iglesias, 1979). It may also decrease the abundance of species that require flat homogenous substrata, such as sand dollars and ocean quahogs. Significant variation at the line-scale was observed for many taxa. American lobsters were most abundant directly under the mussel line, most often associated with anchor blocks, whereas the abundance of rock crabs and sea stars increased with proximity to mussel lines and Northern moon snails and large hermit crabs showed the same trend. However, the relative importance of trophic and physical structure effects on the distribution of taxa within farms is difficult to resolve. Drouin et al. (2015) did an experiment to separate these factors and found that the association of American lobster with mussel lines is likely primarily due to the presence of anchor blocks but suggested that the abundance of food in mussel farm areas further increased their abundance. As American lobsters cannot quickly bury themselves in sand, they seek protection from other shelters in their environment (Hudon and Lamarche, 1989). In contrast, Drouin et al. (2015) also suggested that rock crabs, which are less dependent on shelter and physical structure (Fogarty, 1976; Cobb et al., 1986; Gendron and Fradette, 1995), likely benefit from fallen mussels, thus explaining the line-scale distribution of this species.

Overall, the distribution of winter flounder does not appear to be influenced by mussel leases as no clear trend was observed except for a temporal variation. Similarly, Clynick et al. (2008) found no difference in the abundance of winter flounder in farm sites relative to reference sites. Winter flounder is a habitat generalist that occurs on a variety of shallow substrates and sediment types (Sogard and Able, 1991). According to Worobec (1984), juveniles prey opportunistically on small infaunal organisms, crustaceans, and other appropriate food as they grow. Only winter flounder was observed to vary temporally in the present study. However, the abundance of other taxa also likely vary temporally but the sampling regime employed (sampling twice at the beginning of summer) was not appropriate to detect such temporal variation.

The present study suggests that most epibenthic macrofauna taxa have a positive response to suspended mussel aquaculture in the studied offshore area. By itself, the addition of aquaculture structural features likely increase local productivity, diversity, and biomass, which are typically greater on hard-substrates (Cowles et al., 2009). Mussel fall-off from longlines likely increases the abundance of many predators in the farm site. Beyond the simple aggregative effect of predators due to fall-off, the impact of such a transfer of trophic energy from the pelagic environment (plankton) and intermediary (mussel filtration and fall-off) to the benthic environment on the animals that live there is poorly understood. Observational and modeling work on benthic scallop culture (Kluger et al., 2016a,b) suggests that the addition of farmed bivalves to the bottom increases the abundance of their predators at the bay-scale, thus benefiting fisheries of any such species, whereas this increased abundance of predators also negatively impacts non-predatory species that are normally present. However, the increased abundance of predators in farm sites may also cause farms to act as ecological traps for these animals if fishing effort is directed in or around them (Fernandez-Jover et al., 2008; Dempster et al., 2009). Although a recent review (Barrett et al., 2022) suggests that such aggregations of wild animals may enhance the productivity of farm environments relative to otherwise equivalent environments, the impact of mussel farms on the fitness of these animals is unknown. Wang and McGaw (2016) suggest that a mussel-only diet is not suitable for lobsters because of low levels of amino acids such as asparagine, alanine and glutamic acid and the carotenoid astaxanthin in mussel flesh. Further studies are needed to obtain a more holistic understanding of the interactions between mussel aquaculture and associated macrofauna to ensure the ecological sustainability of the industry and better understand its effects on the fishing industry and the fisheries species themselves.

CONCLUSION

Marine aquaculture of many types is expanding into offshore areas to provide seafood products while limiting environmental impacts and conflict between users of the limited space in coastal zones (Froehlich et al., 2017). In the context of marine spatial planning, a better understanding of the interactions between aquaculture and fisheries is of great importance (Clavelle et al., 2019) and may be used to limit the impacts of these two activities on one another (Gentry et al., 2017; Lester et al., 2018). It is hoped that the clear evidence of increased abundance of many macrobenthic species in the examined îles-de-la-Madeleine offshore mussel farm will contribute to a more holistic (Weitzman, 2019) understanding of offshore mussel farm effects to support logical marine spatial planning.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

A-SS, AD, PA, and CM contributed to the design and concept of the study. A-SS, AD, and CM contributed to field missions, statistical analysis of the data, and writing the manuscript. AD, PA, and CM obtained funding to complete the work. All authors reviewed and edited the manuscript contributing to the final version.

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Effects of Shellfish and Macro-Algae IMTA in North China on the Environment, Inorganic Carbon System, Organic Carbon System, and Sea–Air CO_2 Fluxes

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Shellfish and macro-algae integrated multi-trophic aquaculture (IMTA) contribute greatly to the sustainability of aquaculture. However, the effects of large-scale shellfish and macro-algae aquaculture on the functions of the ocean carbon sink are not clear. To clarify these effects, we studied the spatial and temporal changes of inorganic and organic carbon systems in seawater under different aquaculture modes (monoculture or polyculture of shellfish and macro-algae) in Sanggou Bay, together with the variation of other environmental factors. The results show that the summertime dissolved oxygen (DO) concentration in the shellfish culture zone was significantly lower than other zones ($p < 0.05$), with a minimum value of 7.07 ± 0.25 mg/L. The variation of pH and total alkalinity (TA) were large across different culture modes, and the seawater in the shellfish culture zone had the lowest pH and TA than the other zones. Seasonal environment and aquaculture modes significantly affected the variation of dissolved inorganic carbon (DIC), CO_2 partial pressure (pCO_2), dissolved organic carbon (DOC), and particulate organic carbon (POC) concentrations. The highest values of DIC, pCO_2, and POC appeared in summer, and the lowest appeared in winter. For DOC concentration, the lowest value appeared in autumn. Spatially, DIC and pCO_2 were highest in the shellfish culture zone and lowest in the macro-algae culture zone, DOC was highest in the macro-algae culture zone and lowest in the shellfish culture zone, and POC was lower in the shellfish culture zone and macro-algae culture zone and higher in the remaining zones. The results of sea–air CO_2 fluxes showed that except for the shellfish culture zone during summertime, which released CO_2 to the atmosphere, all culture zones were the sinks of atmospheric CO_2 during the culture period, with the whole bay being a strong CO_2 sink during autumn and winter. In summary, large-scale shellfish–macro-algae IMTA plays an important role in the local carbon cycle and contributes to mitigating ocean acidification and hypoxia.

Keywords: shellfish, macro-algae, aquaculture, carbon sink, Sanggou Bay

INTRODUCTION

Anthropogenic CO_2 emissions have negative impacts on the environment, and the climate change caused by these emissions may be the most important global environmental problem currently facing mankind (Jiang et al., 2015). If there is no change in the status quo, longer heatwaves and more frequent extreme precipitation events are expected to occur (Petrović and Lobanov, 2020). The ocean is the largest sink of anthropogenic CO_2 (Sabine et al., 2004), as it absorbs approximately 30% of anthropogenically released CO_2 (Gruber et al., 2019). The total amount of CO_2 emitted by human activities in 2018 was approximately 11.5 ± 0.9 GtC, and approximately 2.6 ± 0.6 GtC was absorbed by the ocean (Friedlingstein et al., 2019). Consequently, the carbon sink function of the marine ecosystem has received increased attention (Jiang et al., 2015).

The absorption of CO_2 in the atmosphere by the ocean results in an increase in CO_2 on the surface of seawater, which disrupts the balance of the original carbon system and results in ocean acidification (Gattuso et al., 2018), and this affects the survival of calcified organisms and the sensory abilities of fish (Bignami et al., 2013). The large-scale aquaculture, especially seaweed farming, can alter the ability of the marine ecosystem to absorb CO_2 and alleviate ocean acidification at the local scale (Gattuso et al., 2018). Macro-algae can absorb excess CO_2 through photosynthesis and increase the pH of nearby water through physiological processes (Krause-Jensen et al., 2016; Koweek et al., 2017). During their growth stage, they also release a large amount of dissolved organic carbon (DOC), and this DOC can enter the food web or form refractory dissolved organic carbon (RDOC), thus remaining in the seawater for a long time and eventually resulting in carbon sequestration (Zhang et al., 2017; Li et al., 2018). Large-scale seaweed farming can strengthen the ocean carbon sink function and prevent ocean acidification (Duarte et al., 2017; Xiao et al., 2021). However, a large-scale macro-algae culture can also result in the excessive consumption of dissolved inorganic carbon (DIC), nitrogen, phosphorus, and other nutrients (Zou et al., 2004).

Integrated multi-trophic aquaculture (IMTA) is an effective approach for overcoming the problems associated with the excessive consumption of nutrients in macro-algae culture and increasing the sustainability of macro-algae aquaculture (Barrington et al., 2010). The shellfish–macro-algae IMTA model has been highly successful and is the most economically feasible solution for recovering nutrients in the open water (Fang et al., 2020). The nutrients released by shellfish can be fully absorbed and utilized by macro-algae. O_2 and debris produced by macro-algae and the increased water pH promote the survival and growth of shellfish. Shellfish–macro-algae aquaculture is thus an economically efficient and environmentally friendly form of aquaculture (Tang et al., 2011). There is growing evidence that the $CaCO_3$ shells generated during the calcification process of shellfish growth can remove carbon from the coastal ecosystem, thus improving the carbon absorption capacity of the shelf-edge sea, and significantly affect the carbon cycle of coastal ecosystems (Tang et al., 2011; Han et al., 2020). However, the impact of the CO_2 released during shellfish respiration and calcification on the carbon sink function of the marine ecosystem remains controversial. There have been reports that pure shellfish culture may be an important additional source of CO_2 in seawater and disrupt the balance of the local carbonate system, which affects the carbon sink capacity of marine ecosystems (Han et al., 2017).

The ocean is the largest carbon pool in the world; it thus plays an important role in regulating global climate change. However, large-scale aquaculture methods can vary in their effects on the carbon sink function of marine ecosystems (Jiang et al., 2013; Han et al., 2020). The exchange of CO_2 at the sea–air interface is a key ocean carbon cycle process, and its exchange flux is essential for understanding the transfer of CO_2 in seawater, as well as the source and sink effects of the marine ecosystem on atmospheric CO_2 (Li et al., 2005). Most current studies on CO_2 flux have focused on the ocean or macro-algae culture (Jiang et al., 2013; Xiao et al., 2021); research on the effects of the shellfish–macro-algae aquaculture mode on CO_2 exchange flux and the carbon cycle needs further study (Li et al., 2021). Additionally, because the concentration of DIC in seawater is higher than that of particulate organic carbon (POC) and DOC (Eglinton and Repeta, 2003), most studies of the role of shellfish–macro-algae aquaculture in the carbon cycle have focused on the inorganic carbon cycle; by contrast, few studies have examined the effect of shellfish–macro-algae aquaculture on the organic carbon cycle (Jiang et al., 2013; Jiang et al., 2015; Fang and Jiang, 2021; Li et al., 2021). The inorganic carbon pump only mediates the migration of CO_2 from the atmospheric carbon pool to the ocean carbon pool, and the CO_2 that enters the ocean must be stored by the biological pump to achieve long-term storage (Honda, 2003). Therefore, the organic carbon cycle also plays an important role in the marine biological carbon sink, so attention should also be paid to the cycling changes of organic carbon in the breeding process (Liu et al., 2021). Nevertheless, there are few reports on overall effects of aquaculture on inorganic and organic carbon cycles.

In this study, seasonal changes in the inorganic and organic carbon cycles in the kelp aquaculture zone, shellfish culture zone, and shellfish–macro-algae polyculture zone; the CO_2 flux of different aquaculture modes in different seasons; and the effects of large-scale aquaculture on the carbon sink and water environment of the marine ecosystem were examined in Sanggou Bay, which is a typical shellfish–macro-algae IMTA area in northern China. The results of this study provide new insights that aid our understanding of the role of the shellfish–macro-algae aquaculture in the marine carbon cycle and could be used to optimize the marine carbon sink function and the sustainability of aquaculture.

MATERIALS AND METHODS

Study Area and Sampling Stations

Located on the east side of Shandong Peninsula in eastern China (37°01′~37°09′ N, 122°24′~122°35′ E), Sanggou Bay covers an area of approximately 144 km^2 and has an average water depth of 7.5 m (Zhang et al., 2009). Kelp (*Saccharina japonica*), Pacific oysters (*Crassostrea gigas*), and scallops (*Chlamys farreri*) are the main marine species produced in this region (Li et al., 2021). Currently, Sanggou Bay consists of a shellfish culture zone (inside the bay), macro-algae culture zone (outside the bay), and shellfish–macro-algae polyculture zone (in the middle of the bay) (**Figure 1**). The culture cycle of kelp is from autumn to the following summer (harvest usually begins in May and is completed by early July), and the culture cycle of shellfish is divided into one or two years depending on the actual situation, with seedlings sown in spring and harvested in autumn each year.

Four on-site surveys were conducted in Sanggou Bay in April (spring), July (summer), October (autumn), and January (winter) of 2019. Each survey was conducted on a survey ship, and two sections were surveyed at the same time in the direction of the coastal current at peak tide. The survey stations are shown in **Figure 1**; each aquaculture zone contained four stations, and the control area outside the bay also included four stations.

Analysis Method

A water quality analyzer (EXO2, YSI, Yellow Spring, OH, USA) was used on-site to measure the dissolved oxygen (DO), water temperature, pH, salinity, and other indicators. CO_2 partial pressure (pCO_2) on the surface water was measured with a CO_2 partial pressure meter (OceanPack; Csubctech, Kiel, Germany). Water samples were collected with a water sample collector (KC, Silkeborg, Denmark). The Whatman GF/F membrane and cellulose acetate filter membrane were used for suction filtration on-site, and 200 and 1,000 ml surface seawater samples were filtered. After the Whatman GF/F membrane was acidified, the POC in the water was measured with an elemental analyzer (EL; Elementar, Langenselbold, Germany). The filtrate was transferred into a 30 ml jar that had been burned at 450°C for 4 h in advance, and an appropriate amount of saturated $HgCl_2$ solution was added with a syringe to inhibit microbial activity. The jar was then tightly capped and sealed with a sealing film, and the DIC and DOC were determined by a total organic carbon analyzer (Multi N/C; Jena, Jena, Germany). The cellulose acetate filters and water samples after suction filtration were stored in a refrigerator at 4°C for the analysis of chlorophyll (Chl-*a*) and total alkalinity (TA). The fluorescence method was used to determine the Chl-*a* concentration. TA was measured using an automatic potentiometric titration method (848 Titrino Plus; Metrohm, Herisau, Switzerland) with a measurement accuracy of ± 5 mmol/L. All samples collected were processed the same day and stored in a low-temperature freezer. The samples that required instrumental analyses were analyzed within a week of collection.

The sea–air interface CO_2 exchange flux was estimated using the following formula: $F=k\times\alpha s\times\Delta pCO_2$, where F (mmol/m^2·d) is the CO_2 exchange flux at the sea–air interface, which represents the CO_2 exchange flux (the intensity of the atmospheric CO_2 source and sink). The positive and negative signs indicate the direction of the source and sink. When seawater absorbs CO_2 from the atmosphere and acts as a sink, F is a negative value. When seawater releases CO_2 into the atmosphere and acts as a source, F is a positive value. k (cm/h) is the gas transmission speed at the sea–air interface; αs (mol/kg·atm) is the solubility coefficient of CO_2 in seawater, which is a function related to temperature and salinity and is calculated using the Weiss (1974) formula; and ΔpCO_2 is the difference between seawater and pCO_2 in the atmosphere. In this study, the value of pCO_2 in the atmosphere was 410 μatm (https://keelingcurve.ucsd.

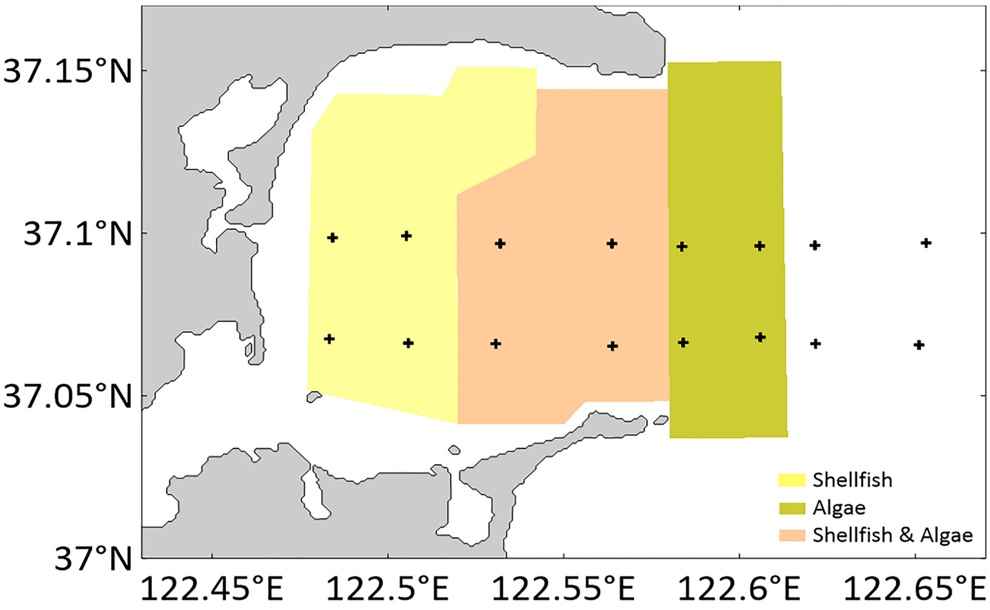

FIGURE 1 | Sampling stations in Sanggou Bay.

edu/), and the gas transmission speed (k) at the sea–air interface was the average value (10.3) of the continental shelf sea area (Jiang et al., 2013).

Data Analysis

Statistical analysis was performed using SPSS 17.0. Analysis of variance (ANOVA) was used to analyze the effects of season and zone on environmental factors, carbon components, $p\mathrm{CO_2}$, and sea–air $\mathrm{CO_2}$ flux. According to the results of the homogeneity test, Tukey's honestly significant difference or Tamhane's T_2 test was used to evaluate the significance of differences between groups ($p<0.05$) after ANOVA.

RESULTS

Spatiotemporal Variability in Environmental Parameters

Table 1 shows the averages for the environmental factors in the study area. The water temperature decreased from the inside of the bay to the outside of the bay in spring and summer, while the opposite was true in autumn and winter. The salinity in the summer was low because of the higher rainfall and land runoff, and salinity in the entire bay in the other seasons did not significantly vary ($p>0.05$). The season ($F=5.10$, $p<0.05$) and aquaculture zone ($F=4.59$, $p<0.05$) significantly affected the DO concentration of surface seawater, and the interaction between the two on the DO concentration was significant (seasonal × zone: $F=8.32$, $p<0.05$). The Chl-*a* concentration showed pronounced seasonal variation ($F=14.59$, $p<0.05$). Although the Chl-*a* concentration of the shellfish culture zone appeared to be lower than the other aquaculture zones, there was no significant effect of the aquaculture zone on the Chl-*a* concentration ($F=2.16$, $p=0.136$). The aquaculture mode significantly ($F=11.79$, $p<0.05$) affected the pH. The pH was lowest in the shellfish culture zone and highest in the macro-algae culture zone. The aquaculture mode ($F=6.32$, $p<0.05$) had a significant impact on TA, and the TA was significantly lower in the shellfish culture zone than in the other regions. Additionally, the season ($F=257.33$, $p<0.05$) had a significant effect on changes in TA. TA decreases with temperature increases.

Spatiotemporal Variability in Dissolved Inorganic Carbon and $p\mathrm{CO_2}$

The season and aquaculture mode significantly affected the spatiotemporal changes in DIC (season: $F=325.75$, $p<0.05$; zone: $F=7.18$, $p<0.05$; **Figure 2**) and $p\mathrm{CO_2}$ (season: $F=38.97$, $p<0.05$; zone: $F=11.79$, $p<0.05$; **Figure 3**) in the surface seawater of Sanggou Bay, and the interaction between the season and aquaculture mode also had a significant effect on DIC and $p\mathrm{CO_2}$ (DIC: season × zone: $F=9.75$, $p<0.05$; $p\mathrm{CO_2}$: season × zone: $F=4.39$, $p<0.05$). DIC in the surface seawater of Sanggou Bay was highest in summer, followed by autumn, spring, and winter; $p\mathrm{CO_2}$ was highest in summer, followed by spring, autumn, and winter. The surface seawater DIC concentration and $p\mathrm{CO_2}$ were highest in the shellfish culture zone and lowest in the macro-algae culture zone. The $p\mathrm{CO_2}$ concentration in the control area did not significantly differ from that in the shellfish–macro-algae polyculture zone ($p>0.05$). In sum, the $p\mathrm{CO_2}$ level in the whole bay increased with temperature.

Spatiotemporal Variability of Dissolved Organic Carbon and Particulate Organic Carbon

Similar to the change law of DIC and $p\mathrm{CO_2}$, different seasons and aquaculture modes also significantly affect the distributions of DOC (season: $F=35.88$, $p<0.05$, zone: $F=4.32$, $p<0.05$, **Figure 4**) and POC (season: $F=11.23$, $p<0.05$, zone: $F=4.59$, $p<0.05$, **Figure 5**) in the surface seawater of Sanggou Bay. The season and aquaculture mode have a significant interaction effect on DOC (season × zone: $F=8.07$, $p<0.05$) and POC (season × zone: $F=8.07$, $p<0.05$). Different from DIC and $p\mathrm{CO_2}$, the DOC concentration in winter and summer is relatively high, the DOC concentration in spring and autumn is relatively low, and the autumn DOC

TABLE 1 | Seasonal variation of average value of different environmental parameters in Sanggou Bay.

Season	Zone	T (°C)	S	DO (mg/L)	Chl-*a* (μg/L)	pH	TA (μmol/L)
Spring	Shellfish	15.58 ± 0.21	31.66 ± 0.05	8.30 ± 0.09	0.50 ± 0.11	8.25 ± 0.08	2,207.87 ± 7.86
	Shellfish and macro-algae	14.98 ± 1.71	31.86 ± 0.12	8.47 ± 0.07	0.48 ± 0.05	8.35 ± 0.09	2,226.54 ± 27.22
	Macro-algae	12.84 ± 1.02	31.87 ± 0.07	8.51 ± 0.15	0.55 ± 0.02	8.53 ± 0.01	2,330.73 ± 26.79
	Control	12.02 ± 0.54	31.91 ± 0.22	8.44 ± 0.12	1.28 ± 0.17	8.39 ± 0.01	2,322.11 ± 36.91
Summer	Shellfish	24.81 ± 0.62	30.49 ± 0.09	7.07 ± 0.25	3.90 ± 0.28	7.73 ± 0.36	2,170.66 ± 9.10
	Shellfish and macro-algae	23.41 ± 0.87	30.78 ± 0.05	7.19 ± 0.04	4.11 ± 0.71	7.97 ± 0.01	2,209.36 ± 9.38
	Macro-algae	21.93 ± 0.13	31.26 ± 0.09	8.13 ± 0.05	5.70 ± 0.02	8.12 ± 0.03	2,231.58 ± 3.09
	Control	20.63 ± 0.44	31.82 ± 0.11	8.13 ± 0.02	5.28 ± 0.45	8.09 ± 0.01	2,243.40 ± 5.81
Autumn	Shellfish	10.88 ± 0.61	31.55 ± 0.10	8.79 ± 0.04	0.58 ± 0.11	8.12 ± 0.01	2,257.62 ± 16.84
	Shellfish and macro-algae	12.36 ± 0.25	31.60 ± 0.06	8.75 ± 0.19	0.61 ± 0.05	8.27 ± 0.06	2,283.06 ± 21.57
	Macro-algae	12.85 ± 0.07	31.58 ± 0.05	8.82 ± 0.19	0.42 ± 0.02	8.40 ± 0.02	2,317.02 ± 35.09
	Control	13.47 ± 0.12	31.61 ± 0.02	8.29 ± 0.06	0.62 ± 0.11	8.30 ± 0.06	2,336.18 ± 17.83
Winter	Shellfish	3.48 ± 0.14	31.17 ± 0.03	8.46 ± 0.17	0.59 ± 0.13	8.03 ± 0.07	2,301.41 ± 99.66
	Shellfish and macro-algae	3.83 ± 0.04	31.18 ± 0.06	8.50 ± 0.21	0.72 ± 0.02	8.19 ± 0.06	2,311.75 ± 24.88
	Macro-algae	4.58 ± 0.24	31.20 ± 0.07	8.53 ± 0.04	0.61 ± 0.56	8.37 ± 0.03	2,354.02 ± 48.78
	Control	6.53 ± 1.13	32.19 ± 0.05	8.11 ± 0.14	0.82 ± 0.55	8.25 ± 0.01	2,377.44 ± 20.35

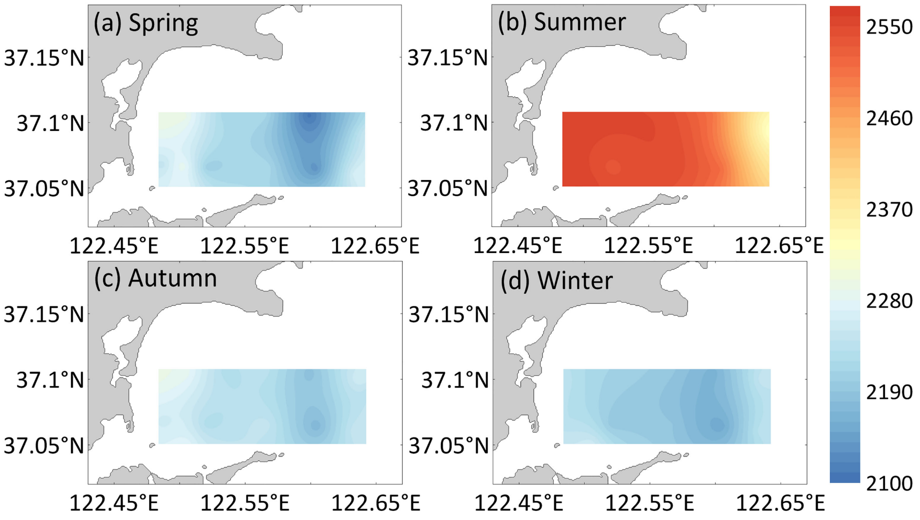

FIGURE 2 | Horizontal distribution of DIC (μmol/L) for the **(A)** spring, **(B)** summer, **(C)** autumn, and **(D)** winter surface water in Sanggou Bay.

concentration is the lowest. From a spatial point of view, the overall performance of different aquaculture zones is that the DOC concentration in the macro-algae culture zone is the highest, and the DOC concentration in the shellfish culture zone is the lowest. Nevertheless, the DOC concentration in the shellfish culture zone in summer was significantly higher than those in other areas ($p<0.05$). The POC concentration in the surface seawater of Sanggou Bay follows the sequence of summer > spring > autumn > winter. From the perspective of spatial changes, the POC concentration in the shellfish culture zone and macro-algae culture zone surface seawater is lower, and the POC concentration in the shellfish–macro-algae polyculture zone and control area is higher.

Spatiotemporal Variability in Sea–Air CO_2 Flux

Figure 6 shows the annual sea–air CO_2 flux in Sanggou Bay. In spring, the shellfish culture zone was a weak sink of atmospheric CO_2. In summer, the shellfish culture zone and shellfish–macro-algae polyculture zone were the sources of atmospheric CO_2. In autumn and winter, the entire bay was a strong sink of atmospheric CO_2, and the macro-algae culture zone played the

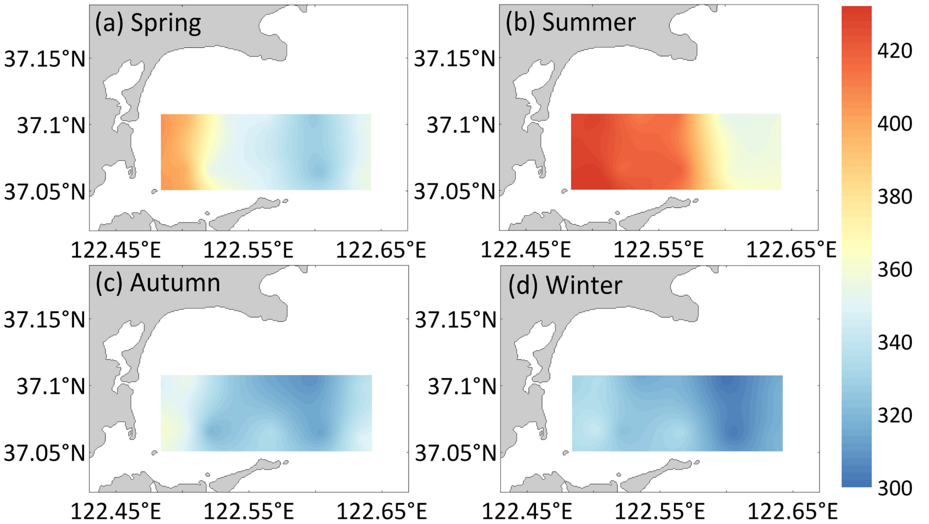

FIGURE 3 | Horizontal distribution of pCO_2 (μatm) for the **(A)** spring, **(B)** summer, **(C)** autumn, and **(D)** winter surface water in Sanggou Bay.

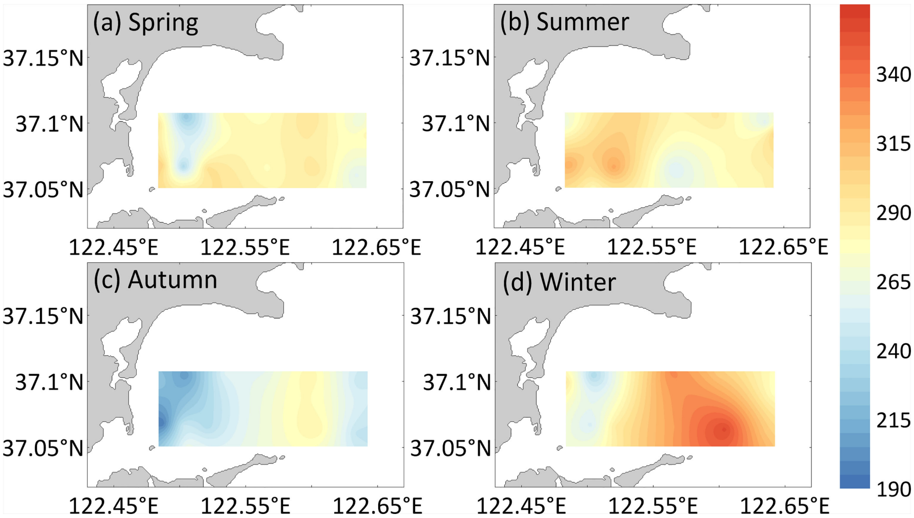

FIGURE 4 | Horizontal distribution of DOC (μmol/L) for the **(A)** spring, **(B)** summer, **(C)** autumn, and **(D)** winter surface water in Sanggou Bay.

largest role in gathering atmospheric CO_2. The season (F=210.43, $p<0.05$) and aquaculture mode (F=44.14, $p<0.05$) had significant effects on the CO_2 flux, and the interaction between the two was significant (season × zone: F=7.396, $p<0.05$). The sea–air CO_2 flux in Sanggou Bay varies differently between seasons, where a significant difference was concluded between spring and winter and between summer and autumn, and a highly significant difference was found between summer and winter. (**Table 2**). Pearson correlation analysis revealed that changes in the CO_2 exchange flux at the sea–air interface in different seasons were affected by different environmental factors (**Table 3**). DIC and Chl-*a* were the most important factors in spring. In autumn and winter, pH and DIC were the most important factors. In summer, the sea–air CO_2 flux was most significantly affected by Chl-*a*, TA, and DO. Throughout the year, water temperature and DIC were the key factors affecting sea–air CO_2 exchange flux.

DISCUSSION

Effects of Shellfish–Macro-Algae Aquaculture on Environmental Factors

Macro-algae can absorb DIC in seawater through photosynthesis, reduce pCO_2 levels, and increase the DO and pH of seawater. By contrast, the respiration and calcification of shellfish result in the

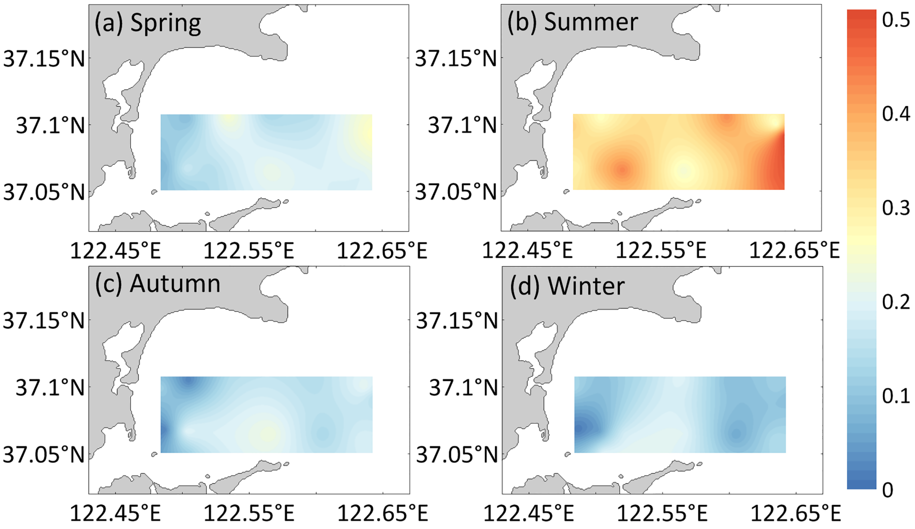

FIGURE 5 | Horizontal distribution of POC (mg/L) for the **(A)** spring, **(B)** summer, **(C)** autumn, **(D)** winter surface water in Sanggou Bay.

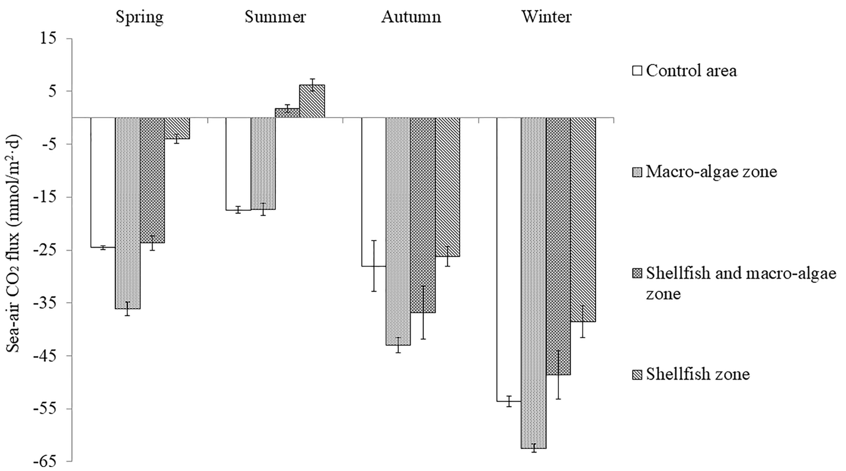

FIGURE 6 | Seasonal variation in sea–air CO_2 flux in Sanggou Bay.

release of CO_2 into seawater, increase DIC and pCO_2, and reduce the pH (Delille et al., 2009). A macro-algae culture can effectively alleviate ocean acidification (Xiao et al., 2021). Calcified organisms (e.g., bivalves, corals) that are particularly susceptible to ocean acidification can benefit from these effects (Kroeker et al., 2013). Thus, the co-cultivation of bivalves and seaweeds in the IMTA system can protect bivalves from the effects of ocean acidification and provide them with sufficient oxygen. The IMTA system can also alleviate the pressure associated with the release of nutrients by bivalves into the environment, as the macro-algae in the system absorb nutrients (Han et al., 2020). It is important to note that the shellfish–macro-algae IMTA model in the survey area has been based on the expansion of kelp culture. Because of the poor high-temperature resistance of kelp, large-scale shellfish farming is carried out for up to 4 months in the study area after kelp is harvested in summer. According to previous studies, the metabolism of shellfish is highest in July and August, which corresponds to the period when shellfish grow most vigorously. In addition, bacteria are most active under high-temperature conditions in summer, and the excrement of cultured shellfish is quickly decomposed, which increases the vulnerability of the shellfish culture zone to hypoxia and eutrophication (Li et al., 2018). Therefore, the large-scale shellfish culture in summer may impose substantial ecological pressures (Liu et al., 2021), and this was confirmed in this study. The pH and DO of the shellfish culture zone and shellfish–macro-algae polyculture zone (actually, only shellfish is cultured in the polyculture zone) decreased to varying degrees in summer; the average pH of the shellfish culture zone was only 7.73, and the DO was 7.07 mg/L. The calcification rate of shellfish decreased by 13.5% when the pH decreased by 0.1 units, and shellfish are not able to synthesize shells when the pH is 7.3 (Zhang et al., 2011; Wahl et al., 2018). The growth of fish is also inhibited when the DO decreases to 5 mg/L, and the large-scale death of crustaceans may occur when DO decreases to 4 mg/L (Sugden, 2017). Therefore, changes in environmental factors associated with the aquaculture mode of Sanggou Bay in summer pose risks to cultured organisms such as shellfish and fish. The pH and DO of the shellfish culture zone in summer were 0.12-0.39 units and 0.94-1.06 mg/L lower compared with values from surrounding areas, respectively. The pH and DO in the macro-algae culture zone were 0.10-0.34 units and 0.03-0.53 mg/L higher compared with values in adjacent seas, respectively. Therefore, macro-algae culture can theoretically offset the impact of CO_2 produced by shellfish respiration and calcification on the pH and DO of the sea. Because of the high temperature of water in summer, kelp cannot grow. Thus, large macro-algae that can withstand high temperatures (e.g., *Gracilaria lemaneiformis*) needs to be bred to reduce the environmental impact of summer shellfish farming (Liu et al., 2021).

In other seasons, the environmental parameters tend to better because of the cultivation of macro-algae in the study area. Macro-algae culture can not only improve the environmental parameters but can also absorb the nutrients in water, which alleviates eutrophication (Mao et al., 2009). Previous studies have

TABLE 2 | *Post-hoc* Tukey HSD tests for the seasonal data of sea–air co_2 flux.

Variables	Source of Variability	Summer	Autumn	Winter
CO_2 flux	Spring	0.256	0.490	0.015*
	Summer		0.023*	0.001**
	Autumn			0.177

**means significant difference, p<0.05, **means highly significant difference, p<0.01.*

TABLE 3 | Pearson correlation coefficients between CO_2 sea–air flux and environmental factors.

Season	Source of variability	SST	pH	DIC	Chl-*a*	TA	DO
The whole year	CO_2 flux	0.773**	-0.233	0.488*	0.264	0.437	-0.427
Spring		0.686	0.713	0.954*	-0.913*	0.634	-0.632
Summer		0.550	-0.385	0.688	-0.975**	0.869*	-0.942**
Autumn		-0.064	-0.935*	0.959**	-0.782	0.160	-0.695
Winter		0.143	-0.905*	0.994**	-0.785	0.867	-0.733

**means significant difference, $p<0.05$; **means highly significant difference, $p<0.01$.*

confirmed that the buffering effect of macro-algae culture is sufficient to partially offset the decrease in pH caused by ocean acidification in recent decades. Compared with natural seaweed systems, macro-algae culture has a greater potential to adapt to future fluctuations in the ocean pH (Krause-Jensen et al., 2016). However, the water exchange capacity in Sanggou Bay was weak because of the blocking effect of breeding facilities and cultured organisms (Liu et al., 2017); there is thus a need to ensure that the scale of macro-algae culture is not excessive to prevent the excessive consumption of nutrients. In autumn, some of the shellfish are harvested, which causes some nutrients to be removed, and kelp absorbs large amounts of nutrients in the early stages of growth. Therefore, the Chl-*a* concentration was lowest in autumn, indicating that even if shellfish ingestion is reduced at this time, macro-algae cultivation in the sea prevents the growth of phytoplankton. Therefore, the excessive expansion of macro-algae culture will restrict the growth of macro-algae; an appropriate amount of macro-algae culture is required for aquaculture to be environmentally friendly (Fang et al., 2020).

Effects of Shellfish–Macro-Algae Aquaculture on the Inorganic Carbon Cycle in Seawater

The inorganic carbon system in the aquaculture zone undergoes various changes due to the physiological activities of shellfish and macro-algae. Large-scale macro-algae and bivalve farming has affected the sea–air CO_2 exchange flux in Sanggou Bay. The shellfish and macro-algae biomass was highest in the winter, and this is when the sea–air CO_2 exchange flux was the highest in the study area. The CO_2 exchange flux between the ocean and the atmosphere is the most important process determining the marine inorganic carbon budget (Jiang et al., 2015). Therefore, DIC is an important factor affecting the annual sea–air CO_2 exchange flux in Sanggou Bay.

The physiological activities of some calcified organisms in their natural habitats can significantly affect the inorganic carbon cycle of the surrounding water (Page et al., 2019). Changes in the inorganic carbon cycle induced by shellfish culture are usually more pronounced than those in natural habitats because the density of cultured shellfish is much higher than that of naturally occurring species (Li et al., 2021). Longer seawater residence times can also substantially affect changes in the marine inorganic carbon system (Page et al., 2019). The weak water exchange capacity of Sanggou Bay induces major changes in the inorganic carbon cycle in the shellfish culture zone (Li et al., 2021). In all seasons, the lowest pH and TA and highest DIC and pCO_2 were observed in the shellfish culture zone. Although the CO_2 emitted by shellfish respiration does not affect the TA in seawater (Zhang et al., 2011), the calcification process of shellfish leads to a decrease in seawater TA, which limits the ability of seawater to absorb CO_2 and affects the carbonate balance (Han et al., 2020). Bivalves are the main cause of the decline in TA in Sanggou Bay, which may lead to an increase in the pCO_2 in the shellfish culture zone. Although bivalves absorb a certain amount of DIC during the calcification of synthetic shells, the DIC released during respiration is significantly higher (Jiang et al., 2015). Therefore, the respiration of a large number of bivalves in the bay may increase the accumulation and emission of inorganic carbon in the water environment (Li et al., 2021). The sedimentation of organisms aggravates the bioburden of sediments, and the remineralization of sediments is an important source of DIC (Jiang et al., 2015). When the bivalves experience high summer temperatures, the DIC concentration in the shellfish culture zone is the highest due to physiological processes such as respiration and excretion. However, some studies have shown that the calcification process of bivalves can regulate the inorganic carbon cycle more than respiration under certain conditions. For example, oysters can remove inorganic carbon from seawater through calcification (Ren, 2014). The respiration of bivalves is weakened by decreases in temperature (e.g., winter), which explains the lack of differences in the concentration of DIC in the shellfish culture zone compared with that of other regions. This might also be one of the reasons why the sources and sinks of the sea–air CO_2 flux in the shellfish culture zone change seasonally in this study. The effects of water temperature on the bay sea–air CO_2 flux may not be mediated through physical effects, such as solubility, but instead through indirect effects, such as the physiological activities of cultured organisms.

The results regarding the sea–air CO_2 flux in the shellfish culture zone in this study differed from those of previous studies. This might be caused by the different methods used for calculating the sea–air CO_2 flux, including the gas transmission speed, the pCO_2 in the atmosphere, and the pCO_2 in the surface water. The acquisition of pCO_2 in the surface water is usually automatically obtained by software by monitoring parameters such as pH, temperature, TA, and salinity. Therefore, a small error in a certain parameter may have a substantial impact on the results. Studies of the same aquaculture zone in the same season have found that differences in pCO_2 in the surface water were greater than 50 μatm (Li et al., 2021; Xiao et al., 2021). Therefore, pCO_2 in the water was directly measured by a CO_2 partial pressure meter, albeit some error in the absolute value is possible. The general patterns of $p\mathrm{CO}_2$ in this study were relatively clear. Hence, we mainly focused on the different patterns of CO_2 flux and $p\mathrm{CO}_2$ and did not discuss absolute values.

Macro-algae uses bicarbonate ions as an external carbon source for photosynthesis, ingests a large amount of DIC during growth, and affects the $p\mathrm{CO}_2$ and TA in surface seawater (Axelsson et al., 2000). The net primary production of macro-algae aquaculture can promote the absorption of atmospheric CO_2, and the difference in the macro-algae growth rate in different seasons significantly affects the level of the sea–air CO_2 exchange flux (Jiang et al., 2013; Xiao et al., 2021), which was verified in this study. Compared with the natural seaweed system, a macro-algae aquaculture has more flexibility and scalability in terms of space planning (Xiao et al., 2021). Macro-algae culture also responded positively to increases in CO_2, which can increase the productivity of macro-algae culture, result in the absorption of additional CO_2 (Krause-Jensen et al., 2016), promote a continuous increase in pH, and provide marine life with a refuge for ocean acidification (Hofmann et al., 2011). Previous studies have shown that the effects of the proliferation of coastal macro-algae on surface seawater $p\mathrm{CO}_2$ can last up to 3 months following the end of a water bloom (Gazeau et al., 2005). However, this was not a pattern that was observed in our study. The $p\mathrm{CO}_2$ in the surface seawater of the study area increased significantly after macro-algae was harvested. This might be explained by the fact that the continuous impact of macro-algae on natural water cannot offset the effects of shellfish and other cultured organisms.

Additionally, after the macro-algae culture was initiated, the sea–air CO_2 flux in Sanggou Bay was significantly improved. Therefore, whether shellfish culture acts as carbon sink or source is greatly dependent on the participation of the macro-algae aquaculture, which may be another reason why the sources and sinks of the sea–air CO_2 flux in the shellfish culture zone change seasonally in this study. Furthermore, we speculate that in bays or waters with weak hydrodynamics, the effect of macro-algae culture could potentially increase the water residence time locally, which may affect the biological process regarding the inorganic carbon cycle. The studies of the effect of macro-algae on the inorganic carbon cycle at the mesocosm scale in the laboratory have been carried out, and the effects of macro-algae on the inorganic carbon cycle in Sanggou Bay are weaker than the effects observed in the laboratory (Jiang et al., 2015; Han et al., 2017; Fang et al., 2020; Han et al., 2020; Liu et al., 2021). Characterizing changes in the inorganic carbon system might be difficult if the time that seawater remains in the macro-algae culture zone is relatively short (Li et al., 2021). Therefore, changes in environmental factors such as ocean currents, as well as multiple effects such as the carbonate balance and biological metabolic processes affect changes in the inorganic carbon cycle of the marine ecosystem (Koweek et al., 2017; Wahl et al., 2018).

Effects of Shellfish–Macro-Algae Aquaculture on the Organic Carbon Cycle in Seawater

The concentrations of DOC and POC significantly varied among seasons and regions in the surface seawater of Sanggou Bay. In winter, the biomass of macro-algae farmed in Sanggou Bay was the largest, and the macro-algae released DOC into the water during their growth (Li et al., 2018). Hence, a higher DOC concentration was observed in the macro-algae culture zone in winter. Jiao et al. (2018) suggested that microbial carbon fixation is a major part of the missing carbon sink, as it involves the conversion of labile DOC into RDOC and its storage in seawater for a long time. Zhang et al. (2017) found that the RDOC generated by macro-algae culture is almost equal to the amount of carbon that it uses during its growth. Therefore, macro-algae culture can not only effectively improve the inorganic carbon cycle but can also effectively improve the organic carbon cycle, thereby increasing the strength of the marine carbon sink. The biomass of macro-algae was lowest in Sanggou Bay in summer. However, the DOC concentration in Sanggou Bay was higher in summer than in spring and autumn. This can be explained by the growth of phytoplankton in summer, which results in the release of a large amount of DOC (Mou et al., 2017). The manure produced by shellfish culture in summer also increases the organic carbon pool and DOC (Ning et al., 2016). A large amount of organic debris is produced when kelp is harvested in summer. The decaying organic debris and shellfish feces can release a large amount of DOC into the seawater *via* decomposition by microorganisms (Mahmood et al., 2017). The DOC concentration was lowest in autumn because the macro-algae were recently cultivated, and the growth of phytoplankton was inhibited by macro-algae. Additionally, some of the shellfish are harvested, and a large amount of carbon is removed from the system in autumn (Li et al., 2018). Therefore, the DOC concentration was lowest in autumn. The DOC concentration in the shellfish culture zone was low in the rest of the seasons except for summer. This suggests that the DOC released by shellfish culture can be quickly reused and thus may not be easily stored in seawater (Zhang et al., 2017; Li et al., 2018).

POC is the main form of carbon solidification and migration output in seawater (Hung et al., 2000). Although it only accounts for approximately 10% of marine organic carbon, the ratio of POC to DOC in marine primary production products is 5:1 (Jiao and Wang, 1994). POC is closely related to life processes and the primary productivity of organisms; it is thus an important material in the food chain of marine organisms. The source of POC in seawater in Sanggou Bay is mainly live phytoplankton, which is the main food source for shellfish (Xia et al., 2013). Therefore, the POC concentration in the shellfish culture zone was low. The shellfish culture can effectively remove POC through filter-feeding activity and generate biological deposits such as feces, which has a substantial impact on carbon biogeochemical processes (Jiang et al., 2015). The POC concentration in the macro-algae culture zone was similarly low because of competition with phytoplankton for nutrients. The POC concentration was higher in the shellfish–macro-algae polyculture zone than in the macro-algae culture zone and shellfish culture zone. On the one hand, this indicates that shellfish–macro-algae IMTA has little effect on the nutrient structure of the sea area and does not inhibit the reproduction of phytoplankton. On the other hand, this suggests that shellfish–macro-algae IMTA can better promote the migration and transformation of particulate carbon.

Overall, DOC and POC play an important role in the marine carbon cycle, and the shellfish and macro-algae in the shellfish–macro-algae aquaculture can affect the organic carbon cycle through their physiological activities. Therefore, the cycle of organic carbon requires consideration in future studies of the carbon sink function of marine ecosystems.

CONCLUSIONS

Large-scale aquaculture alters the carbon sink function and carbon cycle of marine ecosystems, and aquaculture modes vary in their effects. The macro-algae culture and shellfish culture have substantial effects on the sea–air CO_2 flux in Sanggou Bay and inorganic and organic carbon cycles in water. The macro-algae culture can effectively improve various environmental characteristics, promote the absorption of atmospheric CO_2, and provide calcified organisms with refuges from ocean acidification. Although shellfish culture reduces the absorption capacity of seawater for CO_2, its efficient filtration capacity can also effectively influence the organic carbon cycle. We believe that the carbon sink function of shellfish culture must be restricted to certain conditions to be realized (e.g., temperature, co-culture of macro-algae). Large-scale shellfish–macro-algae IMTA plays an important role in the promotion of carbon cycling.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Conceptualization, YL and JZ. Methodology, YL. Experience operimental, WW. Sample analysis, WW and JY. Data curation, XW and YZhong. Writing—original draft preparation, YL. Writing—review and editing, YL. Revision process, HL and YZhang. All authors have read and agreed to the published version of the manuscript.

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The Potential of Kelp *Saccharina japonica* in Shielding Pacific Oyster *Crassostrea gigas* From Elevated Seawater pCO_2 Stress

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Ocean acidification (OA) caused by elevated atmospheric CO_2 concentration is predicted to have negative impacts on marine bivalves in aquaculture. However, to date, most of our knowledge is derived from short-term laboratory-based experiments, which are difficult to scale to real-world production. Therefore, field experiments, such as this study, are critical for improving ecological relevance. Due to the ability of seaweed to absorb dissolved carbon dioxide from the surrounding seawater through photosynthesis, seaweed has gained theoretical attention as a potential partner of bivalves in integrated aquaculture to help mitigate the adverse effects of OA. Consequently, this study investigates the impact of elevated pCO_2 on the physiological responses of the Pacific oyster *Crassostrea gigas* in the presence and absence of kelp (*Saccharina japonica*) using *in situ* mesocosms. For 30 days, mesocosms were exposed to six treatments, consisting of two pCO_2 treatments (500 and 900 µatm) combined with three biotic treatments (oyster alone, kelp alone, and integrated kelp and oyster aquaculture). Results showed that the clearance rate (CR) and scope for growth (SfG) of *C. gigas* were significantly reduced by elevated pCO_2, whereas respiration rates (MO_2) and ammonium excretion rates (ER) were significantly increased. However, food absorption efficiency (AE) was not significantly affected by elevated pCO_2. The presence of *S. japonica* changed the daytime pH_{NBS} of experimental units by ~0.16 units in the elevated pCO_2 treatment. As a consequence, CR and SfG significantly increased and MO_2 and ER decreased compared to *C. gigas* exposed to elevated pCO_2 without *S. japonica.* These findings indicate that the presence of *S. japonica* in integrated aquaculture may help shield *C. gigas* from the negative effects of elevated seawater pCO_2.

Keywords: *Saccharina japonica*, *Crassostrea gigas*, elevated seawater pCO_2, mesocosm, physiological responses

INTRODUCTION

Ocean acidification (OA) caused by elevated concentrations of atmospheric CO_2 is one of the most serious environmental issues facing the world in the 21st century. Multiple studies have indicated that this more acidic oceanic environment will have negative consequences for marine life, such as decreasing calcification rates and impairing feeding, respiration, and physiological energetics (Gazeau et al., 2007; Rastrick et al., 2018a; Jiang et al., 2021). Damage to marine organisms by OA is likely to alter coastal biodiversity, ecosystem functioning and services, and global marine harvests (Cooley et al., 2009; Barry et al., 2011). Calcifying marine organisms such as molluscs, crustaceans, corals, and planktonic calcifies are predicted to be particularly sensitive due to the additional costs associated with calcification and maintenance of calcified structures (Fabry et al., 2008; Hofmann et al., 2010; Findlay et al., 2011; Rastrick et al., 2014). Marine bivalves are calcifying ecosystem engineers, which are important in ecosystem functioning and aquaculture (Cranford et al., 2012). In accordance with their ecological and commercial relevance, the interest in OA research on bivalves is growing steadily and has increased particularly in recent years (e.g., Berge et al., 2006; Cummings et al., 2011; Li et al., 2017; Zhao et al., 2017; Rastrick et al., 2018a; Tan and Zheng, 2019). Evidence from several previous studies suggests that elevated seawater pCO_2 affects calcification (e.g., Ries et al., 2009; Melzner et al., 2011), growth (e.g., Thomsen et al., 2010; Kroeker et al., 2013), burrowing behavior (e.g., Clements et al., 2015; Peng et al., 2017), energetics (e.g., Wang et al., 2015; Zhao et al., 2017; Rastrick et al., 2018a) and immune response (e.g., Bibby et al., 2008; Zha et al., 2017) in bivalves such as oysters, mussels, and clams. However, much of the published information on bivalves comes from short-term laboratory-based manipulative experiments, which reduces the generalizability of results to real-world situations. With the increasing demand for improving the ecological validity of OA studies, it is necessary to scale up the methodology from laboratory- to field-level (Riebesell et al., 2008; Rastrick et al., 2018b).

The Pacific oyster, *Crassostrea gigas*, is one of the most important ecologically and economically cultured bivalves worldwide, with a global mariculture production of 643 thousand tons in 2018 (FAO, 2020). The effects of OA on the Pacific oyster have been well documented. Previous studies show that early developmental stages of oysters are more susceptible to increased seawater pCO_2 (Kurihara et al., 2007; Gazeau et al., 2011). (Barton et al. 2012; 2015) reported that larval production and mid-stage growth (~120 to ~150 mm) of *C. gigas* showed a significantly negative correlation to naturally elevated carbon dioxide levels and clearly linked increased CO_2 to the cause of severe loss of production at the Whiskey Creek Shellfish Hatchery on the Oregon coast. The calcification rates of *C. gigas* have been predicted to decline by 10% following the IPCC IS92a scenario (~740 ppmv in 2,100) (Gazeau et al., 2007). Negative impacts of elevated pCO_2 to *C. gigas* acid–base status and immune response have also been reported by Lannig et al. (2010) and Wang et al. (2016). Despite increased understanding of the potential impacts of OA on Pacific oysters, studies regarding the physiological effects of OA on *C. gigas* under natural systems are scarce. Furthermore, there is still a lack of effective ways for the oyster aquaculture industry to help mitigate the potential negative impacts of OA.

Previous studies have postulated that Integrated Multi-Trophic Aquaculture (IMTA) may be a possible and sustainable solution to help mitigate some of the effects of climate change on production (Clements and Chopin, 2016; Tan and Zheng, 2020). The term "IMTA" refers to the combined aquaculture of various organisms at different trophic levels with complementary ecosystem functions in which the wastes and by-products from one species serve as the food source for one or a number of other species (Chopin, 2013). The seaweed aquaculture sector may be one of the key components of IMTA systems (Chopin, 2014). Seaweed within IMTA is traditionally regarded as a source of nutrient removal from the seawater of the nearby bivalve farms and plays a role in the bioremediation and ecological regulation of the aquaculture environment. Seaweed also has the ability to add oxygen and absorb dissolved carbon dioxide from the surrounding seawater through photosynthesis, producing biomass and contributing to carbon sequestration. It has been reported that ~0.34 million t carbon is removed from the coastal ecosystems each year by seaweed harvesting (Tang et al., 2011). The sea–air CO_2 flux is enhanced by seaweed aquaculture (Jiang et al., 2013). Bay scale dissolved inorganic carbon (DIC) budget research showed that the annual DIC uptake by seaweed *Saccharina japonica* and *Gracilaria lemaneiformis* was about 1.0×10^5 t in Sanggou Bay, which drove the bay as a net DIC sink (Jiang et al., 2015). By sequestering carbon dioxide dissolved in seawater, seaweed plays a potential role in reducing OA and its biological effects on a local scale. An interesting study on the effect of dissolved CO_2 levels (0, 100, 200, 250, and 300 ppm) on the dissolution rate of the outer shells of dead mollusks and spines of sea urchins in the presence and absence of seaweed, *Chaetomorpha antennina*, showed that the weight loss of the samples was curbed when seaweed was introduced (Kaladharan et al., 2019). In the coastal regions of southern Korea, an innovative research approach called the Coastal CO_2 Removal Belt (CCRB) has established a pilot farm of perennial brown alga, *Eklonia calva*, which sequesters ~10 t of CO_2 per ha per year (Chung et al., 2013). Jiang et al. (2014) carried out a mesocosm experiment to monitor the function of seaweed *G. lemaneiformis* in eliminating dissolved inorganic carbon released from the calcification and respiration processes of the scallop *Chlamys farreri*. The results showed that seaweed and bivalve integrated aquaculture practice can not only effectively balance the seawater carbonate system but also buffer acidification. However, although the ecological and economic benefits of IMTA have been highlighted, the role of seaweed within IMTA in mitigating the impacts of OA on bivalves (e.g., physiological activities, energy allocation and growth, etc.) needs further investigation, especially within natural systems.

The goal of this research was to examine: (1) whether the physiological processes and net energy balance of *C. gigas* were affected after medium-term exposure (30 days) to elevated $p\mathrm{CO}_2$ and (2) whether the presence of *S. japonica* could help mitigate these biological effects of elevated $p\mathrm{CO}_2$ on *C. gigas* within a natural system.

MATERIALS AND METHODS

Animal Collection and Acclimatization

Experimental oysters (56.37 ± 8.20 mm shell length, 29.67 ± 3.91 g wet weight) and kelps (70–80 cm length) were collected on 7 May 2017 from a large-scale commercial aquaculture area (37° 03′52.17″N, 122°33′11.54″E) in Sanggou Bay, Yellow Sea, China. All specimens were transported on ice to a small semi-enclosed harbor (37°02′14.71″N, 122°33′02.09″E; area 7900 m^2; mean depth 1.5 m). The oysters were cleaned and maintained in flowing seawater in *in situ* mesocosms at ambient conditions (temperature ~19.0°C, salinity ~32, and seawater $\mathrm{pH}_{\mathrm{NBS}}$ ~8.10) for one week before the start of the experiment. One hundred and thirty healthy oysters were selected and individually labeled with a plastic-laminated number on the upper valve.

Experimental Setup and Procedure

Experiments were conducted using *in situ* mesocosms with six treatments: three biotic treatments (namely "oyster alone", "kelp alone," and "integrated aquaculture"), crossed with two $p\mathrm{CO}_2$ treatments (ambient, ~500 µatm and elevated, ~900 µatm). Each of the six treatments was replicated three times, for a total of 18 mesocosms. The mesocosms were suspended from a floating platform. Each sealed mesocosm was made of translucent polyethylene and was cylinder-shaped (0.5 m × 1.2 m, diameter × height) (**Figure 1**). Inlet and outlet valves allow for the control of seawater flow through each mesocosm. The top of each mesocosm was immersed to a depth of ~30 cm with the inlet and outlet pipes (Φ ~3 cm) extending ~50 cm above the water surface. The inlet pipe extended to the bottom of each mesocosm, allowing water mixing and preventing gradients from forming within the mesocosm.

Elevated $p\mathrm{CO}_2$ treatment was controlled by enriching natural seawater with CO_2 (from a CO_2 gas cylinder) in a submerged 1,000 L reservoir tank suspended from the same floating platform as the mesocosms (modified from Rastrick et al., 2014; Jiang et al., 2019; Jiang et al., 2021). The flow of pure CO_2 to the reservoir tank was controlled *via* a pH controller (Aqua Medic GmbH, Germany) by switching on or off a valve when pH readings in the tank deviated from the predetermined set-points by ±0.05 pH units. The acidified seawater was then pumped to each elevated $p\mathrm{CO}_2$ mesocosm *via* flow rate controllers (60 L h^{-1}). As the CO_2-adjusted seawater was pumped out of the reservoir tank, it was replaced with natural ambient seawater using an automatic pump triggered by changes in volume within the tank. This gave a seawater residence time in the reservoir tank of just under an hour. Pumps in the reservoir tank prevented the formation of CO_2 or temperature gradients, and the ambient temperature was maintained due to the submersion of the reservoir tank and the mesocosms.

Each "oyster alone" treatment contained 8 oysters hugged along a rope tied at the top of the mesocosm to simulate farm conditions. The kelp alone treatment contained 1 blade with holdfasts (70–80 cm length) that were tied at the top of the mesocosm to keep the orientation vertical as in the farm environment. An integrated aquaculture treatment contained both oysters (n = 8) and kelp (n = 1) hung in the same way. The oyster number and the ratio of oyster and kelp were determined based on our modeling research which was published in Lin et al. (2020). The experiment started on the 14 May 2017 (experiment day 1) and continued until 12 June 2017.

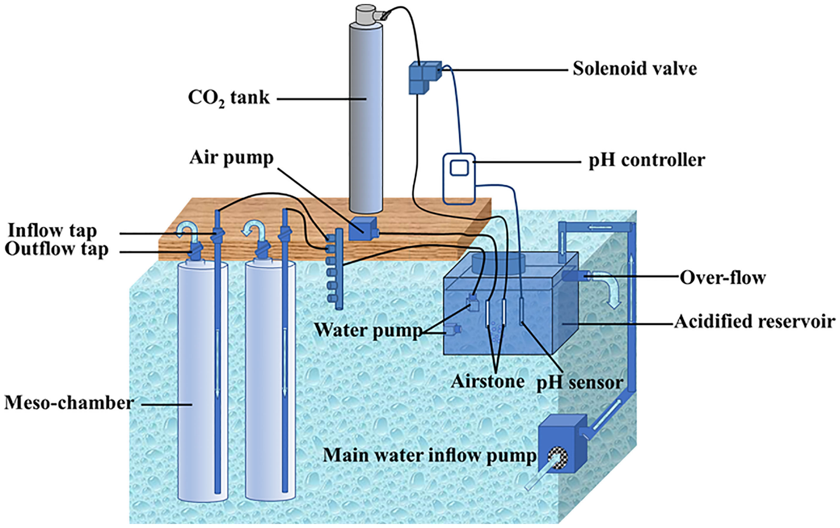

FIGURE 1 | Schematic diagram of the pH controlling and mesocosm system.

Monitoring of the Key Parameters of Seawater

During the 30-day experiment, seawater temperature, salinity, and dissolved oxygen concentration (DO) in each mesocosm were measured three times a day. The pH in the reservoir tank was also measured three times a day, and the pH in the inflow and outflow of each mesocosm was measured only at 12:00 daily. Seawater temperature, salinity, and dissolved oxygen concentration (DO) were measured using a Vernier's LabQuest® 2 (Vernier Software & Technology, USA) equipped with the corresponding probes. The pH level was measured using a commercial combination electrode (Ross type, Orion) calibrated daily using a 3-point calibration procedure on the U.S. National Bureau of Standards (NBS) scale with a precision of ± 0.001 pH units. The concentration of chlorophyll-a in the ambient seawater was continuously monitored using the ACLW-RS chlorophyll turbidity temperature sensor (ALEC Electronics Co., Ltd., Japan) with a precision of ±0.1 µg L^{-1}. Total alkalinity (A_T) was analysed weekly *via* a Metrohm 848 Titrino plus automatic titrator (Metrohm, USA) on 100 ml of GF/F filtered seawater with an accuracy of ±5 mmol L^{-1}. Carbonate system parameters, such as total dissolved inorganic carbon, aqueous partial pressure of CO_2 (pCO_2), the $CaCO_3$ saturation state for calcite (Ω_{cal}), and aragonite (Ω_{arag}), were calculated from pH_{NBS} and A_T by the CO2SYS Package (Pierrot et al., 2006) using the dissociation constants of Mehrbach et al. (1973) and refit by Dickson and Millero (1987). No mortality of oysters was observed in both ambient and elevated pCO_2 treatments throughout the experiment.

Physiological Measurements

The physiological measurements of oysters under different treatments were performed on 12 June 2017 (experiment day 30) by flow-through system after Jiang et al. (2017). Feeding experiments were performed by transferring 9 labeled individual oysters (3 randomly selected from each of the 3 replicate mesocosms per treatment) to individual flow-through chambers which were continuously supplied with the same water as supplied to the respective treatment mesocosms. The flow rates of each chamber were adjusted to 180–200 ml min^{-1} and determined by simultaneously measuring the volume of water collected from each outflow. Three identical chambers without oysters served as the control. The oysters were left undisturbed in chambers for 1 h to resume feeding before sampling water from the outlet of the chambers. The particle number in each chamber was measured by PAMAS S4031GO particle counter equipped with a tube of 50 µm aperture and set to count all particles >2 µm diameter in a 0.5 ml sub-sample. Three replicate counts are made on each sample and the mean calculated. This measurement was repeated at 60 min intervals over a period of 3 h. Clearance rate (CR) is then calculated as follows:

$$\mathrm{CR}\left(\mathrm{L\,h^{-1}}\right) = \text{Flow rate}\left(\text{in } \mathrm{L\,h^{-1}}\right) \times (C_1 - C_0)/C_1$$

where C_1 is the particulate concentration in control chamber (particle number ml^{-1}), and C_0 is the particulate concentration from each experimental chamber (particle number ml^{-1}).

The absorption efficiency (AE) was determined using the following equation (Conover, 1966):

$$\mathrm{AE}(\%) = (\mathrm{F} - \mathrm{E})/[(1 - \mathrm{E}) \times \mathrm{F}]$$

Where F represents the ash-free dry weight: dry weight ratio of food, and E represents the ash-free dry weight: dry weight ratio of the feces. Approximately 4 L of food samples were taken from the seawater being supplied to each treatment, and 4 sub-samples were filtered with pre-ashed and weighted glass fiber filters of 1.2 µm (Whatman GF/C 47mm). These filters were rinsed with isotonic ammonium formate (3%) to remove salts and dried to a constant weight (~48 h) at 65°C and weighed (W_{60}), then ashed at 450°C for 6 h to get the ash-free dry weight W_{450}. The particulate organic matter concentration (POM, mg L^{-1}) is calculated by POM = (W_{60} − W_{450})/v. Each oyster was placed in the individual feces collector at the respective treatment conditions for ~24 h after the CR measurements. The feces collector (*Φ* ~10 cm) was made of the casing pipe equipped with an 80 µm mesh screen (after, Rastrick et al., 2018a). Feces were collected by a pipette and filtered with the organic content of the feces measured as described above.

The respiration rate (MO_2) of individual oysters was measured in ~200 ml stop-flow glass respirometers held in a water bath at the same ambient temperature as the mesocosms and supplied with the same amount of water. Twelve respirometers were run simultaneously, with three chambers without oysters being used as controls. The inflow of each respirometer came from corresponding treatments, and constant flow rates were maintained by a multichannel peristaltic pump. Thirty minutes were allowed for the oysters to recover from handling stress, open and resume pumping, then the flow of water was stopped and MO_2 was measured over the next 90 min. The oxygen concentration in each respirometer was not allowed to drop below 70% saturation throughout the experiment. The rate of decline in oxygen saturation in each respirometer was measured by a calibrated optical oxygen probe connected to a Vernier's LabQuest® 2 (Vernier Software & Technology, USA). The initial and final oxygen concentrations in each respirometer were measured. The respiration rate (MO_2) was then calculated using the following equation:

$$\dot{\mathrm{M}}\mathrm{O_2}\left(\mu\mathrm{mol\ O_2h^{-1}}\right) = \left[\mathrm{C_{(t0)}} - \mathrm{C_{(t1)}}\right] \times \mathrm{V_r} \times 60/(\mathrm{t_1} - \mathrm{t_0})$$

Where t_0 and t_1 represent the initial and finish times (min) of the measurement period, C_t represents the concentration of oxygen in the water (µmol O_2 L^{-1}) at time t; V_r represents the volume of the respirometer minus the animal.

After MO_2 measurement, the ammonia excretion rate (ER) of the same group of oysters was measured. Water samples were taken from each respirometer, filtered by 0.45 µm cellulose acetate membrane filters, and then analysed for ammonia using the phenol-hypochlorite method. ER was calculated using the following equation:

$$\mathrm{ER}\left(\mu\mathrm{mol\ NH_4 - N\ h^{-1}}\right) = [C_{test} - C_{control}] \times (V_r/\ 1000) \times\ 1/t$$

Where C_{test} and $C_{control}$ represent the ammonia concentration in the sample and control, respectively, V_r represents the volume of the respirometer minus the animal; t is the exposure time.

After all the physiological measurements, the oyster tissues were dissected from the shell and dried to a constant weight at 65°C. The CR, MO_2, and ER were then corrected to a 'standard body size' of 1 g of dry weight mass-specific rates using the following equation: $Y_s = (W_s/W_e)^b \times Y_e$, where Y_s is the physiological rate for an animal of standard weight, W_s is the standard weight (1 g), W_e is the observed weight of the animal (g), Y_e is the uncorrected (measured) physiological rate, and b is the weight exponent for the physiological rate function (b = 0.67). Each physiological rate was then converted into energy equivalents (J h^{-1}) and used to calculate the energy available for scope for growth (SfG). Values for MO_2 were transformed into J h^{-1} using a conversion factor of 0.456 J μmol^{-1} O_2 (Gnaiger, 1983), ER was converted to J h^{-1} using the conversion factor: 1 μmol NH_4-N h^{-1} = 0.349 J h^{-1}. SfG (J h^{-1} g^{-1}) was calculated by SfG = A_b – R – U, where A_b is the total absorbed energy (J h^{-1} g^{-1}), R is the energy lost in respiration (J h^{-1} g^{-1}), and U is the energy lost in ammonia excretion (J h^{-1} g^{-1}). A_b = CR (L g^{-1} h^{-1}) × (mg POM L^{-1}) × AE (%). The POM concentration was converted to joules using a conversion factor of 23 J mg^{-1} ash-free dry weight (Widdows and Johnson, 1988).

Specific Growth Rate

The wet weight-specific growth rate (SGR, % d^{-1}) of each oyster was measured as follows: SGR = $(\ln(W_t/W_0)/t) \times 100\%$, where W_t and W_0 are the wet weight (g) of the oyster at time t and at time 0, respectively, and t is the sampling time (d).

Data Analysis

Statistical analyses were conducted using the software SPSS 22.0 for Windows. The general linear mixed model (GLMM) was used to test the effects of culture treatments (oyster alone and integrated aquaculture) or $p\mathrm{CO}_2$ treatments (ambient, ~500 μatm and elevated, ~900 μatm) on physiological parameters with a mesocosm as a random variable nested within fixed factors (culture or $p\mathrm{CO}_2$ treatments). This design considers that 3 replicate mesocosms in each treatment were supplied with the same seawater, and therefore, mesocosms may not be considered true replicates. Differences between culture or $p\mathrm{CO}_2$ treatments were further tested by *F*-tests with a significance threshold of α = 0.05. All data are represented as means ± SD.

RESULTS

Seawater Carbonate Chemistry

The daily variations in temperature, salinity, and pH during the experiment are shown in **Figure 2**. The ambient temperature and salinity were 19.08 ± 1.27°C and 32.86 ± 0.23, respectively.

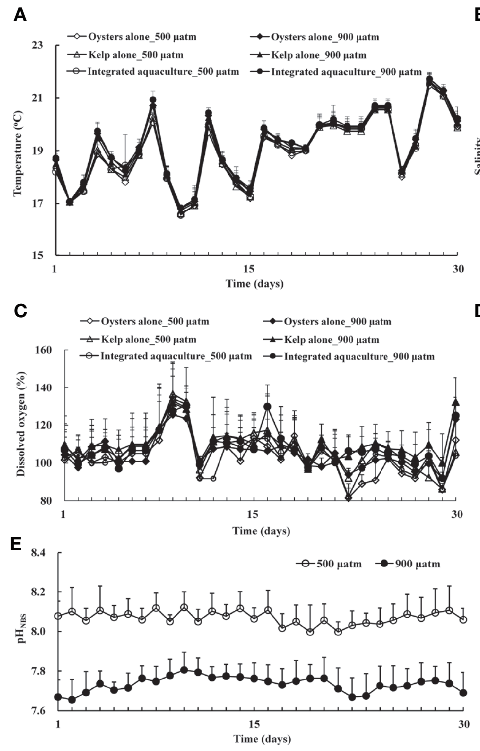

FIGURE 2 | Daily variations of environmental conditions during the 30-day exposure from 14 May to 12 June 2017. **(A)** Temperature; **(B)** salinity; **(C)** dissolved oxygen; **(D)** chlorophyll a in the ambient seawater; and **(E)** $\mathrm{pH_{NBS}}$ in the inflow of experimental units across the treatments. Means ± SD are shown.

The controlled injection of CO_2 separates the treatments successfully (pH_{NBS} ~7.83 in elevated pCO_2 treatments and 8.07 ± 0.03 in the ambient treatment) and keeps them relatively stable during the 30 days of exposure. Seawater chemistry variables over the 30-day experimental period for each pCO_2 treatment are summarized in **Table 1**. There was no significant difference in temperature, salinity, chlorophyll a, DIC, Ω_{cal}, Ω_{arag}, and HCO_3^- between treatments (P >0.05). The saturation percentage of dissolved oxygen in the exposure containers consistently exceeded 100% and was similar across treatments during the experiment. There was a significant difference between pH_{NBS} and pCO_2 between treatments (P <0.05).

The average change in daytime pH_{NBS} between the inflow and outflow of the mesocosms for the different treatments during the 30 days of exposure is shown in **Figure 3**. Results showed that the seaweed alone treatment increased the daytime pH_{NBS} by ~0.08 units, while oyster alone decreased ~0.05 units compared to the inflow under ambient conditions. The magnitude of change in daytime pH_{NBS} was significantly greater in kelp alone treatment under elevated pCO_2 condition than the ambient pCO_2 condition. The kelp alone treatment increased daytime pH_{NBS} by ~0.11 units, while the oyster and kelp integrated aquaculture treatment increased the daytime pH_{NBS} by ~0.06 units.

Clearance Rate and Absorption Efficiency

A significant effect of elevated pCO_2 on the clearance rate of oysters was observed. After 30 days of exposure, the clearance rate of oysters was significantly reduced in the elevated pCO_2 treatments and in the absence of kelp, as compared with ambient pCO_2 treatments (P <0.05) (**Figure 4A**). The presence of kelp in elevated pCO_2 treatment increased the clearance rate of oysters compared with oysters alone treated at the same elevated pCO_2 level (P <0.01). Additionally, no significant difference in CR was found between oysters incubated with seaweed at elevated pCO_2 and oysters incubated at ambient pCO_2 levels (P >0.05).

Absorption efficiency varied between 41 and 72% and was not affected significantly by elevated pCO_2 during the experiment. The absorption efficiency was lower in the oyster alone treatment at elevated pCO_2 treatment but showed no significant difference from the integrated aquaculture treatment (P >0.05) (**Figure 4B**).

TABLE 1 | Seawater chemistry variables over the 30 days experimental period for each pCO_2 treatment.

	Ambient pCO_2	Elevated pCO_2
Measured		
Temperature (°C)	19.00 ± 1.26	19.12 ± 1.25
Salinity	32.83 ± 0.24	32.87 ± 0.23
Chlorophyll a	2.69 ± 1.30	2.89 ± 1.09
pH_{NBS}	8.07 ± 0.03^a	7.83 ± 0.04^b
A_T (µmol kg^{-1})	2,201 ± 42	2,150 ± 76
Calculated		
pCO_2 (µatm)	514 ± 18^a	927 ± 57^b
DIC (µmol kg^{-1})	2,017 ± 43	2,066 ± 78
Ω_{cal}	3.27 ± 0.08	1.97 ± 0.07
Ω_{arag}	2.12 ± 0.05	1.28 ± 0.05
HCO_3^- (µmol kg^{-1})	1,865 ± 38	1,946 ± 72

A_T, total alkalinity; pCO_2, aqueous partial pressure of CO_2; DIC, dissolved inorganic carbon; Ω_{cal}, the $CaCO_3$ saturation state for aragonite; Ω_{arag}, the $CaCO_3$ saturation state for aragonite. Means ± SD are presented. Different letters indicate significant variation between treatments (P < 0.05).

Respiration Rate and Ammonia Excretion Rate

After 30 days of exposure, the respiration rate of oysters was significantly higher in the absence of kelp at elevated pCO_2 compared with oysters in ambient pCO_2 treatments (P <0.05). The presence of kelp in elevated pCO_2 treatment lowered the respiration rate and ammonia excretion rate of oysters compared to ambient conditions, but no significant difference was found (P >0.05). Compared with all other treatments, the respiration rate and ammonia excretion rate were significantly increased in the oyster alone treatment (P <0.05) (**Figure 5**).

Scope for Growth and Specific Growth Rate

Elevated pCO_2 exposure significantly reduced the SfG of *C. gigas* when unintegrated with kelp (**Figure 6A**). Compared to other treatments, SfG was negative (−9.45 J g^{-1} h^{-1}) in the elevated pCO_2 treatment in the absence of kelp (P <0.01). However, in the elevated pCO_2 treatment in the presence of kelp, SfG (35.82 J g^{-1} h^{-1}) was not significantly different to that of oysters in ambient pCO_2 treatments (P >0.05). Moreover, after 30 days of exposure, the wet weight-specific growth rates of *C. gigas* were also observed to be dramatically reduced in the oyster alone treatment compared with the integrated aquaculture treatment at elevated pCO_2 levels (**Figure 6B**).

DISCUSSION

The field mesocosms used in this study followed fluctuations in the natural environment, with CO_2 controlled to give two natural but distinct treatments, maintaining a difference of about 0.25 pH units over time (**Figure 2**). Using this novel approach, our results indicate that medium-term elevated pCO_2 exposure reduces the CR of Pacific oyster, *C. gigas*, while increasing the MO_2 and ER, reducing energy available for growth. Importantly, the presence of kelp, *S. japonica*, in the mesocosms mitigates this negative physiological impact of OA on oysters.

CR is generally considered the most sensitive parameter for the feeding activity and energy acquisition of bivalves. In this study, elevated pCO_2 induced a reduction in CR, limiting the capacity of energy acquisition from food sources. Zhao et al. (2017) and Xu et al. (2016) also demonstrated reductions of CR by the blood clam *Tegillarca granosa* and Manila clam *R. philippinarum* under similar acidified conditions. The gills are an important organ serving both a filter-feeding and respiration function in bivalves. The accumulation of GABA in the gill under elevated pCO_2 conditions may be a potential explanation for the suppression of feeding activity (Jiang et al., 2019). However, Liu and He (2012) reported that CR of pearl oyster *Pinctada fucata* increased at extreme pH reductions of up to 0.7 units, and Sui et al. (2016) found that the physiological energetics of thick shell

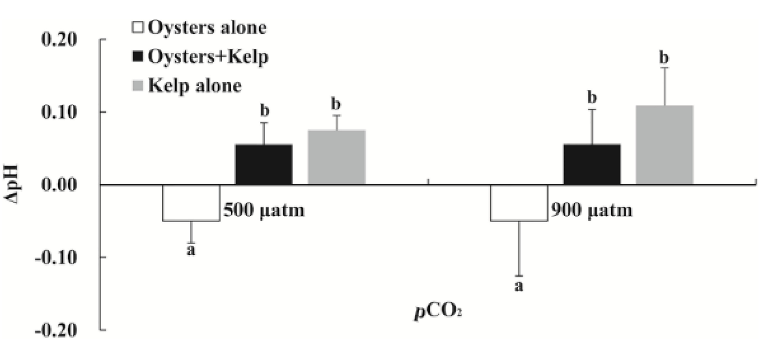

FIGURE 3 | Average change in daytime pH_{NBS} in the inflow and outflow of experimental units across the treatments during the 30 days. Means ± SD are presented. Different letters indicate significant variation between culture treatments ($P < 0.05$).

mussel *Mytilus coruscus* in terms of feeding, absorption and ultimately the energy available for growth remained unchanged by low pH conditions, perhaps due to the species-specific responses or the different experimental methods.

Here, AE was also not affected by acidified conditions, indicating the stable function of the digestive system, at least under the specific conditions and exposure time of this study. However, changes in CR, combined with increased MO_2 and ER in response to elevated pCO_2, resulted in a significant deficit in energy supply for *C. gigas* and ultimately less energy available for growth. For the mariculture industry, the significant reduction in energy available for growth (SfG) and actual growth in *C. gigas* under elevated pCO_2 represents a potential challenge to oyster farming operations, which could lead to economic loss. Particularly during the season of this study, when spat is first introduced to the farm environment and when most growth occurs.

The wide variety of species and exposure-dependent responses reported for bivalves to elevated pCO_2 (Navarro et al., 2013; Sui et al., 2016; Wang et al., 2016) may, in part, be due to differences in methodologies (e.g., measurement of CR *via* static or flow-through systems), which makes it difficult to compare results between studies. Most studies to date are also laboratory-based experiments, making it difficult to scale responses to the ecosystem level. Given the complexity of natural environments (such as food source and availability, daily fluctuation of environmental factors, etc.), particularly in coastal areas where bivalve aquaculture occurs, some recent studies, as here, have used field mesocosms (Jiang et al., 2019; Jiang et al., 2021) to advance the use of field experiments and improve the ecological relevance of data collected (e.g., Rastrick et al., 2018b).

Our results indicated that the presence of *S. japonica* under farm conditions helped to mitigate the negative effects of elevated pCO_2 on *C. gigas*, at least at the mesocosm scale. Being highly effective autotrophic, *S. japonica* use dissolved CO_2 in the seawater as the primary carbon source for photosynthesis. The rate of *S. japonica* photosynthetic carbon fixation was reported to 30 $\mu mol\ g^{-1}\ h^{-1}$ (Han, 2013; Xu et al., 2016). Consequently, it may benefit from a future elevation in pCO_2. For example, Xu et al. (2018) reported that projected pCO_2 (1,000 µatm in 2,100 under RCP 8.5) may increase the growth of *S. japonica*. The increased photosynthesis rates of *S. japonica* improve the growing conditions for nearby *C. gigas* by removing sufficient amounts of CO_2, reducing local seawater acidification, increasing pH, and providing dissolved oxygen at the local scale. Giving the CO_2 released from the calcification and respiration processes of bivalves into account (Chauvaud et al., 2003; Mistri and Munari, 2013), *S. japonica* may also alleviate the potential self-

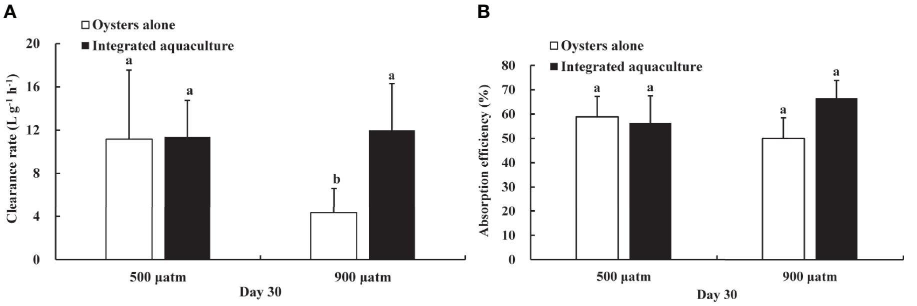

FIGURE 4 | Clearance rate **(A)** and absorption efficiency **(B)** of *C. gigas* exposed to different treatments for 30 days. Means ± SD are shown. Different letters indicate significant variation between treatments ($P < 0.05$).

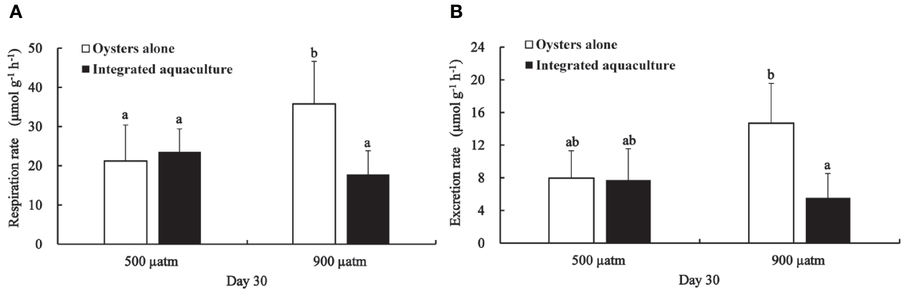

FIGURE 5 | Respiration rate **(A)** and ammonia excretion rate **(B)** of *C. gigas* exposed to different treatments for 30 days. Means ± SD are shown. Different letters indicate significant variation between treatments ($P < 0.05$).

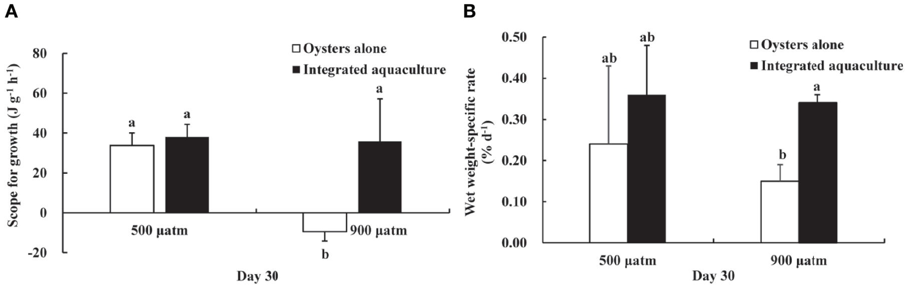

FIGURE 6 | Scope for growth **(A)** and wet weight-specific rate **(B)** of *C. gigas* exposed to different treatments for 30 days. Means ± SD are shown. Different letters indicate significant variation between treatments ($P < 0.05$).

acidification risk caused by *C. gigas* itself in smaller or more enclosed aquaculture settings. Here, *S. japonica* increased the mean daytime pH of the seawater by ~0.11 units in the elevated $p\mathrm{CO}_2$ treatment (**Figure 3**). Such biogenic pH fluctuations are important to the function of bivalves. Wahl et al. (2018) demonstrated that the blue mussel *M. edulis* can take advantage of these fluctuations by shifting most of their costly physiological activities to the daytime when the surrounding chemical conditions are more favorable. Microprofiling studies have shown that kelp *E. radiata* can modify the chemical environment in a micro-zone called the diffusive boundary layer (DBL), and DBL microenvironments at the blade surface may create potential refuges from OA for calcifying epifauna (Noisette and Hurd, 2018).

By using a novel *in situ* field mesocosm approach, this study demonstrates that elevated $p\mathrm{CO}_2$ can negatively affect the physiological energetics of *C. gigas*. Exposure to elevated $p\mathrm{CO}_2$ significantly reduced CR while increasing MO_2 and ER, thereby reducing the energy allocated for reproduction and growth, presumably affecting production. However, similar energetic responses between *C. gigas* incubated at ambient CO_2 levels and those incubated at elevated CO_2 levels in the presence of *S. japonica* indicate that in integrated aquaculture systems, *S. japonica* may benefit the production of *C. gigas* by, in part, mitigating the negative physiological impact of elevated $p\mathrm{CO}_2$. However, further *in situ* investigations are needed to elucidate the mutual benefit mechanisms and quantify context-dependency, particularly at larger farm scales.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

ZJ: Conceptualization, methodology, writing—original draft, writing—review & editing, and funding acquisition. WJ: Investigation, data curation, formal analysis, writing—original draft, and writing—review & editing. SR: Conceptualization, methodology, investigation, and writing–review & editing. XW, JinF, MD, and YG: Investigation and formal analysis. ØS and JiaF: Supervision, resources, funding acquisition, and writing—review & editing. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

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Increased Biodiversity Associated With Abandoned Benthic Oyster Farms Highlight Ecosystem Benefits of Both Oyster Reefs and Traditional Aquaculture

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Oyster reefs are structurally complex habitats which are increasingly recognized for their importance in estuarine systems. With over 85% of oyster reefs lost to human activities globally, there is increasing interest in aquaculture to not only meet the growing need for food worldwide, but also enhance ecological functions and services. Prime among these services is the provision of habitat for marine biodiversity. We assessed the biodiversity associated with an abandoned benthic oyster farm to investigate the potential ecological benefits of benthic farming techniques used in traditional oyster farms. In addition, we made note of any invasive species which may use these abandoned structures as habitat. The macrobenthic assemblage in the oyster habitat formed by the farm was different from the surrounding mudflats, containing 61 and 26 species, respectively. Density of invertebrates was similar in both habitats (~ 5,777 ± 981 indiv·m^{-2}), but biomass in the oyster farm over ten times greater than the mudflat substrate, which had a fauna dominated by small polychaetes (<5 mm long). Molluscs and crustaceans were especially abundant in the oyster habitat and contributed to 90% of the enhanced biomass associated with oyster habitat (excluding oyster biomass). Only one invasive species was documented, the mussel *Xenostrobus secures*, but at densities lower than nearby artificial substrates (e.g., concrete shorelines). Oyster development on this abandoned farm appears to be self-sustaining, provide habitat and trophic support for associated benthic macrofauna. In addition to supporting higher biodiversity and biomass of associated species, traditional oyster aquaculture could improve the restoration potential of oyster reefs by supporting broodstock of native oysters, thus maintaining larval supply.

Keywords: shellfish aquaculture, biodiversity enhancement, habitat provisioning, oyster reef restoration, restorative aquaculture

INTRODUCTION

Coastal environments have suffered from centuries of overexploitation, habitat degradation and pollution, with estuaries being the most degraded of marine ecosystems (Jackson et al., 2001). Despite declines in ecosystem functioning and biodiversity across marine environments globally, different habitats often receive strikingly disproportionate media attention and research effort due to the human propensity to focus on charismatic ecosystems such as coral reefs (Duarte et al., 2008). As a result, the loss of less charismatic but ecologically important coastal habitats continues. Oyster reefs were historically common and extensive in estuarine habitats globally but have experienced an 85% loss over recent centuries (Beck et al., 2011; Zu Ermgassen et al., 2012). Natural oyster reefs form complex three-dimensional structures and create habitats that maintain secondary production, trophic transfer, and coastal biodiversity (Grabowski and Powers, 2004; Tolley and Volety, 2005; Walles et al., 2016). The ecosystem goods and services provided by oyster reefs underpin the health and sustainability of subtropical and temperate estuarine waters (reviewed by Smaal et al., 2019). Therefore, loss at such a global scale significantly impacts not only the function of estuarine ecosystems but also the quality of services provided by oyster reefs to human communities. In addition to food provisioning through fisheries, oyster reefs perform a wide range of supporting, regulating, and cultural ecosystem services, including water filtration, nutrient cycling (Kellogg et al., 2014), shoreline protection, (Ysebaert et al., 2019) fish production (Zu Ermgassen et al., 2016) and tourism (Rusher, 2004). These services are primarily delivered in the nearshore and estuarine areas most impacted by human activities.

Increasing efforts in oyster reef conservation and restoration has meant that attention has largely focused on the role of mature oyster reefs in coastal systems. In contrast, the potential role of aquaculture in providing ecosystem functions and services within an integrated seascape has received relatively little attention (Theuerkauf et al., 2019; Gentry et al., 2020). Notably, benthic oyster aquaculture may provide some of the benefits of oyster reefs to coastal ecosystems (e.g., Coen et al., 2007; Kellogg et al., 2014; Humphries et al., 2016). Yet substantial knowledge gaps on the ecological benefits provided by aquaculture have hampered the development of restorative aquaculture in Asian coastal cities. For example, China is the largest global producer of oysters, which produced 5.14 million tonnes of oysters and contributed about 86% of global oyster aquaculture by weight in 2018 (FAO, 2021), yet there is no assessment of the ecological value of this oyster aquaculture. Given that oyster production in China has continued to increase at ~5% per annum from 1950 to 2016, compared to ~2% per annum for the rest of the world (Botta et al., 2020), there is huge incentive to understand how these oyster farms interact with natural systems, and provide guidance to direct future production growth to not only meet our consumption demand, but also to enhance ecosystem services in the most practical and sustainable way possible.

Hong Kong is a coastal megacity located at the lower basin of Pearl River Delta (PRD) in southern China and has a subtropical monsoonal climate which creates estuarine conditions that are particularly suitable for natural oyster reefs and aquaculture. The full historical extent of natural oyster reefs in Hong Kong is unknown, but archaeological records provide evidence of a natural community of oysters supporting the once-flourishing shell-based lime industry with nearly 30 kilns spread across the territory during the Tang Dynasty (618 to 907 AD) (Meacham 1979). It is evident that historically extensive harvesting and recent, rapid coastal development in southern China (Fang et al., 2007) have caused extensive loss of oyster reefs, leaving only sparse, degraded natural oyster beds in Hong Kong (Lau et al., 2020). Changes in land use practices, including extensive deforestation and coastal reclamation for agriculture, have dramatically increased sediment transport and deposition into the PRD and Greater Bay Area over past millennium (Weng, 2000; Weng, 2007), with increased sedimentation reducing the extent and persistence of sessile hard structured habitats such as oyster reefs (Colden and Lipcius, 2015). Oyster cultivation was first practiced in the PRD about 1,000 years ago during the Song Dynasty (Cheung, 2019) and has a continuous record of cultivation in the region even as harvest of natural oyster reefs declined.

The Hong Kong oyster *Magallana hongkongensis* has been cultivated on the mudflats of Deep Bay, northwest Hong Kong in the past 150–700 years for their meat and the extraction of lime from the shells (Morton and Wong, 1975). The traditional method of farming involved placing hard substrate such as rocks, concrete tiles, and posts on the soft mud to promote settlement of oyster larvae, a practice that is synonymous with the use of artificial substrate in oyster reef restoration. Farms are then managed until harvest, for example, young oysters could be lifted from a breeding ground and transferred to a more suitable growing or fattening grounds or concrete posts could be relocated to avoid sedimentation. One of the ancillary benefits of this method of benthic farming is that it provides hard substrate among soft sediment mudflats, allowing the aggregation of sessile and mobile benthic organisms. The only assessment of the fauna associated with oyster farms in Deep Bay found over 30 species inhabiting the traditional concrete posts (Morton and Morton, 1983). However, over the last few decades there has been a transition of oyster aquaculture in the region from benthic culture techniques to rope-suspended raft culture techniques introduced from Japan to enhance production (Botta et al., 2020). As a result, many traditional oyster farms are now left fallow and have been allowed to develop into more extensive habitats over several years. These fallow benthic farms are inherently different from natural oyster reefs which accumulate generations of oysters that settle, grow and form three-dimensional structures. The design and layout of substrate adopted in traditional oyster farms intentionally discourages the establishment of such complex structure. When abandoned or left fallow, however, these farms can form complex habitats as continuous recruitment and the formation of small oyster clusters suggests they could provide substrate and shelter for other species which enhance biodiversity of intertidal mudflats, even if not to the extent of natural oyster reefs. With the slow expansion of oyster reef restoration in the region, and as yet uncertain outcomes based on heavy harvest pressure (Lau et al., 2020), benthic oyster farms which are allowed to become fallow, but are still protected from recreational harvest, may provide some of the ecosystem benefits of oyster reefs which have previously been overlooked.

Most studies that have investigated fauna associated with aquaculture have focused on assessing mobile species that gather around fish farms (Gentry et al., 2020) meaning that there is limited information on the biodiversity benefits provided by bivalve aquaculture (e.g., D'Amours et al., 2008), especially oyster aquaculture which is closely associated with benthic habitats (e.g., soft-sediment shores). Yet, fallow benthic oyster farms may provide ecosystem functions and services akin to oyster reefs. Therefore, we investigated the macrobenthic biodiversity on an abandoned traditional oyster farm in Hong Kong. As oyster reefs are known to support greater density, biomass and diversity of resident macrobenthic invertebrates than unstructured mudflats (Shervette and Gelwick, 2008; Stunz et al., 2010; Humphries et al., 2011; Quan et al., 2013), we hypothesized that the resident benthic macroinvertebrate communities in the abandoned oyster farm would be more diverse than, and distinct from, the adjacent mudflat habitats. As a result, we aim to provide the first insights into the biodiversity value of the structured habitat of benthic aquaculture production, expand the discussion around the benefits of traditional aquaculture and its role in producing ecosystem services, and inform the potential to enhance the ecosystem services associated with traditional aquaculture practices.

MATERIALS AND METHODS

Site Description

Biodiversity surveys were conducted at an abandoned oyster farm ("oyster habitat") and adjacent mudflats ("control") at Pak Nai (**Figure 1**, 22° 26' N 113° 56' E), Deep Bay, Hong Kong. Deep Bay is subjected to a wide range of seasonal salinity (10.5 - 28.7‰) and temperature (17.8 - 31.1°C) (EPD, 2018). Contrary to its name, Deep Bay is shallow, with an average water depth of 2.9 m and a mean tidal range of 1.4 m (Young and Melville, 1993). As a result, extensive mudflat habitats are exposed during low tide, including the study site.

The site is composed of multiple patchy oyster habitats, formed by the remnant oyster populations from the abandoned farm, with mudflats interspersed in between. The oyster habitat is created by an abandoned benthic oyster farm which is characterized by lines of concrete posts with oysters attached, each spaced 30 – 50 cm apart (**Figure 2** Traditionally, the short concrete posts (5 cm × 5 cm × 60 cm) were embedded in the sediment prior to the initial settlement peak (April in Deep Bay; Lau et al., 2020), leaving ~ 20 cm above the sediment to act as substrate for the natural settlement of oyster spat which were then allowed to grow for 1 – 2 years before harvest. This benthic method of oyster cultivation creates a unique habitat that is absent in modern suspended (e.g., line, basket or raft) oyster aquaculture. At Pak Nai, the farm areas have been left fallow – they have effectively abandoned but are protected from harvest by being within an aquaculture lease area. Without regular maintenance or harvesting, multiple generations of oysters have overgrown the concrete posts and become a habitat that share some resemblance to dispersed restored oyster beds. While farm abandonment was between 5–7 years ago, the oyster

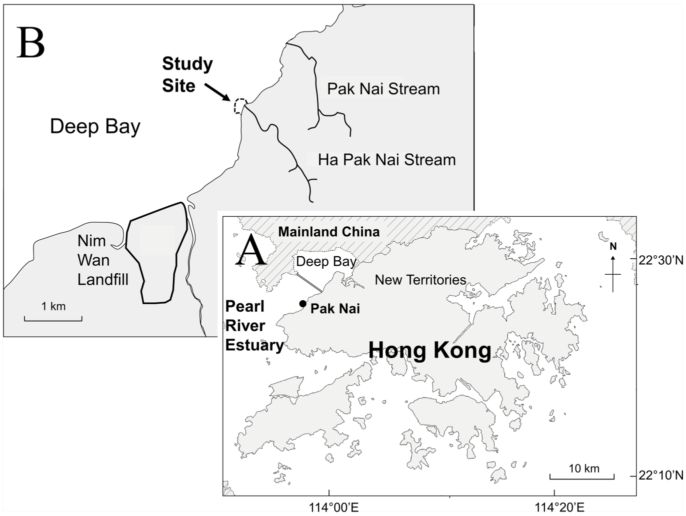

FIGURE 1 | **(A)** A map of Hong Kong showing the location of Pak Nai in Deep Bay, north-western Hong Kong. **(B)** Close-up map showing the location of the study site in Pak Nai. Black lines represent major streams in the study area.

FIGURE 2 | (Left) Regular spatial arrangement of the concrete posts used in traditional oyster culture in the Pearl River Delta and southern China, and (right) oysters growing on concrete posts forming a unique habitat at the abandoned farm of the study site. Note the more modern and intensively managed rafts now used for oyster aquaculture in the background of the left panel.

habitats in this study have yet to fuse together and form the extensive three-dimensional structures found in natural reefs. However, this site remains as potentially the best indication of the diversity of macrobenthic communities associated with historically natural oyster reef in Hong Kong due to the protection from recreational harvesting; even though the aquaculture lease is no longer managed or harvested, it still has legal protection and the absence of intervention over several years has allowed these oyster habitats to establish.

Sampling Methods

Biodiversity surveys took place in November 2018 and May 2019 during daylight low-tide period (<1.0 m Chart Datum) to sample both wet (summer) and dry (winter) months. To fully assess macrofauna associated with the oyster farm, both quadrat sampling and sediment core sampling were used to collect epifauna and infauna, respectively.

A total of six samples (n = 6) were haphazardly located across the abandoned farm using 0.25 m^2 quadrats (0.5 m × 0.5 m) and adjacent, interspersed mudflat (controls). This size of quadrat was determined appropriate for moderate oyster densities of 100 to 500 oyster m^2 (Baggett et al., 2014; Baggett et al., 2015). First, all oysters within the sampled quadrat were collected and recorded for their abundance and shell length to obtain size-frequency distributions. Shell length (the distance from the umbo to the distal margin of the shell) were measured to the nearest 0.1 mm using dial callipers.

All fauna were then removed from the live and dead oyster shells within each quadrat and were collected for later identification and enumeration in the laboratory. Sediment core samples were then taken to a depth of 10 cm below the substrate surface using a 10 cm diameter PVC pipe core sampler. The same sampling protocol was used for nearby mudflats as control (n = 6), but as there were no oysters or structured habitat in any of these samples, no epifauna were collected from the quadrats (i.e. all fauna were in the sediment cores). All samples were washed and sieved through a 500 µm stainless steel mesh sieves on the day of collection and preserved in 70% ethanol until processed in the laboratory. All samples were further sorted in the laboratory under dissecting microscope for macrobenthos before final enumeration and identification to the lowest possible taxonomic level. Samples with substantial volume of detritus and/or sediment were subsampled at fixed fraction (50%) prior to sorting and identification of organisms, yielding cost-effective estimate of species richness and abundance (Barbour and Gerritsen, 1996; Cox et al., 2017).

Statistical Analysis

Data produced from the samples were abundance (number of individuals), biomass, density (number of individuals per unit surface area), species richness (number of species), Shannon diversity index (Shannon, 1948) and J-evenness.

The effect of habitat type and season on macrobenthic assemblages (community composition) was examined with permutational multivariate analysis of variance (PERMANOVA) using Bray–Curtis dissimilarity. Non-parametric multidimensional scaling (nMDS) was then employed to visualize dissimilarities between habitats based on their corresponding benthic assemblages. Abundance data were square-root transformed prior to analysis to reduce the influence of the numerically dominant species (Clarke and Warwick, 2001).

Following nMDS, the means and standard deviation of species density, richness, Shannon diversity index and evenness were determined for each habitat type and season. All parameters were tested by two-way ANOVA with season (wet vs. dry) and habitat type (oyster vs. mudflat control) and their interactions as treatment variables for potential seasonal and spatial differences. The effect of season on oyster density and shell lengths were tested using one-way ANOVA because there were no oysters in control mudflats. Data sets with significant difference between habitat types which did not show homoscedasticity were tested additionally by non-parametric Kruskal–Wallis test by rank.

Data on overall density for higher taxonomic groups of interest were also tested for differences using the same statistical methods (above), however the data are presented as pooled values only showing the effect of habitat for precise and comparative purposes.

All statistics were performed using R 3.6.3 (R Core Team, 2020), using *vegan* (v2.5-6; Oksanen et al., 2019) and *BiodiversityR* (v2.11-3; Kindt et al., 2005) packages *via* the R-Commander. All tests were assessed at the $\alpha = 0.05$ significance level.

RESULTS

Oyster Population in the Abandoned Farm

The abandoned farm allowed multiple generations of oysters to create relatively new but complex habitat. Oysters were young overall, with a mean length of 31.3 ± 0.8 mm and 26.8 ± 0.6 mm (mean ± SE) in the dry and wet seasons, respectively. Oyster shell length was greater in dry season than that of wet season (**Figure 3**, Kruskal–Wallis, $H_1 = 19.89$, $p < 0.001$), probably representing the older juveniles from the previous spawning season. There were very few individuals > 100 mm in length, with none > 150 mm, meaning that the population is mostly comprised of individuals less than ~3 years old. In contrast to size, density of oysters was similar between seasons (ANOVA, $F_{1,10} = 0.002$, $p > 0.05$), with the mean density being 329 ± 103 ind·m^{-2} and 336 ± 138 ind·m^{-2} (mean ± SE) in the dry and wet seasons, respectively.

Macrobenthic Fauna

Habitat-forming oysters (*Magallana* spp.) were excluded from the analysis of the associated microbenthic fauna as they formed the habitat and they were not present in the control mudflat plots. There was a distinct macrobenthic assemblage present in oyster habitat created by the abandoned oyster farm compared to mudflat (**Figure 4**). Habitat type had a large effect on community assemblages. Differences in the benthic community likely exist during wet and dry seasons but would require long-term monitoring to confirm (**Table 1**; p = 0.09). The separation of macrobenthic assemblages between habitat types is clear based on density data.

Pak Nai harbours a diverse macrobenthic community. A total of 6,112 individuals were recorded in the biodiversity surveys across both oyster habitats and mudflats, representing 70 taxa, in which 52 (74%) were uniquely found in the oyster habitats surveyed in this study (**Table S1**). The mean density of macrobenthic invertebrates in oyster habitat (5,777 ± 981 indiv·m^{-2}) and in mudflat (5,694 ± 1173 indiv·m^{-2}) were similar and are comparable to the density of invertebrates reported from both natural and restored oyster reefs in China, the USA, and France (**Table 2**).

Species richness (**Figure 5A**) and diversity (Shannon diversity index) were greater in oyster habitat than mudflats (**Table 3**). The maximum value of Shannon diversity index (2.73) was found in the oyster habitat during wet season, with the mean values being 1.85 and 1.35 in oyster habitat and mudflats, respectively. Biomass of fauna was up to 10 times greater on oyster habitats than mudflats (**Figure 5B**, Kruskal–Wallis, $H_1 = 10.83$, $p < 0.01$). Overall, season did not affect species richness or diversity in either habitat type (**Table 3**), though there was a trend towards greater faunal density in the dry season (**Figure 5C**; **Table 3**, p = 0.069). Polychaetes were the most numerically dominant taxa (up to 96%) and obscured abundance patterns in other taxa. When polychaetes are excluded, density of other taxa was greater in oyster habitat than on mudflats (**Figure 5D**, Kruskal–Wallis, $H_1 = 11.34$, $p < 0.001$). Differences in species evenness across seasons

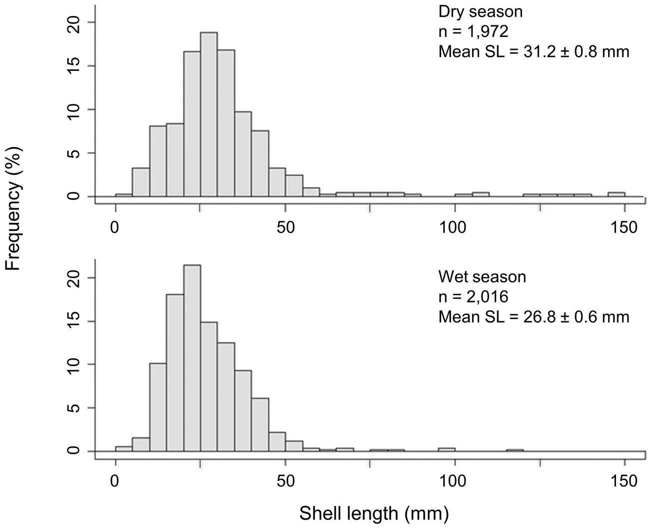

FIGURE 3 | Percentage frequency distributions of the shell length (SL) of oysters growing on the abandoned oyster farm in Deep Bay, Hong Kong, in both dry and wet season. (n = number of individuals sampled, mean ± SE).

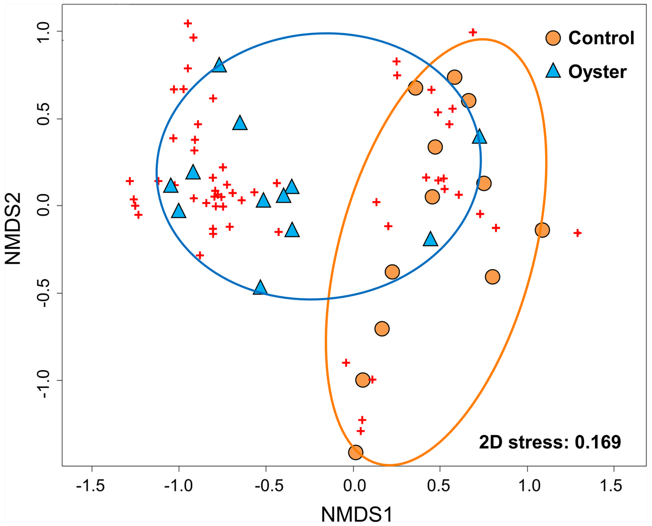

FIGURE 4 | Nonparametric multidimensional scaling (nMDS) ordination plot of dissimilarities between sampling sites based on Bray–Curtis distance metric of square-root transformed abundance of 70 invertebrate taxa in a total of 24 sampling units (n = 6 from two intertidal sites during two sampling times) in Pak Nai. Symbols represent the placement of the faunal communities (labelled as 'control' and 'oyster') and the individual species which comprise those communities (red crosses) within the multidimensional space.

TABLE 1 | Results of a two-factor PERMANOVA testing for the effects of Habitat (oyster farm vs. mudflat control) and Season (wet vs. dry) on macrobenthic assemblages (70 Taxa) from Pak Nai, based on square-root transformed relative abundances and Bray–Curtis dissimilarities.

Source	df	SS	MS	Pseudo F	p-value
Habitat	1	9719	9719	4.532	0.002
Season	1	3737	3737	1.742	0.090
Habitat × Season	1	1828	1828	0.853	0.552
Residual	20	42894	2144		
Total	23	58179			

TABLE 2 | Comparisons of species richness, biomass, and density of macrobenthos inhabiting various natural or restored oyster reefs and the abandoned benthic oyster farm in this study.

		Benthic assemblage parameters			
Location	Reef description (age)	Species richness	Biomass (g/m^{-2})	Density (indiv./m^{-2})	Citation
Yangtze River estuary, China	Artificially restored, intertidal (5)	45	46.7 ± 12.8	765 ± 241	Quan et al., 2012a
Jiangsu, China	Natural, intertidal	66	499.6 ± 35.4	2830 ± 182	Quan et al., 2012b
South Carolina, USA	Natural, intertidal	37	N/A	2476 – 4077	Dame, 1979
Bay of Brest, France	Natural, intertidal	73	30.09 ± 4.84	7409 ± 3860	Lejart and Hily, 2011
Deep Bay, Hong Kong SAR	Abandoned intertidal oyster farm (5 – 7)	61	108.7 ± 28.6	5777 ± 981	Current study

N/A, Not assessed.

may be present but undetectable due to low sample sizes (**Table 3**, p = 0.16). In general, both oyster habitat and mudflat exhibited a moderately even community with mean values ranging from 0.61 to 0.75, respectively.

Three taxonomic groups accounted for the majority of the fauna found in oyster habitats; polychaetes (57%; 3,302 ± 556 ind·m^{-2}), molluscs (23% excluding oysters; 1324 ± 352 ind·m^{-2}) and crustaceans (19%; 1,098 ± 365 ind·m^{-2}). The remaining 1% were numerically rare taxa such as Sipuncula (*Phascolosoma agassizii*) and barnacles. The same three taxonomic groups were present for the mudflat controls, but polychaetes accounted for ~96% of individuals in the mudflat. All species encountered in

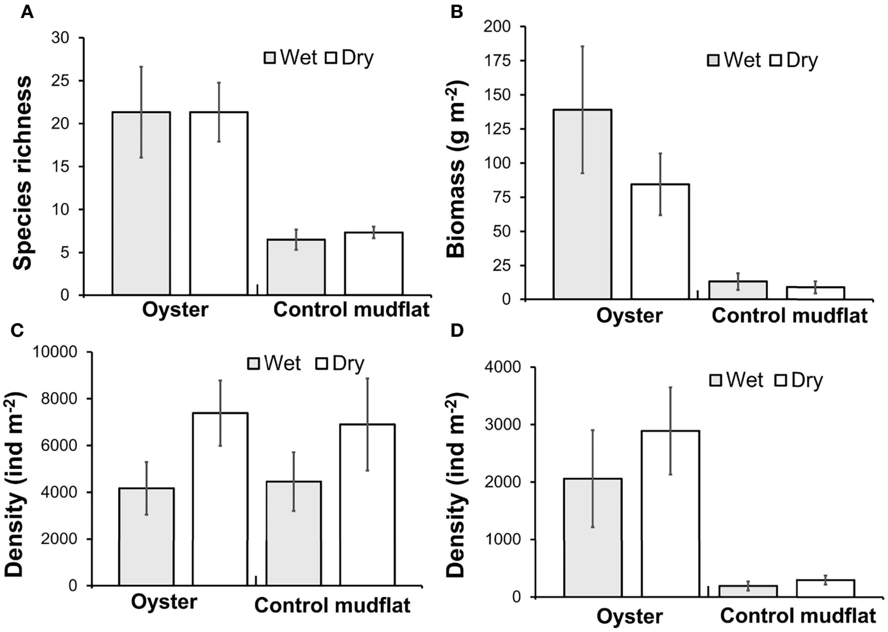

FIGURE 5 | Biodiversity survey for resident macrobenthic invertebrates conducted on oyster habitats and control mudflats in 2018 and 2019 in Pak Nai, Hong Kong. **(A)** Species richness; **(B)** Biomass (g·m^{-2}); **(C)** Species density (indiv·m^{-2}); and **(D)** Species density without polychaetes (indiv·m^{-2}) of the sampling sites among habitat types across wet and dry seasons. All values are means ± SE.

the surveys are known to either be native to Hong Kong, or occur within their natural Indo-Pacific range, except for the invasive mussel *Xenostrobus securis* which is native to Australia and was first recorded in the eastern waters of Hong Kong in 2010, from where it spread across Hong Kong (Morton and Leung, 2015). It is also worth noting that some fish (Blennidae) were occasionally observed among the oysters during surveys but were not encountered in the quadrats.

The density of the different taxonomic groups of interest varied between habitats and possibly differ among seasons (**Figure 6**; **Table 4**). The seasonal effects were on the margins of significance (**Table 4**, p = 0.07 to 0.1) potentially due to low sample sizes. Oyster habitats contained densities of molluscs and crustaceans one to two orders of magnitude greater than mudflats (**Figure 6**; **Table 4**). In contrast, the Sedentaria, a taxonomic group typified by sediment burrowing or tube-dwelling polychaete worms, were approximately twice as abundant in mudflats than oyster habitat (**Figure 6**, $F_{1,20}$ = 5.15, $p < 0.05$). But, at higher taxonomic rank, the density of Polychaeta was considered equally abundant in both habitats. The small invasive mussel *Xenostrobus securis* (mean shell length 7.8 ± 0.3 mm) only occurred in oyster habitat (882 ± 253 indiv·m^{-2}) and comprised approximately two-thirds of the total molluscan abundance, excluding oysters (Kruskal–Wallis, H_1 = 12.8, $p < 0.001$). This density is two orders of magnitude less than on concrete structures (e.g. protective seawalls) on which *X. securis* dominates in other parts of Hong Kong (20,900 - 76,000 indiv·m^{-2}; Morton and Leung, 2015) and an order of magnitude less than found on more modern suspended rope (raft) oyster aquaculture which is actively managed (up to 3,500 indiv·m^{-2}; Lau et al., 2018).

DISCUSSION

Benefits of Benthic Aquaculture for Biodiversity

Oyster reefs are well known to provide structured habitat for diverse assemblages of fauna (Meyer and Townsend, 2000; Tolley and Volety, 2005; Boudreaux et al., 2006; Shervette and Gelwick, 2008; Stunz et al., 2010). Here, we show that abandoned benthic oyster farms can provide habitat for a rich diversity of fauna and enhance the biodiversity of intertidal mudflats from which oyster reefs have been lost through anthropogenic activities. The macrobenthic assemblages in oyster habitats were distinct from that of the surrounding mudflat habitats. For instance, species richness of the benthic assemblages found in oyster habitat was substantially greater than unstructured mudflats, in particular for groups of epifauna such as crustaceans and molluscs (6 and 18 times greater, respectively). While both mudflat control and the oyster habitat had similar density of macroinvertebrates, the biomass of macrofauna on the mudflats was 9 - 10 times less than that of oyster habitats, given that 96% of individuals in the mudflat were small (2 – 5 mm) polychaetes found as infauna in the sediment matrix. These polychaetes were also present within the oyster habitat, while the other taxa were much more abundant, thus oyster habitats substantially increased biodiversity and biomass of organisms on the intertidal shore.

The only other biodiversity assessment of oyster farms within the region documented roughly half the number of species found in our study (Morton and Morton, 1983). That farm was, however, actively managed meaning that the benthic poles were cleaned of

TABLE 3 | Summary of two-way ANOVAs testing the effects of habitats and seasons on six benthic assemblage parameters.

Source	*df*	SS	MS	*F*	p-value
Shannon Diversity Index					
Habitat	1	1.47	1.47	6.5873	0.0184*
Season	1	0.08	0.08	0.3578	0.5564
Habitat × Season	1	0.05	0.05	0.2294	0.6372
Error	20	4.46	0.22		
Evenness					
Habitat	1	0.01	0.01	0.4377	0.5158
Season	1	0.05	0.05	2.1557	0.1576
Habitat × Season	1	0.003	0.003	0.1344	0.7178
Error	20	0.47	0.02		
Species Richness					
Habitat	1	1247.04	1247.04	20.0570	0.0023*
Season	1	1.04	1.04	0.0168	0.8983
Habitat × Season	1	1.04	1.04	0.0168	0.8983
Error	20	1243.5	62.18		
Biomass					
Habitat	1	60932	60932	14.9205	0.0009*
Season	1	5171	5171	1.2662	0.2738
Habitat × Season	1	3806	3806	0.9319	0.3459
Error	20	81675	4083		
Density					
Habitat	1	56664	56664	0.0044	0.9480
Season	1	48000924	48000924	3.6940	0.0689
Habitat × Season	1	897875	897875	0.0691	0.7954
Error	20	259889060	12994453		
Density excluding polychaetes					
Habitat	1	29827685	29827685	15.31	0.0007*
Season	1	1318141	1318141	0.68	0.4204
Habitat × Season	1	788673	788673	0.40	0.5318
Error	20	38964445	1948222.3		

** indicates significance terms.*

organisms other than large oysters at least annually and lacked the extra time that our abandoned farm had to develop greater structural complexity and the associated faunal community. Furthermore, the abundance of benthic species in the abandoned farm habitat is comparable to the established macrofaunal populations on remnant natural oyster reefs in the national marine park in Jiangsu Province, China, though a different species composition because they are in a different biogeographic region (Quan et al., 2012b). Accretion of oyster shells over longer periods is the single most unique component of natural oyster reefs, which are

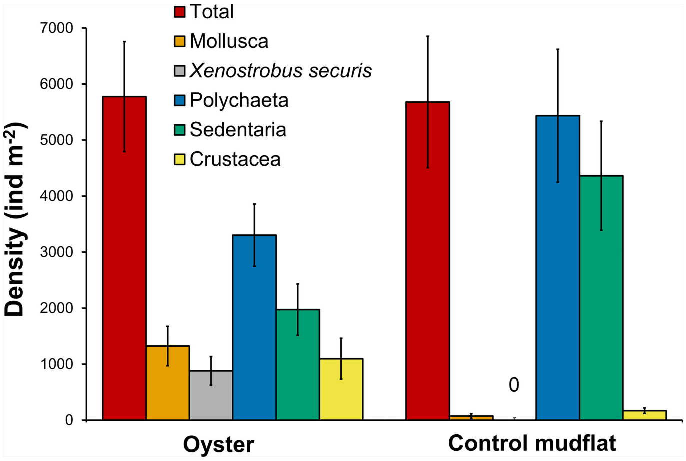

FIGURE 6 | The mean density of different taxonomic groups sampled on oyster habitats and control mudflats pooled across seasons. All values are means ± SE.

TABLE 4 | Summary of two-way ANOVAs testing for the effects of habitats and seasons on the density of individuals (m^{-2}) in different taxonomic groups.

Source	df	SS	MS	F	p-value
Mollusca					
Habitat	1	9370765	9370765	11.3751	0.0030*
Season	1	70086	70086	0.0851	0.7735
Habitat × Season	1	45255	45255	0.0549	0.8171
Residuals	20	16475873	823793		
Xenostrobis securis					
Habitat	1	4667544	4667544	11.0936	0.0033*
Season	1	26667	26667	0.0634	0.8038
Habitat × Season	1	26667	26667	0.0634	0.8038
Error	20	8414843	420742		
Polychaeta					
Habitat	1	27284224	27284224	2.8240	0.1084
Season	1	33410325	33410325	3.4581	0.0777
Habitat × Season	1	3539	3539	0.0004	0.9849
Error	20	193228266	9661413		
Sedentaria					
Habitat	1	34257805	34257805	5.1465	0.0345*
Season	1	19360972	19360972	2.9086	0.1036
Habitat × Season	1	417	417	0.0001	0.9938
Error	20	133131247	6656562		
Crustacea					
Habitat	1	5169918	5169918	7.3058	0.0137*
Season	1	2285046	2285046	3.2291	0.0875
Habitat × Season	1	1439040	1439040	2.0336	0.1693
Error	20	14152967	707648		

** indicates significance terms.*

formed by high-density shell aggregations that introduce high complexity into benthic ecosystems akin to coral reefs (Gutiérrez et al., 2003). Therefore, while abandoned benthic oyster farms do not provide the same level of complexity as natural oyster reefs, they illustrate the potential for regenerative aquaculture in enhancing species diversity, biomass and abundance of both oyster reef restoration and by using the habitat provided by an abandoned oyster farm.

The presence of structurally complex habitats has a positive relationship with increased macrobenthic density and species richness (Hosack et al., 2006; Humphries et al., 2011). Increased diversity associated with biogenic habitats is well known; the structure and complexity created by seagrass beds (Wells et al., 1985; Hosack et al., 2006; McSkimming et al., 2016), algal mats (Dean and Connell, 1987), kelp forests (Fowler-Walker and Connell, 2002), and empty bivalve shells (Tolley and Volety, 2005) are known to be inhabited by greater densities and diversity of marine invertebrates compared to unstructured habitats. Therefore, the presence of oyster reef structure *per se* is likely a dominant factor determining nekton assemblages (Humphries et al., 2011). While the density and configuration of habitat can determine species diversity (Goodsell and Connell, 2002; Russell et al., 2005), the provision of structure on previously structureless mudflats will cause a rapid increase in diversity and abundance of organisms. Therefore, although the abandoned oyster farm habitat in this study lacks the vertical relief and consolidated structures found in natural oyster reefs, basic structures such as shell valves and clusters formed by several generations of oysters have already increased habitat complexity, surface area and interstitial spaces, which differentiates this oyster habitat from structurally simple habitats like mudflats.

Increased Ecosystem Function

Not only do structurally complex habitats provide structure *per se*, but they also provide enhanced resources and trophic flows thus increasing the number of species that utilize a habitat (Hiwatari et al., 2002; Grabowski and Peterson, 2007). In addition to offering refugia for macrofauna, the presence of dense epibenthic invertebrates may indicate that the oyster habitats, formed by the remnant oyster populations from the abandoned farm, has the capability to provide trophic support as a valuable foraging resource for intermediate predators (Dame, 1979; Meyer and Townsend, 2000; Grabowski et al., 2005; Humphries et al., 2011; Hanke et al., 2017). For example, high densities of crustaceans, resident crabs and polychaetes can provide important sources of prey for fishes, gulls and terns, including the vulnerable Saunders's Gull (*Chroicocephalus saundersi*; BirdLife International, 2018) and the endangered Black-faced spoonbill (*Platalea minor*; BirdLife International, 2017), both of which are regularly recorded using Deep Bay as their wintering site. Indeed, wildlife taking advantage of open-system aquaculture operations is well-documented (Barrett et al., 2019), generally due to increased food availability either by direct feeding on the cultured species or indirect trophic subsidies from pelagic-benthic coupling due to filter feeding, transferring energy into the benthic environment in the form of faeces and undigested material (pseudofaeces). This deposition subsequently drives a series of trophic resources as the abundant benthic detritivores attracts higher order consumers, contributing to the higher biodiversity and biomass associated with oyster habitats (Rodney and Paynter, 2006; Grabowski and Peterson, 2007; Hancock and zu Ermgassen, 2019). Therefore, the structural complexity created by oysters not only increases abundance and biomass of resident fauna, but it also increases the functional roles of oyster habitats (and therefore abandoned oyster farms) as refugia and feeding areas for macrobenthic organisms.

Habitat-forming species can perform ecosystem functions differently depending on whether they are native to the community or not, and whether they are in natural configurations (Kochmann et al., 2008). When habitat-forming species, such as oysters, are associated with other hard substrate (e.g., concrete) or not allowed to form naturally (e.g., managed aquaculture farms) the resulting habitat can affect the relative abundances of associated organisms (Kochmann et al., 2008). Therefore, in soft sediment estuaries where hard substrate is a limiting resource, oyster aquaculture selects for organisms that utilize hard substrate, thus potentially providing habitat for invasive species (Heiman et al., 2008). In our surveys, we documented an invasive mussel, *Xenostrobus securis*, which was recently introduced to Hong Kong (Morton and Leung, 2015), in what may initially seem to be relatively high density (882 indiv·m^{-2}) in the abandoned oyster farm. While the presence of the abandoned farm clearly allowed for the settlement of this mussel, because they weren't recorded on the mudflat, the density in the farm is two orders of magnitude less than documented on manmade concrete structures (e.g., concrete foreshore; 20,900 - 76,000 indiv·m^{-2}; Morton and Leung, 2015) and an order of magnitude less than on more modern, and actively managed, suspended rope (raft) oyster aquaculture (up to 3,500 indiv·m^{-2}; Lau et al., 2018) in Hong Kong. Oyster reefs were historically extensive along the coastline in this region (Meacham 1979) and while some invasive species may inhabit restored reefs where they cannot occupy soft-sediment, restored reefs enhance local biodiversity and ecosystem services above what mudflats provide. Therefore, while there is potential for abandoned oyster farms to provide habitat for invasive species, the relatively low density of *Xenostrobus securis* suggests that the high density of native oysters (*Magallana* spp.) which form the basis of the habitat inhibits mussel populations (Gestoso et al., 2014), although further study is required to validate such a link.

Implications of Benthic Aquaculture as a Complement to Restoration

Oyster reefs were historically an important estuarine habitat providing multiple ecosystem functions and services (Grabowski and Peterson, 2007; Gregalis et al., 2009; Grabowski et al., 2012). Native oyster reefs have been overharvested globally and are functionally extinct along many coastlines of the world (Beck et al., 2011). As a result, the restoration of oyster reefs is increasing globally (Fitzsimons et al., 2020). Restoring marine habitats can be expensive, requires both social and political will, and has had varying success rates in many parts of the world (Bayraktarov et al., 2016). Within southern China, oyster restoration is in its infancy, progressing slowly, and outcomes that are uncertain because of high levels of recreational harvest (Lau et al., 2020). In contrast, there is a history of benthic oyster aquaculture in the Pearl River Delta (which includes Hong Kong) stretching back over 1,000 years. This continuous aquaculture practice means that not only native oyster populations been prevented from functional extinction in the region, but these habitats may also have provided the key to cost-effective local reef restoration. Natural recruitment is extremely high in the western waters of Hong Kong (up to 197,550 indiv·m^{-2}) despite natural oyster beds in the region being degraded and having sparse densities of individuals (2.3 indiv·m^{-2}), suggesting an additional source of larval supply from local oyster farms (Lau et al., 2020). In contrast, the collapse of the oyster fisheries on the east coast of the USA since the 1800s has led to recruitment limitation in many areas, meaning that restoration needs to begin by re-establishing a breeding population to provide the larvae for recruitment. Therefore, even though Hong Kong is one of the most intensively developed coastal cities in Asia, providing multiple challenges for marine ecosystems such as habitat loss, eutrophication and coastal acidification (Williams et al., 2016), benthic oyster aquaculture has likely maintained breeding populations of native oysters and helped sustain reef-associated biodiversity over recent centuries even as oyster reefs have been in decline. Although implemented with seafood production in mind, the potential role of traditional oyster aquaculture in facilitating and sustaining native biodiversity and future ecosystem services should be considered. As inshore shellfish aquaculture continues to transition into offshore operations around the world, the potential for transforming abandoned intertidal oyster farms into protected reef habitat may provide opportunities in the future. These traditional farms could be revitalized to complement restoration and showcase an example of 'restorative aquaculture', which describes the intentional design of aquaculture to deliver direct ecological benefits to the surrounding area, in addition to the providing seafood or other commercial products and livelihoods through economic opportunities (Theuerkauf et al., 2019). When abandoned or left unmanaged, they might also provide an opportunity to consolidate material and biogenic structure to speed restoration.

Capturing this concept, ecologically conscious aquaculture techniques and infrastructure could effectively be integrated into restoration efforts and enhance the rebuilding of native oyster populations (Powers et al., 2009). Developing good relationships and the support of industry stakeholders is essential in this process, since local knowledge and collaborations must be involved in aligning farming practices with conservation objectives in a specific local context (Froehlich et al., 2017). Importantly, both environmental (e.g., anthropogenic pollution, heavy sedimentation) and economic (e.g. low return on investment) challenges will need to be addressed to create an opportunity for ongoing traditional oyster farming in Hong Kong if it is to be effective in providing support to restoration activities.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors.

AUTHOR CONTRIBUTIONS

SC: Formal analysis, Investigation, Writing - Original Draft. HTW: Methodology, Investigation, Writing - Review & Editing.

MT: Conceptualization, Methodology, Investigation, Writing - Review & Editing. HA: Conceptualization, Writing - Review & Editing. BH: Conceptualization, Methodology, Investigation, Writing - Review & Editing. BR: Conceptualization, Methodology, Investigation, Writing - Original Draft, Review & Editing, Funding acquisition. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.862548/full#supplementary-material

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Nutrient-Loading Mitigation by Shellfish Aquaculture in Semi-Enclosed Estuaries

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Bivalve aquaculture may provide a variety of ecosystem services including nitrogen extraction from estuaries, which are often subject to excess nutrient loading from various land activities, causing eutrophication. This nitrogen extraction may be affected by a combination of various non-linear interactions between the cultured organisms and the receiving ecosystem. The present study used a coupled hydro-biogeochemical model to examine the interactive effects of various factors on the degree of estuarine nutrient mitigation by farmed bivalves. These factors included bay geomorphology (leaky, restricted and choked systems), river size (small and large rivers leading to moderate (105.9 Mt N yr^{-1}) and high (529.6 Mt N yr^{-1}) nutrient discharges), bivalve species (blue mussel (*Mytilus edulis*) and eastern oyster (*Crassostrea virginica*)), farmed bivalve area (0, 10, 25 and 40% of estuarine surface area) and climate change (water temperature, sea level and precipitation reflecting either present or future (Horizon 2050) conditions). Model outputs indicated that bivalve culture was associated with the retention of nitrogen within estuaries, but that this alteration of nitrogen exchange between estuaries and the open ocean was not uniform across all tested variables and it depended on the nature of their interaction with the bivalves as well as their own dynamics. When nitrogen extraction resulting from harvest was factored in, however, bivalve culture was shown to provide a net nitrogen removal in the majority of the tested model scenarios. Mussels provided more nutrient mitigation than oysters, open systems were more resilient to change than closed ones, and mitigation potential was shown to generally increase with increasing bivalve biomass. Under projected future temperature conditions, nutrient mitigation from mussel farms was predicted to increase, while interactions with the oyster reproductive cycle led to both reduced harvested biomass and nutrient mitigation potential. This study presents the first quantification of the effects of various biological, physical, geomorphological and hydrodynamical processes on nutrient mitigation by bivalve aquaculture and will be critical in addressing questions related to eutrophication mitigation by bivalves and prediction of possible nutrient trading credits.

Keywords: eutrophication, nutrient loading, shellfish aquaculture, ecosystem services, estuary model, climate change

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INTRODUCTION

Bivalve aquaculture is increasingly being acknowledged for the ecosystem services it provides beyond its primary goal of food provisioning (van der Schatte Olivier et al., 2018; Alleway et al., 2019; Smaal et al., 2019). Among these services, nitrogen extraction from near-shore ecosystems (*e.g.* estuaries, lagoons and bays) is of particular interest as these regions are often subject to excess nutrient loading from various land activities (*e.g.* agricultural runoff, waste-water treatment effluent) resulting in regional eutrophication (Cloern, 2001; Bricker et al., 2007). Owing to their capacity to clear particles from large volumes of water, bivalves have been proposed to mitigate these excess nutrients and be included in nitrogen-trading programs (Lindahl et al., 2005; Lindahl, 2011; Nielsen et al., 2016; Petersen et al., 2019). While bivalve harvest clearly contributes to a net extraction of nutrients, the net effect of wild or cultured bivalves on nutrient dynamics while present in the coastal environment is not as obvious. These organisms may potentially interact with several biogeochemical processes that regulate ecosystem functioning. Their direct consumption of food particles can lead to changes such as increased intra- and inter-specific competition for phytoplankton (Cloern, 2005), enhanced water clarity (Meeuwig et al., 1998; Newell, 2004), and alterations in plankton community size structure as a consequence of their size-selective retention efficiency (Strohmeier et al., 2012; Sonier et al., 2016). In addition, non-assimilated food can also affect ecosystem dynamics. Particulate wastes (*e.g.* faeces and pseudo-faeces) sink faster than original food particles (Callier et al., 2006), which may lead to a net transfer of material from the water column to the benthos. This process could result in particulate organic material retention within near-shore ecosystems (Cranford et al., 2007). Part of the assimilated food is also excreted in dissolved organic and inorganic forms that can be more readily exported to the open ocean. These same dissolved wastes (*e.g.* dissolved organic nitrogen and ammonia) may also stimulate local planktonic primary production (Prins et al., 1995) and consequently increase the residence time of nutrients in the system. The net role played by cultured bivalves in material exchange between near-shore systems and the coastal ocean is difficult to predict *a priori* as it involves several non-linear interactions between the bivalves and the supporting ecosystem and can be influenced by various environmental, physical and farming conditions.

Climate change has the potential to increase nutrient loading, through increased precipitation (Nazari-Sharabian et al., 2018) and to intensify eutrophication, through increased water temperature and its effects on many biogeochemical processes in coastal waters (Rabalais et al., 2009). The ultimate effect climate change could have on the role of bivalves in nutrient cycling through coastal ecosystems remains, however, highly uncertain as competing influences are at play. For instance, some bivalve species may grow faster at higher temperatures (Guyondet et al., 2015; Steeves et al., 2018). Temperature-associated changes in primary production (Brown et al., 2010) and planktonic communities (Allen and Wolfe, 2013; Mackas et al., 2013), however, may alter bivalve food availability. Temporal shifts in the seasonal temperature cycle may also affect the interaction between bivalves and the pelagic ecosystem by changing the phenology of planktonic species and/or the phenology of bivalves, especially their reproductive cycle (Filgueira et al., 2015).

Intrinsic features of near-shore regions may also greatly influence their response to pressures such as nutrient loading, bivalve aquaculture and climate change. Specifically, inlet morphology plays a major part in controlling the intensity of exchange with the open ocean and, consequently, strongly influences the prevailing internal conditions and the sensitivity to external stressors (Filgueira et al., 2013; Panda et al., 2013; Roselli et al., 2013). The objective of the present study was to determine the overall net nitrogen retention versus extraction of bivalve aquaculture in semi-enclosed estuaries and explore some of the driving factors of this relationship. Specifically, we focused on the exchange between various types of embayments and the open ocean under various levels of nutrient loading, different shellfish culture species/biomass and climate-change-induced temperature increase as drivers of coastal ecosystem response. Numerical modelling provides an adequate framework for this investigation involving the non-linear intricacies of bivalve-ecosystem interactions, as suggested in previous research using coupled hydro-biogeochemical model simulations (Filgueira et al., 2016). This modelling could help to characterize the eutrophication mitigation potential of bivalve culture in the context of different environmental and biological scenarios relevant to coastal management.

MATERIAL AND METHODS

Coupled Hydro-Biogeochemical Model

The RMA10-11 suite of models was used for this work (King, 1982; King, 2003) in a 2D depth-averaged configuration with a spatial resolution of 100 – 250 m (distance between nodes). This same modelling framework was successfully applied to bivalve-ecosystem interaction studies in the past (Guyondet et al., 2010; Guyondet et al., 2015). Details of the model structure are given in these previous reports and the latest configuration, used in the present study, was similar to Filgueira et al. (2016) and provided in **Appendix 1**. Briefly, the model simulates the nitrogen cycle through a coastal pelagic ecosystem consisting of dissolved inorganic nitrogen (DIN), phytoplankton (Phyto), zooplankton (Zoo) and organic detritus (D). Cultured bivalves are fully integrated within this pelagic structure and their physiology including feeding, somatic growth and reproductive cycle is simulated using a dynamic energy budget (Kooijman, 2010) with parametrizations from (Filgueira et al., 2011; Filgueira et al., 2014) for mussels and oysters, respectively. The model also accounts for the effect of water temperature on all simulated biological processes. The benthic nitrogen cycle is not explicitly simulated, rather temperature-dependent rates of DIN efflux from the sediment are prescribed outside and inside commercial bivalve farms according to observations made in a coastal lagoon of the Magdalen Islands, Atlantic Canada

(Richard et al., 2007). River, atmospheric, and open-boundary conditions were constructed from observations in the southern Gulf of Saint Lawrence (GSL), Atlantic Canada (Guyondet et al., 2015) and are characterized by weak mixed diurnal and semi-diurnal tides (0.15 – 0.60 m amplitude). Further forcing details are provided in **Appendix 1**.

Model Factors: Bay Geomorphology

In order to draw general conclusions, we chose to carry out numerical experiments on geomorphologically-idealized coastal systems, which are representative of shallow temperate estuaries (model mean depth of 4.6 m). Three geomorphologies were tested that only differed by their inlet configuration, leading to leaky, restricted and choked systems in increasing order of tide and exchange attenuation (Kjerfve, 1986). This strategy allowed spanning over a wide range of systems that real estuaries could be compared to and provided a full control on the degree of exchange between the near-shore area and the open ocean as reflected by the differences in water renewal time among the three systems (**Figure 1**), computed following Koutitonsky et al. (2004).

Model Factors: Environmental Drivers

A factorial design was developed to test the effects of several factors and their interactions on the response of the coastal ecosystem. In addition to the system's geomorphology, the following factors were tested. The first was nutrient loading, with two levels, where nutrient inputs were obtained from small and large river discharges resulting in moderate (105.9 Mt N yr^{-1}) and high (529.6 Mt N yr^{-1}) loading, respectively (Coffin et al., 2018). This factor also influences the overall water exchange between the embayment and the open ocean. The second factor was bivalve species, with two levels, being either the blue mussel (*Mytilus edulis*) or the eastern oyster (*Crassostrea virginica*). These species are two of the most commonly cultured bivalves in Atlantic Canada. The third factor, with four levels, was the surface area of the embayment covered by bivalve aquaculture (no aquaculture and low, medium and high aquaculture), which was based on the farm surface area coverage of the embayment (0, 10, 25 and 40%, respectively, **Figure 1**). The "no aquaculture" scenario (stocking coverage = 0%) served as a reference to test the effects induced by the presence of the bivalve farms. The density of bivalves in farmed areas was typical of culture practices in southern GSL with 94.1 mussels m^{-2} and 30.0 oysters m^{-2} (Drapeau et al., 2006; Comeau, 2013). The remaining three factors were related to climate change, with two levels each. Water temperature, sea level and precipitation reflected either present or future (Horizon 2050) conditions. Current conditions were obtained from observations in the southern GSL (Guyondet et al., 2015) while future conditions were derived from predictions at the regional scale based on observed trends (Vasseur and Catto, 2008; Taboada and Anadón, 2012) and corresponding to an annual mean 2°C increase in temperature [with warmer summer peak and earlier Spring **Appendix 1**)], a

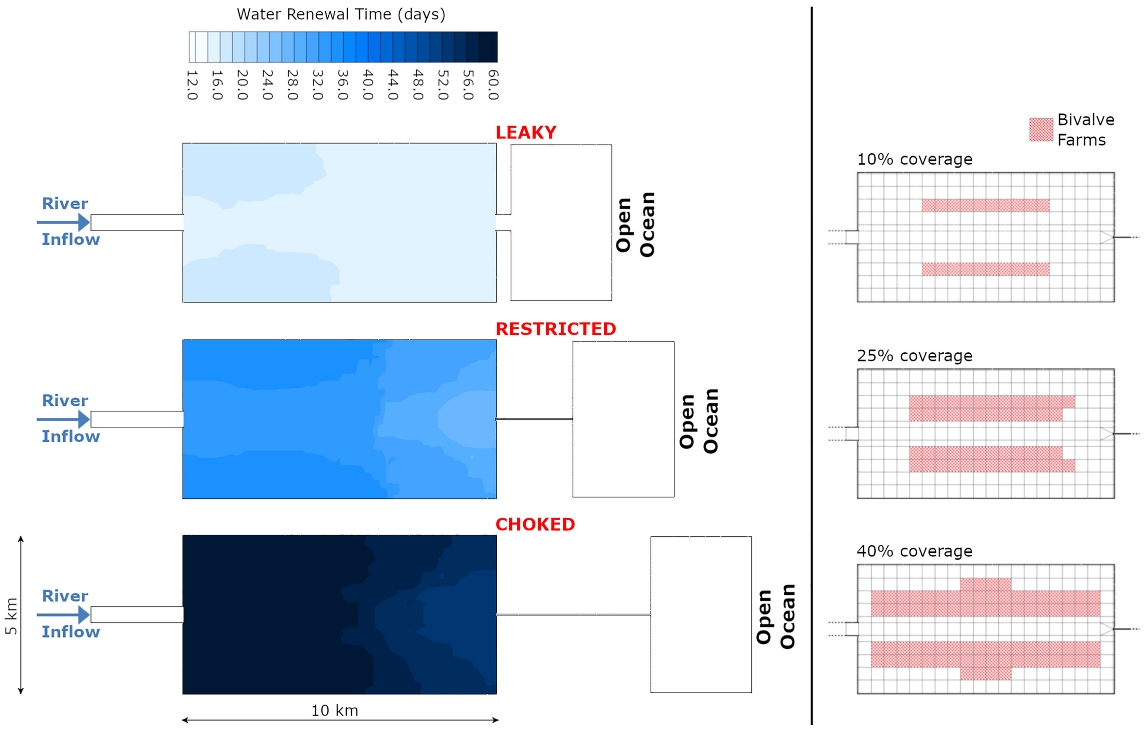

FIGURE 1 | Inlet configuration and water renewal time for the three bay geomorphology types (left) and bivalve farm stocking areas for the three aquaculture scenarios (right).

0.5 m sea level rise and a 10% precipitation increase, forced through increased river discharge.

The combination of all the factors and their respective levels led to a total of 336 scenarios, which were simulated over a full year from June to June, preceded by a 90-day spin-up period in order for the system to reach an equilibrium state. For the purpose of the present study, exchange fluxes of all variables across an inlet section were recorded and then integrated over the year to estimate their net annual import/export balance. These net figures for all variables were also combined into a single estimate of the overall net import/export balance of the coastal ecosystems using the conversion coefficients reported in **Table 1**. Bivalve production was computed as the difference in individual biomass between the end and beginning of the simulation, scaled up to the total cultured population of each scenario. Among the climate factors tested, Filgueira et al. (2016) noted that temperature had by far the largest influence on the ecosystem response. Therefore, temperature is the only climate factor explicitly discussed in this study.

Eutrophication Mitigation Potential

As mentioned in the Introduction, some of the feedbacks from cultured bivalves to the ecosystem can lead to retention of inorganic/organic material within the near-shore area (*i.e.* estuary or bay). This retention was quantified by comparing the net annual exchange of material for a given aquaculture scenario with the exchange for the corresponding scenario without aquaculture. The eutrophication mitigation potential is defined here as the difference between the bivalve production, representing the material that can actually be extracted through harvest, and the retention of material defined above. Bivalve production had to be estimated at a fixed date (end of simulation), which makes the evaluation of the mitigation potential sensitivity to the various factors somewhat tributary to the phenology of the biological processes, in particular the bivalve reproductive cycle (see Discussion).

Statistics

Statistical analyses were conducted using R version 4.0.2 (R Core Team, 2020-06-22) operating in the RStudio version 1.2.1335 environment. Significance for all statistical tests was set at $p < 0.05$.

TABLE 1 | Conversion coefficients used to express the different model variables in a common nitrogen currency.

Parameter	Value	Units	Reference
Phytoplankton (Carbon to Nitrogen)	0.176	mg N mg C^{-1}	Redfield, 1934
Zooplankton (Carbon to Nitrogen)	0.194	mg N mg C^{-1}	Omori, 1969; Tang and Dam, 1999; Walve and Larsson, 1999
Detritus (Carbon to Nitrogen)	0.176	mg N mg C^{-1}	Redfield, 1934
Bivalve (Energy to Carbon)	0.182	mg C J^{-1}	van der Veer et al., 2006
Bivalve (Carbon to Nitrogen)	0.185	mg N mg C^{-1}	Smaal and Vonck, 1997; Higgins et al., 2011

Our first goal with the application of statistics was to understand how three independent variables of interest (bivalve area coverage, bay morphology and species) interact to predict changes in response variables, namely primary production (PP) and export to the open sea of phytoplankton biomass (Phyto), detritus biomass (Detritus), dissolved inorganic nitrogen (DIN), zooplankton biomass (Zoo), and total nitrogen (TN). Response variables were expressed as a percentage change when compared to non-aquaculture scenarios. In order to meet normality assumptions prior to analyses, all response variables were subjected to Yeo-Johnson transformations (Yeo and Johnson, 2000) using the recipe() function in the recipes package (Kuhn and Wickham, 2022). QQ plots were produced to visually assess the normality assumption for all groups together and also for each group separately. Residuals versus fits plots were produced to check the homogeneity of variances. The absence of extreme outliers was confirmed using the identify_outliers() function from the rstatix package (Kassambara, 2021). Finally, three-way ANOVAs were performed using the Anova() function from the car package (Fox and Weisberg, 2019).

Our second statistical objective focused on the extractive capacity of bivalve aquaculture. Our null hypothesis was that the annual amount of nitrogen extracted from the system due to bivalve harvest was equal to the annual amount of nitrogen further retained within the system (export reduction) due to the presence of bivalve farms. A comparison of scenarios 'with' and 'without' cultured bivalves revealed that differences between paired scenarios were not distributed normally, nor symmetrically around the median. Hence the paired samples sign test was used to determine whether differences were consistent across scenarios. The same approach was utilized for assessing whether differences were consistent across species.

All statistical results, raw data, and annotated R-script can be found in the **Supplementary Material** (**Appendices 2–4**).

RESULTS

Embayments Without Aquaculture

As expected, all coastal system configurations tested in this study under "natural", *i.e.* without bivalve farms, and both present and future conditions, showed a net export of material (all forms of N combined) to the open sea (**Figure 2**). The magnitude of this export is mainly driven by the inlet morphology and the river discharge – the more open the system and the larger the river, the greater the nitrogen exports to the open sea. The total N export ranged from 13.1 to 316.7 Mt N yr^{-1} for small rivers and 27.5 to 532.5 Mt N yr^{-1} for large rivers, while these systems received a total N river loading of 105.9 Mt N yr^{-1} and 529.6 Mt N yr^{-1}, respectively, of which 60% was of inorganic form (DIN) and 40% of organic form (Detritus) for both river sizes.

There are some obvious associations that can be made between nutrients and primary biomass/production within bays (**Table 2**). High nutrient loading from rivers are associated with high concentration of all pelagic variables (Phyto, Zoo and DIN), and also with elevated rates of PP. With respect to bay morphology, there is a tendency for

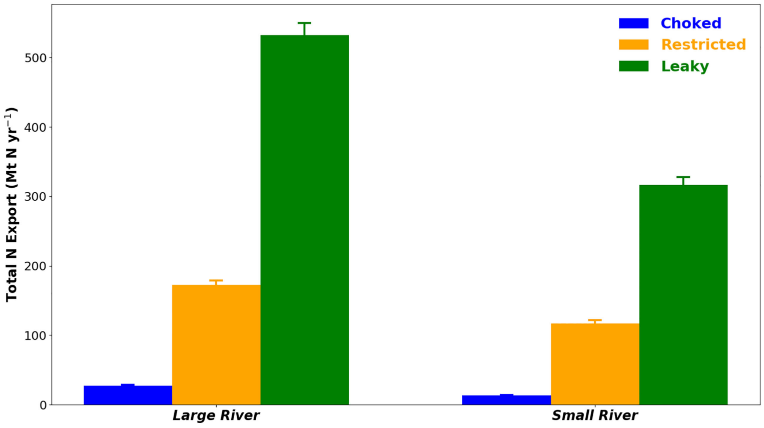

FIGURE 2 | Mean (± SD) net total nitrogen (N) export to the coastal ocean as a function of river discharge and bay morphology under the "no aquaculture" scenarios. Error bars represent variability introduced by other factors (*i.e.* climate).

TABLE 2 | Main pelagic model variables and rates (annual mean ± SD) in scenarios without aquaculture.

	Large River *(high loading)*				Small River *(moderate loading)*			
	DIN	**Phyto**	**Zoo**	**PP**	**DIN**	**Phyto**	**Zoo**	**PP**
Choked	126.3 ± 9.1	4.3 ± 0.3	49.4 ± 3.4	72.3 ± 4.1	92.4 ± 10.0	2.9 ± 0.2	37.7 ± 2.8	41.1 ± 4.8
Restricted	117.7 ± 7.4	4.1 ± 0.3	42.4 ± 3.8	76.3 ± 4.4	92.5 ± 6.6	3.0 ± 0.2	36.5 ± 4.7	49.4 ± 6.4
Leaky	109.4 ± 3.8	3.9 ± 0.3	36.2 ± 6.3	87.8 ± 8.8	94.4 ± 3.0	3.0 ± 0.2	32.2 ± 5.9	58.7 ± 8.2

DIN, dissolved inorganic nitrogen (µg N L^{-1}); Phyto, phytoplankton (µg Chl a L^{-1}); Zoo: zooplankton (µg C L^{-1}) and PP, net phytoplankton primary production (mg C m^{-2} d^{-1}).

curtailed dissolved nutrient concentrations (but high PP) in open systems. This pattern is more obvious in systems with large rivers as Phyto and DIN remain at the same level independently of the morphology of the bay when a small river is present.

Embayments With Aquaculture

For all scenarios, the addition of bivalve farms consistently led to a decrease in total N export to the open sea (**Figure 3A**), signaling the presence of aquaculture-related retention mechanisms at the bay scale. Export reduction was influenced by a two-way interaction between stocking area and bay morphology, $F(4, 270) = 6.53$, $p < 0.001$, $\eta^2_p = 0.09$ (**Appendix 2**; the three way interaction including the species factor is non-significant). Stocking area has a highly significant effect on export reduction ($p < 0.001$) across all bay morphologies, with a strikingly large effect in choked systems (η^2_p= 0.54 for mussels, 0.53 for oysters). Bay morphology also has a highly significant ($p < 0.001$) and large ($\eta^2_p = 0.13$ to 0.17) effect on export reduction, although only at high and medium stocking area levels; at low stocking area levels, the effects of bay morphology on export reduction are either non-significant (mussels, $p = 0.09$) or significant but small in magnitude (oysters, $p < 0.01$, $\eta^2_p = 0.04$).

The addition of bivalve farms increases grazing pressure (top-down control), which leads to a decrease in primary production in most scenarios (**Figure 3B**). The low stocking area results show however that there is potential for a positive feedback from bivalves on primary production through the excretion of DIN (bottom-up control). As with total N export, PP is influenced by a two-way interaction between stocking area and bay morphology, $F(4, 270) = 4.45$, $p < 0.01$, $\eta^2_p = 0.06$; however, PP is also regulated by a two-way interaction between stocking area and species, $F(4, 270) = 4.29$, $p < 0.05$, $\eta^2_p = 0.03$. In more detail, the effects of stocking area on PP are significant ($p < 0.05$) for all bay morphologies, with the exception of mussels in leaky systems ($p = 0.16$). Where significant the magnitude of the stocking area effect ranged from a low effect size ($\eta^2_p = 0.03$ for oysters in leaky bays) to a large effect size ($\eta^2_p = 0.17$ for oysters in choked bays). Overall, these factorial ANOVAs revealed that both mussels and oysters can decrease PP and reduce total N exports to the open sea, particularly when cultivation activities are carried out at high intensity in choked systems.

The alteration of material exchange induced by bivalve farming is not uniform among pelagic components (**Figure 4**). Despite being the preferred bivalve food source, Phyto export was among the least affected, while Zoo showed the highest relative change. ANOVAs corroborated the data's inference that stocking area coverage increased retention across all pelagic components (**Appendix 3**). However, the nature of the stocking area effect varied across components. A two-way interaction between stocking area and bay morphology regulated Phyto [$F(4, 270) = 2.65$, $p < 0.05$, $\eta^2_p = 0.04$] and DIN [$F(4, 270) = 26.42$, $p < 0.001$, $\eta^2_p = 0.28$]. By contrast there

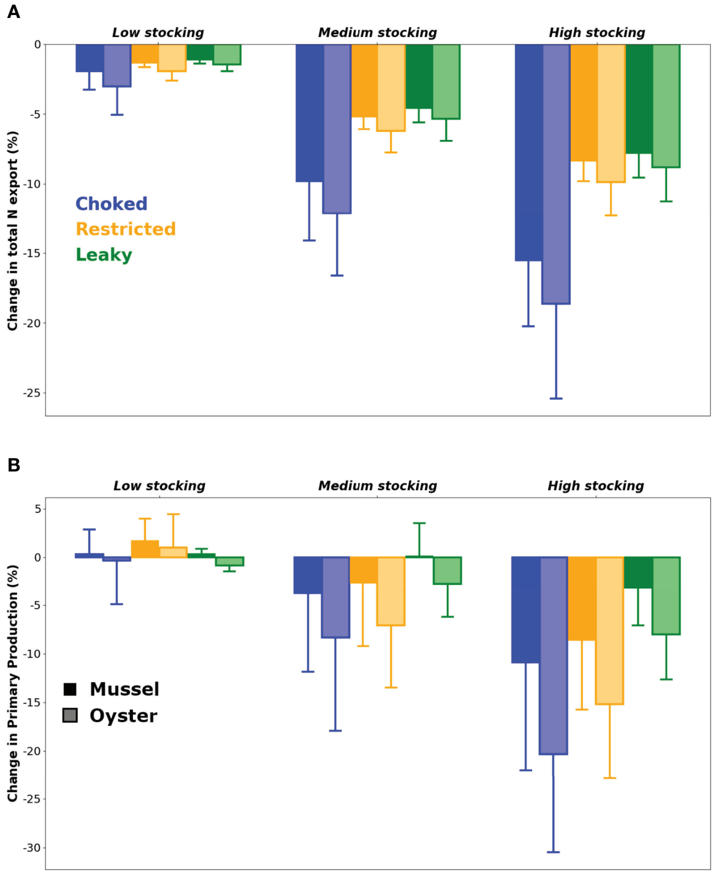

FIGURE 3 | Mean (± SD) relative changes in **(A)** total nitrogen export to the coastal ocean and **(B)** primary production, following the introduction of various cultured bivalve species at various stocking surface areas in the three bay types. Error bars represent variability introduced by other factors (*i.e.* nutrient loading and climate).

are no interactions between predictors for all other pelagic components. Rather, the main effect of stocking area is remarkably large for Detritus [$F(4, 270) = 666.87$, $p < 0.001$, $\eta^2_p = 0.83$] and Zoo [$F(4, 270) = 1152.55$, $p < 0.001$, $\eta^2_p = 0.90$]. The main effect of bay morphology is similarly notable for detritus [$F(4, 270) = 55.00$, $p < 0.001$, $\eta^2_p = 0.29$] and Zoo [$F(4, 270) = 52.76$, $p < 0.001$, $\eta^2_p = 0.29$]. Zooplankton was the only component regulated by the three main effects (stocking area, bay morphology, and species). Lastly, with respect to the direction of effects, there was a clear tendency related to export changes in DIN and Zoo, with leaky systems being more resilient (*i.e.* less impacted by presence of aquaculture) compared to restricted or choked systems.

While bivalve aquaculture reduced exports to the open sea, it may also provide an extraction service due to harvests. It was found that the magnitude of these contrasting effects differs between cultured species (**Figure 5**). Export reduction mechanisms were stronger for oysters than for mussels (**Appendix 4**; $p < 0.05$, paired-samples sign test), with the exception of leaky systems at low stocking density where there was no species effect ($p = 0.077$). By contrast, mussel production provides a much higher extractive potential than oyster production ($p < 0.001$, paired-samples sign test). Consequently, the mitigation potential was stronger for mussel farms (43.8 ± 5.6 – 148.0 ± 35.1 Mt N yr^{-1}) compared to oyster farms (1.1 ± 7.6 – 29.4 ± 2.2 Mt N yr^{-1}). The mitigation potential was highly significant across all culture scenarios ($p < 0.001$, paired-samples sign test), with the exception of oysters cultured under high densities in leaky systems, for which there was no significant (positive or negative) mitigation effect. Net retention (negative mitigation) only occurred in a small subset of scenarios (15 out of 288), corresponding to oyster culture in leaky systems at medium or high stocking area coverage under future temperature conditions.

Water temperature interacted with other factors such as bivalve species, stocking surface area and system morphology (**Figure 6**). The mitigation potential of mussels increased with temperature for the majority of scenarios (54/72 points above the 1:1 line). In contrast, the mitigation by oysters tended to decrease at higher temperature and all cases of negative mitigation potential (*i.e.* net N retention) occurred in leaky systems and future temperature conditions. This result, although counter-intuitive and also contradicting the faster growth of oysters at higher temperature (not shown, but see companion paper Filgueira et al., 2016), is caused by the strong influence of temperature on the reproductive cycle of oysters, which leads

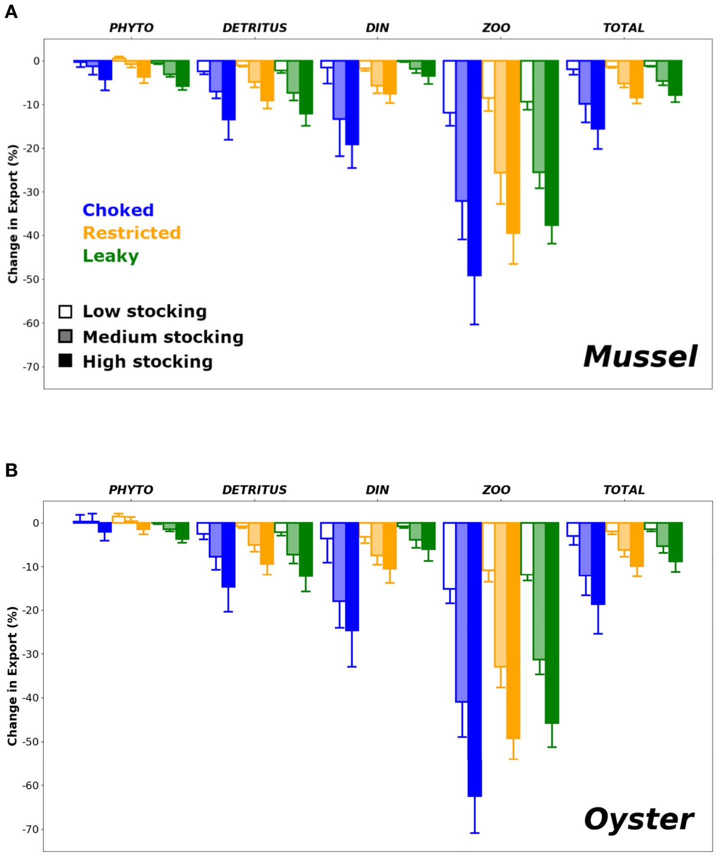

FIGURE 4 | Mean (±SD) relative change in the net export of the different pelagic variables (PHYTO: phytoplankton, DETRITUS: detritus, DIN: dissolved inorganic nitrogen, ZOO: zooplankton and TOTAL: total nitrogen, i.e. all variables combined) to the coastal ocean following the introduction of **(A)** mussel or **(B)** oyster farms and as a function of stocking surface area and bay morphology. Error bars represent variability introduced by other factors (i.e. nutrient loading and climate).

to an increased reproductive output (larger spawning), which in turn may decrease the oyster final biomass and production estimate. The positive relationship between mitigation potential and stocking area for a given morphology is preserved in future temperature scenarios except for oyster farms in leaky systems. The general rule stating that the stronger the flushing the more resilient the system is to changes (points closer to the 1:1 line) is verified for the temperature effect on mitigation potential of mussel farms, leading more enclosed systems to benefit more from mitigation in future climate scenarios. However, mitigation by oyster farms departs from this rule due to the influence of temperature on their reproductive cycle mentioned above. Finally, the clearer temperature response pattern provided by the mussel farm scenarios allows the viewing of a different behaviour between the small and large river systems (only visible for medium and high stocking areas). In systems with large rivers, higher flushing from wider openings tends to lead to a lower mitigation potential, consistent with the higher resilience hypothesis, while the reverse occurs in small river systems, especially at current temperature forcing. Moreover, while increased temperature tends to exacerbate the flushing effect in large river systems (stretch along the y-axis), the range of mitigation potential in small river systems becomes much narrower among flushing scenarios in future conditions (compressed y-axis range).

DISCUSSION

The use of idealized systems prevents the direct extrapolation of our results to existing estuaries, however the general conclusions drawn from these results and discussed in the following sections can be transposed to real systems with similar dimensions, loading, and exchange with the open ocean. The model outputs obtained in this study indicate that for all stocking area scenarios bivalve culture was associated with further retention of material within bays. This alteration of the bay exchange was not uniform across pelagic variables as it depended on the nature of their interaction with bivalves as well as their own dynamics. When the material extraction from harvest was factored in, however, bivalve culture was shown to provide a net nitrogen removal in the majority of scenarios tested. In addition, for the husbandry conditions simulated, mussel rather than

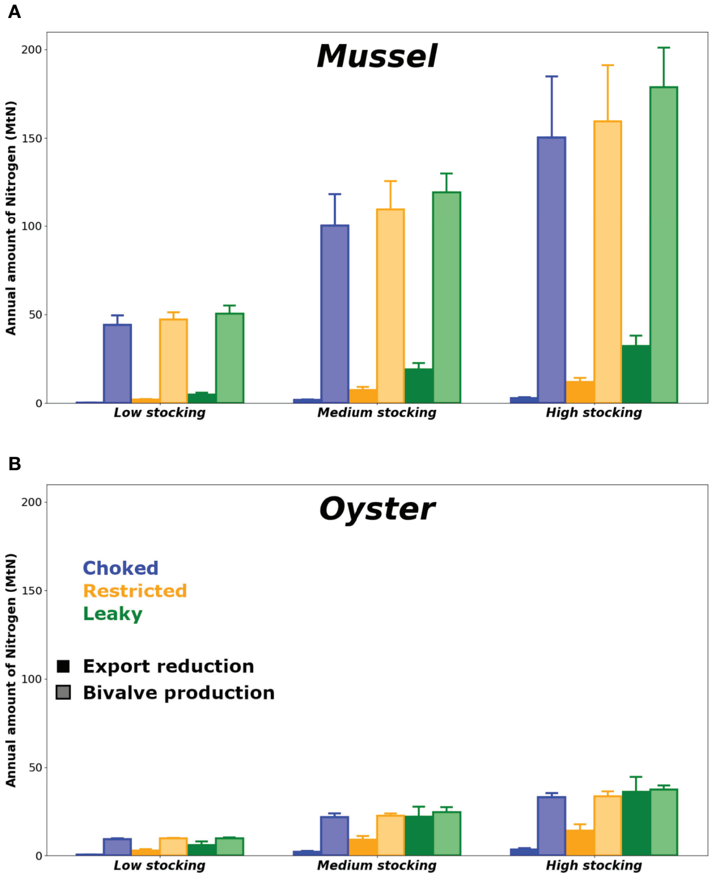

FIGURE 5 | Mean (±SD) nitrogen export reduction (i.e. retention within the bay) and bivalve production (potential nitrogen removal) for **(A)** mussel and **(B)** oyster farming the different shellfish species, and for the different stocking surface areas and bay morphologies tested. Error bars represent variability introduced by other factors (i.e. nutrient loading and climate).

oyster farming provided the strongest potential for nutrient loading mitigation. Our results also support the idea that more open systems are more resilient to change. The corollary stating that more enclosed systems should benefit more from bivalve culture for nutrient mitigation, only holds for oyster farming scenarios. Moreover, the mitigation potential was shown to generally increase with bivalve stocking area. In future temperature conditions, the mitigation from mussel farms was predicted to increase, while interactions with the oyster reproductive cycle led to reduced harvested biomass and nutrient mitigation potential.

Simulated Embayments

The idealized embayments tested are representative of shallow estuarine systems in temperate regions with a potential for moderately to highly eutrophic conditions, with nutrient loading ranging from 21 to 106 kgN estuary ha^{-1} yr^{-1}, depending on the river size (Coffin et al., 2018). As can be expected for such river-driven systems, as opposed to those driven by coastal upwelling, the balance of material exchange at the ocean boundary results in a net export. The more open the system is to the ocean, the higher its export capacity becomes. In the moderate loading (small river) scenarios choked systems could only export 1/10 of the river input, leaky systems being able to export an amount equivalent to three times that same river loading. The apparent imbalance between inputs and export of these leaky scenarios is explained by the ability of the more open systems to also export part of the nitrogen provided by benthic loading. However, the exporting capacity seems limited as shown by the higher river loading scenarios where choked systems could only export 1/20 of the nitrogen received and leaky systems exported the equivalent of only one time what they received from river input. Hence, material retention within the estuary increases in more enclosed systems and for larger river loading. This material retention has been referred to as the filtering capacity of an estuary and for nitrogen has been reported to range from 0 to over 50% of the river loading on an annual basis (Arndt et al., 2009 and references therein). Benthic processes such as burial and denitrification may contribute to the net removal of this retained material (Seitzinger et al., 2006), however, they were not explicitly detailed in the model structure so their

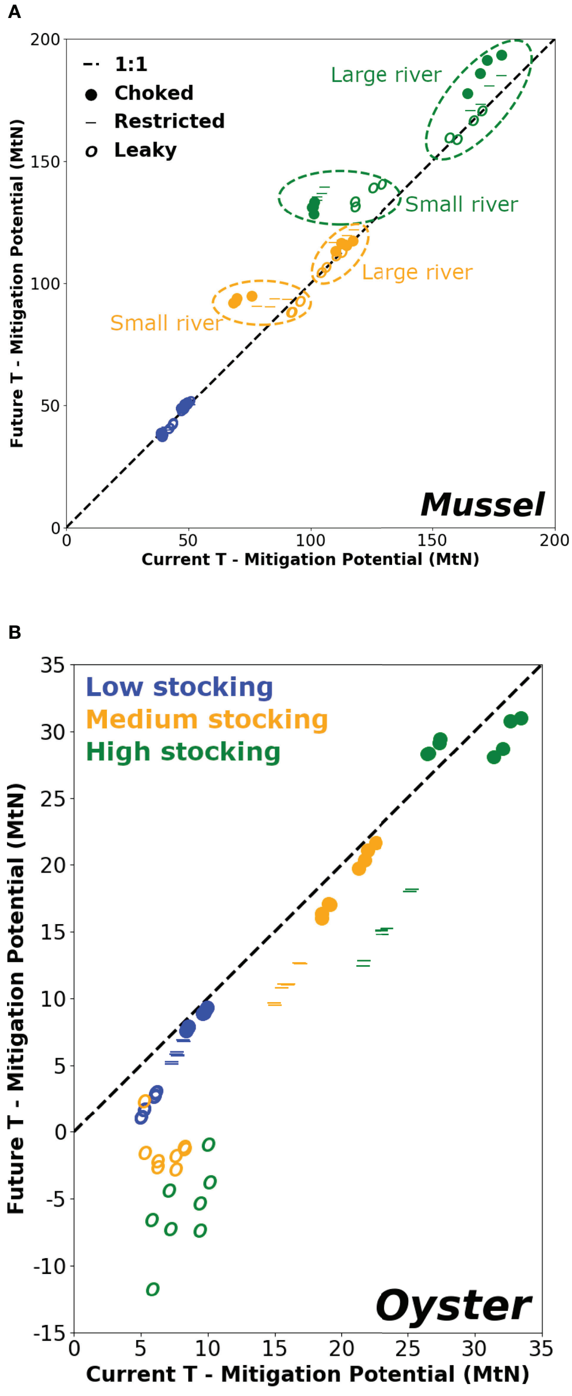

FIGURE 6 | Comparison of current and future nutrient loading mitigation potential provided by **(A)** mussel and **(B)** oyster aquaculture in the various scenarios of stocking area, nutrient loading (river size) and bay morphology.

contribution and change among model scenarios was not evaluated.

In real ecosystems, the retained inorganic/organic material can fuel the internal productivity of higher trophic levels, both pelagic and benthic, as exemplified by the higher Zoo predicted in this study for enclosed embayments. Counter-intuitively, these systems show a smaller PP rate than more open ones (**Table 2**). This result must be regarded from an efficiency perspective as it relates to the importance of the balance between the biological and hydrodynamic time-scales of a coastal system (Officer, 1980;

Kimmerer et al., 1993) that dictates how much of the nutrient loading can be used by local biological processes (Filgueira et al., 2016). In more open bays, the faster exchange dynamics are more favorable to the fast processes of phytoplankton production (Eyre and Twigg, 1997), while in enclosed or restricted-opening bays, slower exchange leads to a more efficient transfer of energy towards secondary producers (*i.e.* zooplankton). Part of the reduced pelagic PP in the latter can also be explained by the overall higher concentration of suspended particles, which can increase light attenuation and potentially decrease PP rates, even in bays with ample nutrient supply (Meeuwig et al., 1998). Moreover, in shallow estuaries, high nutrient loading leading to eutrophic conditions often manifests itself through the proliferation of opportunistic macroalgae, *e.g. Ulva* spp. (Valiela et al., 1997). Accounting for this other type of primary producer would provide a more realistic representation of these system dynamics (Lavaud et al., 2020) as they may affect the filtering capacity of the bay by being likely less easily exported than phytoplankton and by storing large nitrogen pools that would not be directly amenable to mitigation through bivalve farming.

Climate change, leading to warmer future temperatures, could possibly increase metabolism in coastal systems (Angilletta et al., 2002; Brown et al., 2004), resulting in faster biological dynamics, without directly affecting the hydrodynamic time-scale. Consequently, these future conditions could favour an increase in the filtering capacity or material retention of these nearshore regions. However, warming could also alter coastal ecosystems more profoundly as it might not affect all communities and trophic levels in the same way or intensity (Carr and Bruno, 2013; Mertens et al., 2015; Alexander et al., 2016; Ullah, 2018). Hence, future changes in the filtering capacity of estuaries and coastal systems remain highly uncertain and warrant further research at the ecosystem scale.

Bivalve Influences

Cultured bivalves feed preferentially on phytoplankton and consequently build their tightest link with this component of the ecosystem (Filgueira et al., 2011; Saraiva et al., 2012). The net balance of this interaction results, however, from the competition between the top-down feeding pressure and the bottom-up control exerted by the ammonia excretion of these mollusks (Prins et al., 1995; Smaal et al., 2013). The positive response of PP in some of the low stocking area scenarios is a direct manifestation of this bottom-up feedback. Nutrient loading also contributes to the bottom-up side of this balance, with higher loading generally counter-acting more of the feeding pressure from cultured bivalves. Finally, this balance is modulated by the system's morphology and temperature as can be partially seen in **Figure 3B** (see Filgueira et al. (2016) for a detailed analysis).

When considering the impact of bivalve aquaculture on the exchange of material with the open sea, phytoplankton appears as the most resilient variable. The low impact on it likely results from its fast turn-over rates and the feedback mechanism mentioned above. Conversely, zooplankton exchange is the most affected due to its slower dynamics, the competition for food with the bivalves (Gianasi et al in review; Granados et al., 2017a; Granados et al., 2017b) and, although marginal, the direct bivalve predation on zooplankton (Pace et al., 1998; Davenport et al., 2000; Trottet et al., 2008; Peharda et al., 2012). Despite the overall low contribution of zooplankton, representing only 1.8 to 3.9% of total N exported in all scenarios tested, this last result indicates that the bivalve aquaculture signal can be amplified through the trophic levels both in and out of these nearshore systems. Similarly, the retention efficiency of bivalves as a function of food particle size (Riisgard, 1988; Strohmeier et al., 2012; Sonier et al., 2016), coupled with the strong feeding pressure exerted in aquaculture settings, could affect the pelagic community size distribution at a system scale. According to size-spectrum theory (Sheldon et al., 1972; Andersen et al., 2016), bivalve culture could then affect the way energy propagates through trophic levels. Further work is required to better understand how and how much these cascading effects might change coastal ecosystem functioning and the services they provide, and to examine the balance between these negative effects and the positive effects that accrue from added food production and nutrient loading mitigation from bivalve aquaculture.

All tested scenarios showed an increase in estuarine filtering capacity (*i.e.* material retention) in the presence of bivalve culture farms. This increment in retention ranged from 0.3 to 4.3% and from 1.5 to 18.5% of river N loading for large and small rivers, respectively. Despite the lower stocking density typically used in oyster versus mussel farming (30 vs 94 ind. m^{-2} of farm in the present model applications) and the slightly lower individual feeding rate resulting from the mean size and environmental conditions over the simulated period, oyster culture provided a slightly higher increase in retention compared to mussel culture for the same farm coverage. This species difference rests mostly on a feeding efficiency variance at the individual level and in particular a higher feedback by mussels through excretion, which gets amplified by the difference in stocking density. Finally, in absolute value, material retention from bivalve farms is larger in systems with stronger flushing intensity, which can be explained by the retention/export being already high/low in more enclosed systems, thus limiting the potential for further retention from bivalve farms. This result is also in accordance with the negative relation between the fraction of loading exported and freshwater residence time reported by Dettmann (2001).

Mitigation Potential

When the material retention effect is examined in the context of the net material removal provided by the harvest of bivalve production, a positive nutrient loading mitigation potential is predicted for all mussel and most oyster culture scenarios tested in this study. Moreover, such mitigation potential can reach important fractions of the river loading, with values ranging in high loading conditions (large river) from 8.86 to 36.54% and from 0.21 to 6.31% for mussels and oysters, respectively, and in moderate loading conditions (small river) from 35.14 to 132.69% and from 1.57 to 27.80%, respectively. The stronger mitigation potential provided by mussel farming can be attributed, at the individual level, to the faster growth of this species (12 – 18 months and four years to reach harvest size for mussels and oysters, respectively) and, at bay-scale, to the higher densities they are typically reared in (94 mussels vs 30 oysters per square meter of farm in the present study). The higher mitigation efficiency predicted in lower loading conditions reflects the limits to the assimilative capacity that bivalve farms can provide.

The tested mussel culture scenarios show an interesting interaction between loading and flushing intensities. The reversal of the relationship between mitigation potential and flushing, depending on loading intensity, highlights the importance of the absolute loading level for the vulnerability of coastal ecosystems to eutrophication and for the mitigation benefit cultured bivalves can provide, as well as the tight link between hydro- and biological dynamics in these regions (Officer, 1980; Kimmerer et al., 1993; Dame et al., 2000). From this result, it appears that a loading inflection point exists beyond which more flushing becomes detrimental to the mitigation potential of bivalve farms. Further testing with incremental loading levels would be required to refine this relationship. In addition, spatial heterogeneity was not assessed in this study, given the idealized morphology of the test bays used. In natural systems, however, the realized mitigation could depend on the distribution of wild and cultured bivalves in relation to the nutrient loading outfall location and the distribution of water residence time (Gray et al., 2021). A spatially-explicit approach would then be critical in any real-life mitigation potential assessment.

Future warmer temperatures could have competing influences on mitigation potential. As stated earlier, a general increase in biological activity is expected that could lead to increased material retention. At the same time, cultured bivalves could also benefit from increased metabolism to reach higher production rates, which would favour nutrient removal. However, the actual future response of bivalve production in a given region will depend on how future temperatures relate to each species' tolerance range (Steeves et al., 2018), how tightly adapted they are to current conditions and how quickly they can evolve in response to these changes (Pernet et al., 2007; Thomas and Bacher, 2018; Thyrring et al., 2019). According to the scenarios tested, not accounting for potential adaptation effects, mussel culture could provide increased mitigation at these future temperatures. The picture provided by the oyster culture scenarios is much less clear and even tends to indicate movement in the opposite direction despite the tolerance for higher temperatures of that species. Part of this somewhat surprising result can be explained by a method artefact, which comes from the necessity in our modelling framework to evaluate bivalve production at a fixed date at the end of the simulation period and how this can interact with the changes in the phenology of the reproductive cycle of the species considered. The potential for oyster production is actually higher at higher temperature, but the realized production is sometimes lower because of the evaluation at a fixed date. However, this result highlights the importance of the reproductive cycle in optimizing bivalve culture for nutrient loading mitigation as these species can invest large fractions of their total tissue weight in gametes [over 40% for American oysters (Choi et al., 1993) and similar values (35 – 38%) for blue mussels (Sukhotin and Flyachinskaya, 2009; Hennebicq et al., 2013). Such phenological change considerations tie to the broader seasonality of the various processes involved in the filtering capacity of nearshore systems (Brion et al., 2008) and ultimately in the nutrient loading mitigation potential from bivalve culture, especially in temperate regions.

Bivalve culture has been proposed as a mitigation tool in nutrient trading schemes in Europe and the United States with studies evaluating its costs/benefits against more traditional methods (Lindahl et al., 2005; Lindahl, 2011), assessing the removal potential at farm-scale (Clements and Comeau, 2019; Bricker et al., 2020) and operationalizing and optimizing the production for this specific mitigation service (Petersen et al., 2014; Taylor et al., 2019). The results of the present study concur on the ability of bivalve farming to provide such a nitrogen extraction service and detail the influence of various drivers on the realization of this mitigation. In a management context, the seasonal considerations mentioned earlier and the tight and complex links between bivalves and their environment advocate for the use of a dynamic and spatially-explicit approach, such as the modelling tool used in this study, to provide an integrated assessment of the mitigation potential of bivalve culture within the broader coastal ecosystem functioning.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

TG: Conceptualization, Funding acquisition, Methodology, Investigation, Writing - original draft, Writing - review & editing. RF: Conceptualization, Funding acquisition, Methodology, Investigation, Writing – review and editing. CP: Conceptualization, Funding acquisition, Writing – review and editing. RT: Conceptualization, Funding acquisition, Writing – review and editing. LC: Conceptualization, Funding acquisition, Investigation, Writing – review and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.909926/full#supplementary-material

Seven supplementary files are provided:

1. Appendix 1: Model structure and forcing (equations, parameters and river, atmospheric and open ocean physical and biochemical forcing conditions).
2. Appendix 2: Commented R script for statistical analyses related to **Figure 3** and corresponding data (2 files).
3. Appendix 3: Commented R script for statistical analyses related to **Figure 4** and corresponding data (2 files).
4. Appendix 4: Commented R script for statistical analyses related to **Figure 5** and corresponding data (2 files).

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REVIEW
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Ecosystem Services of Ecosystem Approach to Mariculture: Providing an Unprecedented Opportunity for the Reform of China's Sustainable Aquaculture

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China is the biggest provider of mariculture products, and the industry is still growing rapidly. Increasing scientific evidence indicates that mariculture may provide valuable ecosystem goods and services in China. Here, we performed a systematic literature review of studies with the aim of understanding the ecosystem services of mariculture and a comprehensive review of ecosystem approaches that may improve mariculture ecosystem services and goods in China. We highlight four ecosystem services functions in China, including food supply, nutrient extraction, carbon sequestration, and biodiversity conservation. Given the further reform of China's mariculture, we outlined several ecosystem approaches including integrated multi-trophic aquaculture (IMTA), carrying capacity assessment and monitoring, marine spatial planning, and waste treatment and recirculating mariculture system. We conclude that the ecosystem services of the ecosystem approach to mariculture provide an unprecedented opportunity for the reform of China's sustainable aquaculture. Finally, a synthesis of sustainable development of mariculture, along with the five recommendations for future mariculture development in China, is outlined.

Keywords: mariculture, ecosystem service, techniques, management, IMTA, carrying capacity

INTRODUCTION

Globally, there is a growing concern over the sustainable development of mariculture. On September 23, 2021, the UN General held the Food System Summit 2021 to look at the delivery of the Sustainable Development Goals (SDGs) by 2030, and "Blue Foods" has become one of the future directions to achieve the 17 sustainable goals set by the 2030 Agenda, including end poverty and hunger, promote sustainable agriculture, ensure sustainable consumption, and conserve and sustainably use the oceans, seas, and marine resources for sustainable development. In China, the government also anchors sustainable development of mariculture goals in the 14th *Five-Year Plan for the National Economic and Social Development of PRC* (refer to as 14FYP hereafter) by demonstrating "prioritizing ecosystem and promoting green development". However, developing mariculture sustainability remains an ongoing challenge due to the large demand for aquatic products.

China is the world's biggest mariculture country (FAO, 2020) and has been producing more mariculture products than wild catch since 2008, and its mariculture production share in global production reached 65.9% in 2018. Mariculture has substantial impacts on ecosystem health and sustainable use. Along with the unprecedented and continuous expansion, China's mariculture industry has been received many undesirable environmental and ecological impacts in terms of seawater eutrophication (Islam, 2005), chemical's accumulation (Burridge et al., 2010; Liu et al., 2017), microplastic pollution (Chen et al., 2021), habitat destruction (Ahmed and Thompson 2018; Ottinger et al., 2016), biodiversity depletion (Ticina et al., 2020), and space competition between fish farmers and other marine stakeholders (Bostock et al., 2010). However, many peer experts such as Tang (2019) and Jiao (2018) suggested that mariculture can deliver a broad range of ecosystem services functions, and many unexpected environmental effects of mariculture can be alleviated even eliminated by the integration of advanced ecosystem approaches and appropriate management. Nevertheless, several questions arise regarding ecosystem-based techniques and management tools: What are the ecosystem services functions of mariculture in China? How could these diverse characteristics of ecosystem services functions of mariculture contribute to the designing of mariculture activities in meeting ecological and environmental issues? What and how current ecosystem based-management tools can help to achieve a sustainable, healthier mariculture production in a responsible manner better integrated with social-environmental issues? Given that China's aquaculture is transforming into a sustainable development pattern with low and high outputs, and this shift has started to exhibit positive effects, it is time to make a shift toward a better understanding what ecosystem services functions might be expected from mariculture and the ecosystem approach to mariculture—the enabling techniques, measures, and management tools that could ensure that mariculture activities generate substantial ecosystem services to nature and society.

In this paper, we provide a state-of-the-art review of the above considerations as they relate to the mariculture's ecosystem services and ecosystem approaches to mariculture in China, derived from a systematic literature review. Specifically, we first describe the mariculture's ecosystem services in China, including food supply, nutrient extraction, carbon sequestration, biodiversity conservation, and stock enhancement. We then identify ecosystem approaches that could improve the ecosystem functions of mariculture, including integrated multi-trophic aquaculture (IMTA), carrying capacity assessment and monitoring, marine spatial planning (MSP), and waste treatment and recirculating mariculture system. Finally, we provide a synthesis of sustainable development of mariculture lessons from the world, along with the future direction of mariculture in China.

MATERIALS AND METHODS

To obtain an overview of mariculture's ecosystem service functions, and technologies literature in China, we first conducted a systematic review of articles published in Web of Science from 2001 to 2021. In China, four main ecosystem services were proposed: (1) food supply, (2) nutrient extraction, (3) carbon sequestration, and (4) biodiversity conservation and stock enhancement. Hence, articles on studies that reported the ecosystem services functions of mariculture such as food supply, nutrient extraction, carbon sequestration, stock enhancement, and biodiversity conservation were considered. Still, mariculture improvement methods and technologies such as IMTA, carrying capacity assessment, marine spatial planning, and monitoring were also considered. We searched the Web of Science to find articles using the following key terms: "mariculture" OR "marine aquaculture*" OR "integrated multi-trophic aquaculture*" and "food supply*" OR "nutrient extraction*" OR "bioremediation" OR "carbon sequestration" OR "carbon sink" OR "stock enhancement" OR "biodiversity" OR "biodiversity conservation", OR "marine spatial planning" OR "carrying capacity" OR "monitoring" OR "waste treat" OR "sustainable" and "China" or "Chinese". Over 800 articles were manually screened on an individual basis. Most articles were excluded by title alone, while the rest were included or excluded after reviewing the abstract or full text. Specifically, we retained articles that focused on quantitatively assessing the positive effect of nutrient extraction of mariculture, carbon sequestration, quantitatively assessing the biodiversity and stock enhancement function of mariculture carrying capacity of mariculture, monitoring and modeling, MSP of mariculture, and waste treatment and recirculating aquaculture. We then categorized these articles according to their evaluation objectives and findings and retained articles that focus on the ecosystem service of mariculture. According to these articles, six articles examined the food supply function of mariculture, 14 articles quantitatively assessed the nutrient extraction of mariculture, nine articles mentioned the carbon sequestration, five articles quantitative assessed the biodiversity and stock enhancement function of mariculture, nine articles quantitatively assessed the value of ecosystem service of mariculture, 15 articles quantitatively assessed the carrying capacity of mariculture, six articles were related to monitoring of the mariculture ecosystem, nine articles were associated with MSP of mariculture, and five articles were related to waste treatment and recirculating aquaculture. Several articles were considered more than once, as they contained different ecosystem service functions of mariculture.

ECOSYSTEM SERVICES OF MARICULTURE IN CHINA

Mariculture is a double-edged sword in China (Meng and Feagin, 2019), namely, mariculture can have both negative and positive impacts on the ecosystem. Consequently, mariculture cannot be viewed as a risk-free solution to the sustainable development of agriculture in China (Li et al., 2011). However, an increased number of studies indicate that mariculture could provide valuable ecosystem services functions in China, including food supply, nutrient extraction, carbon sequestration, biodiversity conservation, and stock enhancement. Undoubtedly, “ecological priority” and “ecological and environmental sustainability” have been put in the first place for future mariculture initiatives in China. In the following, we give a brief state-of-the-art summary that focuses on the positive effects of mariculture in China to demonstrate the main ecosystem functions of mariculture in China.

Food Supply

China’s developing mariculture has made a significant contribution to ensuring food security and against hunger since the 1980s when the coastal capture fishery of China faced serious over-exploitation issues (Yu and Han, 2020). Aquatic foods of mariculture food supply in China include finfish, crustaceans (mainly crabs and shrimp), cephalopods (mainly octopus and squids), mollusks (mainly bivalve), algae (mainly macroalgae), and other aquatic animals (mainly sea cucumbers). The total mariculture production in China increased 12.5-fold between 1990 and 2020 (**Figure 1A**). China’s mariculture production has exceeded capture fisheries production between 2000 and 2010 (**Figure 1B**). Notably, mollusks and algae mariculture development during the past 30 years have supported the dramatic increase in mariculture since 1990, while the production of finfish and crustaceans keep pace with the increase in mariculture production. However, the finfish and cephalopods produced from capture fishery are higher than that from mariculture production. The finfish and cephalopods food supply are still dependent on the wild catch production (**Figure 1B**). Regardless of the unbalanced supply amount of different aquatic foods, mariculture still became the most important part of the aquatic food supply. For example, the mariculture production supplies over 20 million tons in 2020, about onefold more than capture fishery production. With the flourishing of mariculture production, the mariculture alone supplies more than 15 kg/cap/year of edible aquatic food consumption for China in 2020 (**Figure1C**), which is higher than the world’s average aquatic (including both marine and freshwater food) consumption (14.6 kg/cap/year). In other words, mariculture helps China to get rid of hunger.

In the past decade, multiple reviews have investigated the nutrition of different aquatic foods (mainly animals, plants, and microorganisms) and their functions to improve human health (Golden et al., 2021). Overall, aquatic foods can provide hundreds of nutrients, such as minerals, vitamins, protein, and fatty acids (such as docosahexaenoic acid and eicosapentaenoic acid, DHA, and EPA). Still, aquatic foods are the most important sources of several key micronutrients, omega-3 long-chain polyunsaturated fatty acids, and vitamins, which can reduce meat intake, fill the nutrient gap, and support the vulnerability

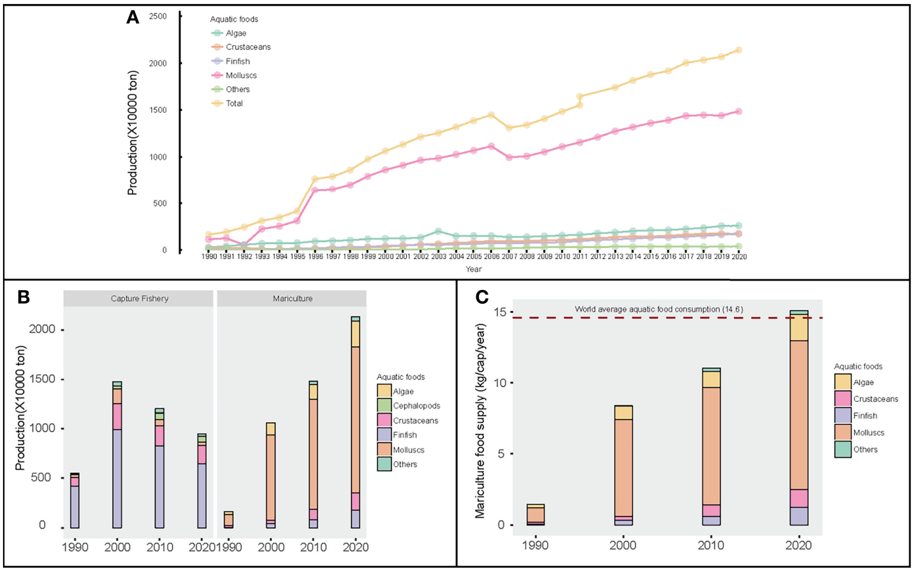

FIGURE 1 | Food supply function of mariculture in China. **(A)** The total and selected group mariculture production of China between 1990 and 2020. **(B)** The production and comparison between capture fishery production and mariculture production of China in 1990, 2000, 2010, and 2020. **(C)**. Per capita aquatic food consumption for selected groups (kg/cap/year) in China. China demonstrates a high level of edible mariculture product per capita in relation to global edible aquatic consumption (14.6 kg/cap/year, including freshwater and marine aquatic foods). By 2020, China’s mariculture food supply alone is higher than the world’s average aquatic consumption.

of human beings (He, 2010). Along with China's policy converted from the past emphasis on increasing production to achieving sustainable development, China's mariculture has replaced capture fishery in nutrient supply in China. Take the finfish, the most high-nutrient-value aquatic food, for example, after reaching about 15 Mt capture fishery production in 1999, China's finfish resources mainly consisted of small and low-value pelagic fish, instead of the previous high-nutrient carnivore fish like large yellow croaker (Liang and Pauly, 2019). Along with the depletion of several high-nutrient value finfish, the mariculture growth rate grew continuously after 2000 and produced more than 1.7 Mt of high-nutrient-value finfish such as large yellow croaker, groupers, and sea bass. The rapid development of mariculture like the finfish cultural system demonstrates the capability to reduce, even fill the nutrient gap under the production reduction in capture fishery background.

Nutrient Extraction

Nutrient extraction is the most widely known ecosystem service of mariculture, and significant progress has been made in mariculture's nutrient extraction in recent years (Naylor et al., 2021). To prevent eutrophication, recent nutrient criteria emphasized the importance of controlling phosphorus and nitrogen due to the increasing link between nitrogen pollution and cultural eutrophication (Xiao et al., 2017; Liang et al., 2019). Globally, molluscan and seaweed mariculture are two well-known ways to remove inorganic nutrients and reduce potential adverse environmental impacts (Rosa et al., 2018; Qi et al., 2019). In China, molluscan and seaweed mariculture production together accounts for 69% and 12% of China's total mariculture production, respectively (Fishery Administration of the Ministry of Agriculture, 2021). Basically, most molluscan and seaweed cultivation in China does not require feed, and it is effective in enhancing water purification and water clarity by assimilation of excess nutrient produced by anthropogenic activities (Duan et al., 2019). The formation processes of mariculture nutrient extraction are complex but through at least four pathways. First, seaweed can absorb dissolved nitrate and phosphate in seawater and convert dissolved inorganic nitrate and phosphate into particle organic matters during their growth (Xie et al., 2020). Second, mollusks, especially filter-feeding bivalves, can filter particle organic matter and then accumulate nitrogen and phosphorous (Mahmood et al., 2016). Third, a large quantity of nitrate and phosphate was removed from the water through harvesting seaweed and mollusk. Ultimately, the sedimentation of seaweed detritus and fecal or pseudo-fecal of mollusk could promote the deposition of nitrogen and phosphate pool (Wu et al., 2015). All of these demonstrate that no-fed species mariculture is an indispensable way of nutrient extraction and their potential ability is species and area specific.

Conversely, fed species such as finfish and shrimp mariculture may release nutrients and cause nutrient pollution eutrophication in the adjacent water (Kang and Xu, 2016; Yang et al., 2021). For example, Cao et al. (2007) assessed the nutrient release of pond-based shrimp mariculture, and the release rate of shrimp is about N of 45.8 kg/t, P of 10.1 kg/t, and chemical oxygen demand (COD) of 49.65 kg/t. In the finfish cage mariculture system, nitrogen excretion quantities of the finfish in Nanshan Bay are about 2.81t–15.59 t/season. However, the nutrient pollution caused by fed species mariculture can be alleviated through the deployment of IMTA. The practice of IMTA in China (Fang et al., 2016) showed that the nutrient pollution caused by 1 kg finfish mariculture can be eliminated by the culture of 6.1–9.2 kg extractive species. Combined with China's mariculture production of fed and non-fed groups, the fed/non-fed mariculture ratio is about 10:1; therefore, China's mariculture can provide nutrient extraction function.

In China, although there is no standard procedure to evaluate the overall nutrient extraction counting system, many scientists have carried out research using a variety of methods for the estimation or assessment of the nutrient extraction potential in the mariculture ecosystem. Collectively, three methods are documented: first, in most studies, the assessment of mariculture nutrient extraction is based on the nitrogen and phosphate content and yield of mariculture products. Second, several studies used laboratory-based assessment to measure the nutrient absorbing rate of cultural species and then establish nutrient-extraction-counting assessment *in situ*. Third, many studies measured the biodeposition rate of cultural species and then assessed nutrient transport to sediment (**Table 1**).

Carbon Sequestration

Like nutrient extraction functions of mariculture, carbon sequestration is widely recognized as another ecosystem services function of mariculture. In the past decade, the Chinese government has stressed on the various occasion that the country aims to facilitate the adoption of an ambitious, and realistic carbon emission peak goal before 2030 and would strive for "carbon neutrality" by 2060 (Gao et al., 2022). In this context, mariculture could be useful to remove excess carbon from the oceans and reduce CO_2 levels indirectly in the atmosphere through two pathways: biological pump and microbial carbon pump (Longhurst and Harrison, 1989; Jiao et al., 2011).

The previously published review on blue carbon sinks calculated that more than half of global carbon is captured by marine life (Nellemann et al., 2009). In 2005, Zhang et al. (2005) pointed out the first systematic assessment of carbon sequestration of mariculture in China and proposed the carbon sequestration fisheries concept—defined as non-fed mariculture that can remove a mass of carbon and has great potential for carbon sequestration and has been argued for more than 15 years because only a small proportion (about 1.4%) of CO_2 should be transported to the seafloor by biological pump (BP) for long-term sequestration, and the removal carbon is mainly respired into CO_2 after consumption (Jiao et al., 2018). Fortunately, recent studies focusing on the mechanisms of carbon cycling, especially microbial carbon pump (MCP) in the marine system, have unveiled the full picture on how mariculture contribute to carbon sequestration. Among different cultural species, seaweed and bivalve could be useful to remove excess carbon from the oceans and reduce CO_2 levels indirectly in the atmosphere

TABLE 1 | Nutrient extraction assessment of mariculture in China.

Method	Cultural species	Nutrient extraction	Reference
Content assessment	Mussel	N: 160.3 mg/ind/a P: 36.7 mg/ind/a	Gao et al. (2008)
Biodeposition rate	fouling communities	N: 0.11 g/m^2 P: 0.98×10^{-2} g/m^2	Qi et al. (2015)
Content assessment	Seaweed	In oyster farm: C: 15,016.90 ± 6,241.78 mg/thallus N: 1,112.45 ± 459.81 mg/thallus P: 134.69 ± 55.46 mg/thallus In finfish cage: C: 2,457.46 ± 1,073.78 mg/thallus N: 180.27 ± 75.23 mg/thallus P: 13.69 ± 5.88 mg/thallus	Yu et al. (2016)
Lab *in situ* assessment	Seaweed–oyster	MC-Control N/P: 38.1 ± 11.0 MC-Seaweed N/P: 92.5 ± 25.6 MC-Bivalve N/P: 36.4 ± 10.9 MC-Integrate N/P: 32.0 ± 16.3	Wang et al. (2017)
Content assessment	Seaweed	China's large scale kelp farm: N: 75,000t P: 9,500t	Xiao et al. (2017).

through two pathways: biological pump and microbial carbon pump (Duarte et al., 2017). In a recent review by Zhang et al. (2017), the carbon fixed in mariculture is characterized by four forms: carbon fixed in cultural organisms (0.677 Tg of C/year), particulate organic carbon (POC, 0.944 Tg of C/year), dissolved organic carbon (0.822–0.915 Tg of C/year) in seawater, and buried carbon in sediments (0.14 Tg of C/year). Over the last decade, trends in the carbon sequestration performance of mariculture have been highly warranted. For example, the *National Science Review* published a review characterizing the blue carbon strategy in China. In this review, Jiao et al. (2018) pointed out that the dissolved organic carbon (DOC) released from cultural seaweed is even higher than the total burial flux of organic carbon from coastal blue carbon in China. Therefore, mariculture could become an important part of blue carbon in the future.

Despite the direct carbon sequestration functions of mariculture, a most recent published article in *Nature* creates a cohesive model that unites terrestrial foods with nearly 3,000 taxa of aquatic foods to understand the future impact of aquatic foods on human nutrition, and results revealed that mariculture fisheries nearly always produce fewer greenhouse gas emissions than terrestrial foods (Golden et al., 2021). In other words, mariculture foods can be considered low-carbon foods compared with other foods, which make a great contribution to carbon mitigation against the background of growing global demand for foods.

Biodiversity Conservation and Stock Enhancement

Since China proposed 13 FYP, China places more emphasis on the restoration and sustainability of mariculture ecosystems and is responsible for maintaining a wide range of ecosystem values, particularly through conservation and sustainable use of biodiversity. Although aquaculture and stock enhancement are two distinctly different areas, mariculture can provide support for biodiversity conservation and stock enhancement functions (Lorenzen et al., 2013; Theuerkauf et al., 2021). Mariculture provides biodiversity and stock enhancement through at least two pathways: first, as of 2015, 167 billion juveniles of nine marine species groups were cultured and released along the Chinese coast. Hence, the success of biodiversity conservation and stock enhancement activities depends upon mariculture-based artificial breeding and cultural juvenile release (Liu et al., 2022). Second, the mariculture system, especially suspended or elevated rafts and cages, can be viewed as three-dimension habitat and can positively influence the structure and function of wild macrofaunal communities through at least three pathways: provision of structured habitat, provision of food resources, and enhanced reproduction and recruitment. In China, field studies showed that mariculture infrastructure can introduce considerable diverse structures into the natural environment and act as a floating aggregation device, and the waste of fish meal can have a positive effect on the benthos polychaete community and provide an additional food source for the fish community (Fang et al., 2017; Zhou et al., 2019). Such positive influence is possible only under the presupposition that mariculture activities cannot cause destructive habitat conversion on the adjacent key habitats, such as mangrove, seagrass, oyster reef, and saltmarsh (Herbeck et al., 2020). Appropriately, a definition of sustainable mariculture development in China should include both supplementations with hatchery-produced seed and promotion of wild populations. Hence, the combination of mariculture and stock enhancement received growing attention in recent years, and China will have increased mariculture-based measures in the future to protect and restore habitats for marine species, as the country will continue to strive for better protection of biodiversity.

Assessment of China's Mariculture Ecosystem Services Value

As mentioned above, the ecosystem service functions supported by mariculture have gained substantial focus. Consequently, the assessment of mariculture ecosystem services value, especially social–economic value is of interest to many ocean-related bodies to guide the policymaking and management of the ecosystem. In China, mariculture's ecosystem service value has been involved in the nationwide and regional ecosystem services value assessment since 2000 (Chen and Zhang, 2000; Lin et al., 2019; You et al., 2019; Gao et al., 2020). For instance, Chen and Zhang (2000) estimated that the marine ecosystem service value is about $21{,}736\times10^8$ yuan/a, and the mariculture ecosystem has been included; You et al. (2019) estimated the ecosystem service value (17.4×10^8 yuan/a to 53.7×10^8 yuan/a) from 1997 to 2015, and using stepwise regression and path analysis methods, mariculture has been identified as a main driving force of ecosystem service value of Quanzhou Bay. Compared with nationwide and regional ecosystem service values assessment, studies on the ecosystem service value of the mariculture system are relatively rare. One representative study is the benefit–cost analysis of the Sanggou bay mariculture ecosystem based on ecosystem services (Zheng et al., 2009); the result showed that the net present value of ecosystem service (including food production, oxygen production, climate regulation, and waste treatment) of mariculture in Sanggou bay is about $1.12–1.33\times10^8$ yuan/a. Another example is the valuation of shrimp ecosystem services in Leizhou; in this study, Liu et al. (2010) used the market method, carbon tax rate method, reforestation cost method, and contingent valuation method to estimate the net value of ecosystem service of the shrimp ecosystem in Leizhou (about 3.9×10^8 yuan/a). However, according to Costanza's calculation method (Costanza et al., 1997; Costanza et al., 2017), several non-marketing ecosystem service values of mariculture are still unknown (such as erosion control and sediment retention, biological control, and refugia) or cannot be quantitatively assessed (such as genetic resources, recreation, carbon sequestration, nutrient extraction, and culture). Therefore, the ecosystem service value of mariculture must be thoroughly studied and assessed quantitatively before the social–economic value of mariculture can be fully integrated into the mariculture ecosystem service value assessment.

ECOSYSTEM APPROACHES TO MARICULTURE IN CHINA

Since the Chinese central government proposed the National 13th Five-Year Plan in 2016, environmental protection has been given national priority on par with economic development, and the conservation of marine ecosystems has attracted unprecedented attention in China (Council, 2016). Consequently, the sustainability of mariculture in China essentially capitalizes on existing favorable features of a marine area and combines the natural attributes of the sea environment of the region with various levels of technology. Ecosystem Approach to Aquaculture (EAA) is defined as "a strategy for the integration of the activity within the wider ecosystem such that it promotes sustainable development, equity, and the resilience of interlinked social-ecological systems" (FAO, 2011). The development of mariculture ecosystem service functions and transformation of China's mariculture industry have resulted in a variety of concepts and EAA to avoid unwanted ecological effects and improve the performance of mariculture deployment, either technique or management tools. In China, the extension of different applied sustainable aquaculture modes and tools, such as polyculture and integrated multi-trophic aquaculture, carrying capacity assessment and marine spatial planning, and waste treatment and recirculating aquaculture is reported.

Integrated Multi-Trophic Aquaculture

The original concept of IMTA was defined as the incorporation of species from different positions or nutrient levels in the same system (Chopin and Robinson, 2004). With the rapid development of the sustainable EAA, which demands both sustainable practices at the level of the target cultural species, and taking responsibility for its interactions in the ecosystem context, the IMTA now refers to a suitable approach to address such concerns, which is to limit aquaculture nutrients and organic matter outputs through bio-mitigation, with both economic and environmental sustainability (Chang et al., 2020).

There are several IMTA modes in China with the local condition and characteristics of cultural organisms (**Figure 2**). The finfish–bivalve–seaweed (**Figure2A**) and finfish–seaweed (**Figure2B**) IMTA mode is popular in Zhejiang, Fujian, and Guangdong provinces; these implementations can alleviate the negative environmental and ecological effects of finfish culture, such as eutrophication. For instance, finfish culture produces waste (uneaten fishmeal, faces, and dissolve nutrients) in the water, and the filtered bivalve can remove the particle organic matter (POM), while the seaweed can absorb the dissolved nutrient in the water (Wei et al., 2017; Xie et al., 2020). The bivalve–seaweed (**Figure 2C**) mode is deployed in nearly all coastal provinces of China. Extractive filter bivalves and seaweed are the main mariculture groups in China; they can improve water quality and contribute to carbon sequestration. However, a large density of bivalve culture has a potential negative influence on the water quality through excreting dissolved inorganic nutrient and feces and pseudofeces; therefore, seaweed mariculture is effective in reducing nitrogen load from bivalve and enhance the beneficial functions of the mariculture system. The pond-based IMTA (**Figures 2D–F**) modes are also well-developed in China, especially in seawater shrimp farming. In the shrimp-centric mariculture system, the coculture of shrimp, fish, crab, and mollusks provides comprehensive benefits at the ecosystem and economic levels. Specifically, the shrimp–finfish–bivalve (**Figure 2D**) IMTA mode links multi-trophic level so that fish can consume diseased shrimp, and benthos mollusk can utilize the uneaten forage of shrimp (Liu, 2010). The shrimp–crab (**Figure 2E**) IMTA mode is popular in China; crab takes up uneaten forage of shrimp and creates more economic benefit. The shrimp–bivalve (**Figure 2F**) IMTA mode is popular in Shandong and Jiangsu province. Shrimp and bivalve are

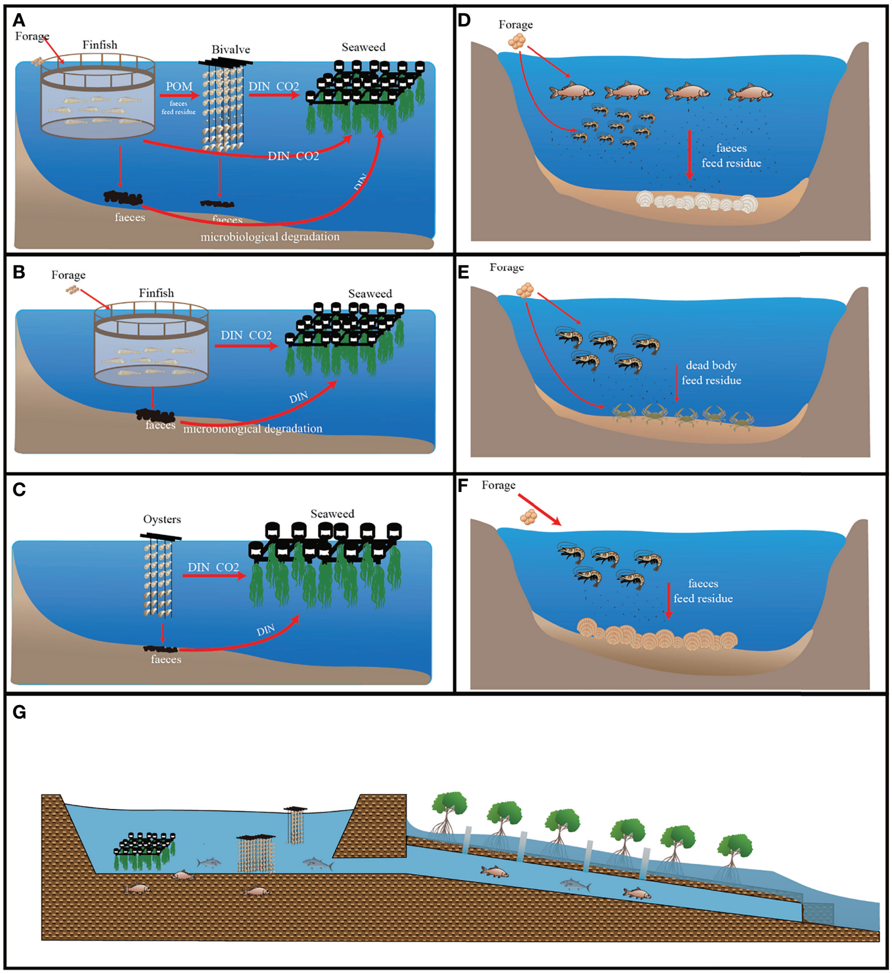

FIGURE 2 | Integrated multi-trophic aquaculture (IMTA) mode in China. **(A)** Cage culture of finfish and long-line culture of bivalve and seaweed. **(B)** Cage culture of finfish and long-line culture of seaweed. **(C)** Long-line culture of bivalve and seaweed. **(D)** Pond culture of finfish, shrimp, and bivalve. **(E)** Pond culture of shrimp and crab. **(F)** Pond culture of shrimp and bivalve. **(G)** Natural habitat (mangrove)-based IMTA mode.

inhabited in different water column and therefore provides combined benefits of food source and waste reduction: shrimp feed on forage and the bivalve utilized the waste of shrimp. In the past decade, natural habitat restoration has been considered an important component of ecological civilization in China. Therefore, scientists in China have improved the cultural technique innovation for integration of natural habitat restoration and eco-friendly IMTA mode. In Guangxi province, scientists have developed a mangrove-restoration-based IMTA mode, which can improve connectivity between the natural habitat and the mariculture system (Xu et al., 2011).

Carrying Capacity Assessment and Monitoring

Quantitative and comprehensive carrying capacity assessment and monitoring were the foundation of the ecosystem approach to aquaculture and executed for mariculture ecosystem management and codified in marine policy worldwide (Brugère et al., 2019). In general, negative environmental and ecological impacts may ensue if the mariculture system is overdense; therefore, carrying capacity assessment can control the density more scientifically and increase the production in the relatively sufficient sea space. In China, the characteristic of carrying capacity fall into five categories: physical carrying capacity, production carrying capacity, ecological carrying capacity, social carrying capacity, and environmental carrying capacity (Fang et al., 2020). The carrying capacity methods in China generally fall into four categories: mass-balanced methods, environmental threshold method, population dynamics with hydrodynamics, and ecological footprint methods (**Table 2**). Collectively, most carrying capacity assessment in China belongs to production carrying capacity, which aims to achieve maximum sustainable mariculture yield of cultural product. Still, many representative mariculture areas have focused on a broad scale assessment of directions and patterns of change to achieve long-term management and planning of comprehensive benefits based on the combination of biological, ecological, economic, and social

TABLE 2 | Carrying capacity assessment of mariculture in China.

Method	Type	Carrying capacity	Reference
Mass-balanced	Production carrying capacity	Fish: 5.8 t/ha	Xu et al. (2011)
Mass-balanced	Ecological carrying capacity	Bivalve: 976 t/km^2	Gao et al. (2020)
Mass-balanced	Production carrying capacity	Bivalve: 118 t/km^2	Han et al. (2017)
Environmental threshold	Environmental carrying capacity	Phosphorus: 0.216 mg/L; nitrogen: 0.039 mg/L	Cai and Sun (2007)
Population dynamics with hydrodynamics	Physical carrying capacity	Bivalve seeding densities: 60,000–105,000 ind/ha	Zhao et al. (2019)
Mass-balanced	Production carrying capacity	Seeding density: Clams:345ind./m^2 Oyster: 60 ind./m^2 Mussel: 165 ind./m^2 Scallops: 80 ind./cage.	Liu et al. (2020)
Population dynamics with hydrodynamics	Production carrying capacity	Scallop:17.6 t/ha Oyster:45.8 t/ha	Nunes et al. (2003).
Energy ecological footprint	Physical carrying capacity	Energy ecological footprint area(1953.19 ha) about 14 times greater than energy carrying capacity (135.88 ha) and about 293 times greater than actual physical area(6.67 ha)	Zhao et al. (2013)
Ecological dynamic system numerical models	Physical carrying capacity	Fish cage: circumference of 40 m and the depth of 8 m	Zhao et al.(2020)
Mass-balanced	Production and physical carrying capacity	Bivalve: 19,881.95 t. Suitable area: 461.83 hm	Zhu et al.(2011)
Population dynamics with hydrodynamics	Ecological carrying capacity	Oyster: 38,564 t	Sequeira et al. (2008).

factors. An example is the ecosystem carrying capacity-based management framework in Sanggou Bay IMTA pilot; despite the carrying capacity, this framework also integrated hydrodynamic modeling while considering ecosystem resilience and redundancy. Meanwhile, other works attempted to provide ecosystem models that incorporate social, economic, and ecological sectors in Sanggou Bay (Shi et al., 2013). Such a form of ecological–economic modeling seems highly warranted and fortunately is ongoing in some northern China's IMTA pilot areas.

In recent decades, different technological tools such as remote sensing and undersea observation net have been used to provide comprehensive social–environmental data for carrying capacity assessment and scientific-based management. Among them, remote sensing can contribute to the quantitative assessment of mariculture areas by giving an instantaneous overview over large areas of the Earth's surface, which has been widely used in mariculture research (Meaden and Aguilar-Manjarrez, 2013), including coastal area monitoring, environmental elements determination, mariculture site selection and mapping, mariculture infrastructure classification, and mariculture dynamic detection (Wong et al., 2019; Duan et al., 2020; Fu et al., 2021). In the context of real-time environmental monitoring, inspired by the successful experience in Japan, Korea and some western countries' marine ecosystem management, undersea observation networks have been integrated into the mariculture system monitoring in China. The undersea observation networks permit the monitoring of the ecosystem in real time and from a great distance. For example, Shandong province has built up 24 real-time seafloor observation stations in IMTA pilots, which contain continuous monitoring of the biotic (ROV and acoustic monitoring) and abiotic (hydrodynamic, current velocity, water quality, temperature, DO, Chla, turbidity) factors in mariculture system. Once the data are obtained, all the data are sent to the big data center for ecological assessment and management. Therefore, the importance of monitoring the mariculture system has been highlighted by the Chinese government.

Marine Spatial Planning

Marine spatial planning is accepted as a robust management tool for EAA. In China, a growing demand for nutrition, foods, and ecosystem services means an expansion of mariculture activities in the oceans and coastal areas and increased pressure on the environment and spatial conflict with other resources. These issues include direct and indirect competition for marine space between different zones and stakeholders, unexpected environmental (water quality, disease risk, fish meal) impacts, and ecological impacts (primarily related to species invasion, erosion of adjacent habitat, and genetic pollution). These issues all negatively impact public perception of mariculture (Ahmed and Thompson 2018; Chopin et al., 2012; Edwards, 2015; Carballeira Braña et al., 2021).

To solve the spatial conflict in marine space and alleviate the unexpected environment–ecological effect of mariculture, the Chinese government has delivered MSP in two ways: the first is named Marine Functional Zoning (MFZ), which governs the utilization of marine space by different stakeholders from the national to the local scale (Council, 2015; Yu and Li, 2020). Meanwhile, the government also formulated Tidal Flat Planning (TFP) in mariculture waters to strengthen the regulation of mariculture space (MOA, 2017). Under the guidance of regulation, policy, scientific advice, and practices, the adoption of mariculture MSP in the Yellow China Sea, the East China Sea, and the South China Sea begins to follow the designed orient frameworks (Teng et al., 2021). Accordingly, the MFZ clarifies the priority of fishers and fish farmers in territorial space including their rights in zones for fishery and mariculture. Still, there are limited rights in zones for recreation and zones for conservation, while the MFZ forbids mariculture activities in zones for industry and urban use, port and shipping, miners and energy utilization, special utilization, and marine protected area (MPA). Since the 13 FYP, China sought to fight and limit the aquaculture activities against MFZ and TFP by conducting a series of inspections, especially Central Environmental Protection Inspection. Such inspections resulted in the local government using a clear-cut approach to remove mariculture activities when mariculture communities violated the MFZ and TFP (Miao and Xue, 2021). Notably, the expansion of MPAs will continue to occupy traditional mariculture areas and cause problems to fish farming communities (especially small-scale communities). The spatial exclusion effect between mariculture and MPA will undoubtedly continue in coastal China, since MPAs in China are permanent and restrict reservation.

Waste Treatment and Recirculating Mariculture System

The ministry of China has been accelerating the establishment of systems for the management of wastewater discharge from mariculture and marine waste governance. In China, the natural-based IMTA and recirculating aquaculture systems are two technologies to tackle the major environmental challenges associated with fed species culture systems and land-based mariculture systems (Fei et al., 2011; Song et al., 2019; Hu et al., 2021). The application of natural-based IMTA has advantages in the nearshore waters by using some low-value biofouling species. For instance, the finfish-polychaete IMTA mode could utilize the particulate organic waste from intensive mariculture and is appropriate for sediment recovery in the culture system (Fang et al., 2017). In the context of recirculating mariculture system, the said system has lower water requirements and could tackle the major environmental issues caused by cage culture systems. Subsequently, some high-value species like salmon have been cultured in the indoor recirculating system in China (Song et al., 2019).

OPPORTUNITY AND RECOMMENDATION FOR THE REFORM OF CHINA'S SUSTAINABLE MARICULTURE

Overall, optimizing the overall arrangement of mariculture green development goal in 14 FYP, together with newly introduced ecosystem service functions of mariculture and innovations in technique or management tools of EAA in our state-of-the-art review, is rapidly providing an opportunity for the reform of China's sustainable mariculture to meet with SDG goals by 2030. Accordingly, we put forward the following specific recommendations.

First, in the last decades, mariculture enhanced the aquatic food supply under capture fishery reduction background. However, as fisheries resource conservation becomes a top priority of marine fishery management in China, China's mariculture industry should produce more finfish to fill the gap of the high-nutrient value aquatic product.

Second, observation data and previous experience have shown that extractive species, particularly molluscan and seaweed cultures, are effective in nutrient extraction and carbon sequestration. However, the nutrient extraction and carbon sequestration functions of mariculture systems are complicated, and standard protocols for both nutrient-accounting and carbon-counting systems of mariculture system are still lacking. Therefore, we suggest that China establish standard protocols and nutrient- and carbon-counting systems for the mariculture system's ecosystem service functions. Such applications can make China's mariculture green development goal, carbon emission peak goal and carbon neutrality goal essentially meaningful.

Third, mariculture activities have always been considered to have negative effects on natural habitats and wild species; however, our reviews show that mariculture can have biodiversity conservation and stock enhancement functions if appropriately managed. In this case, China's scientists should provide more detailed knowledge of how mariculture activities interact and affect the wild species and explore sustainable development modes like mangrove restoration-based IMTA.

Fourth, mariculture is a double-edged sword. To avoid unwanted ecological effects and improve the performance of mariculture deployment, China required the highly efficient and flexible use of different IMTA modes, carrying capacity assessment, monitoring (remote sensing and undersea observation networks), and waste treatment techniques and then regulated the mariculture activities based on MSP.

Ultimately, China should strengthen cooperation with fish farming stakeholders, integrating the government, mariculture community, scholars, and non-government organizations in the mariculture system's management. This will be conducive to reduce management costs and improve the livelihoods of small-scale mariculture communities.

AUTHOR CONTRIBUTIONS

X-JZ, JH-F, and L-FH contributed to the conception and design of the study. X-JZ and JH-F wrote the original draft of the manuscript. BX and LY collected the data. X-JZ and S-YS analyzed the data. L-FH provided funding acquisition and validated the manuscript. All authors contributed to the article and approved the submitted version.

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Temporal Dynamics in Energy Fluxes and Trophic Structure of a Portunus trituberculatus Polyculture Ecosystem During Different Culture Periods

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Swimming crab (*Portunus trituberculatus*) are an important aquaculture species in eastern coastal areas of China. To improve the understanding of *P. trituberculatus* culture ecosystem functioning, the dynamics of energy flow and trophic structure of a *P. trituberculatus* polyculture system (co-culture with white shrimp *Litopenaeus vannamei* and short-necked clam *Ruditapes philippinarum*) were investigated in this study. Three Ecopath models representing the early, middle, and late culture periods of a *P. trituberculatus* polyculture ecosystem, respectively, were constructed to compare ecosystem traits at different culture periods. The results demonstrated that detritus was the main energy source in this polyculture ecosystem, and most of the total system throughput occurred at trophic levels I and II. Artificial food input and consumption by the culture organisms increased from early to middle and late periods, which produced marked impacts on biomass structure and primary production. *R. philippinarum* was considered to have a dominant influence on phytoplankton community dynamics which changed from nano- to pico-phytoplankton predominance, from the middle to the late period. Considering the low utilization efficiency of pico-phytoplankton production, large amounts of detritus accumulated in the sediment in the late period, which may constitute a potential risk for the ecosystem. Ecological network analyses indicated that the total energy flow and level of system organization increased from the early to the middle and late periods, whereas food web complexity and system resilience decreased from early to middle and late periods, which may indicate a trend of decreasing ecosystem stability. The system may be further optimized by increased stocking density of *R. philippinarum* and by introducing macro-algae at a suitable biomass to increase ecosystem stability, energy utilization efficiency, and aquaculture production.

Keywords: *Portunus trituberculatus*, polyculture, trophic structure, dynamics, Ecopath, ecological network analysis

1 INTRODUCTION

Most of the world's fishing areas have reached their maximal potential for capture fishery production, while demand for seafood worldwide is steadily increasing (Pauly et al., 2002; FAO, 2014). Global production from aquaculture has been increasing steadily over the past decade (Soto-Zarazúa et al., 2014), and the constantly increasing demand for seafood can only be met by aquaculturing (Troell et al., 2003). However, potential increase in aquaculture production is limited by environmental challenges and resource constraints (Klinger and Naylor, 2012; FAO, 2020). Integrated multi-trophic aquaculture, which involves the cultivation of fed species (e.g., finfish or shrimps fed sustainable commercial diets) with extractive species (e.g., suspension- and deposit-feeders, or seaweeds or other aquatic vegetation), is considered an effective solution to meet the demands of aquaculture production and achieve sustainable aquaculture development (Chopin et al., 2001; Abreu et al., 2011; Dong et al., 2022).

The swimming crab *Portunus trituberculatus* is an important marine-culture species in China (Song et al., 2006), with a production of 100,895 tons in 2020 [FDAMC (Fisheries Department of Agriculture Ministry of China), 2021]. This species was typically cultured in ponds by small-holders or family farms in eastern coastal areas of China. Polyculture of *P. trituberculatus*, with shrimp (e. g. white shrimp *Litopenaeus vannamei*) and clam (e. g. short-necked clam *Ruditapes philippinarum*) in ponds is considered effective integrated multi-trophic aquaculture of this species (Zhang et al., 2016). *P. trituberculatus* consume large particles of food input (e.g., residue of imported fish and shellfish), and considerable amounts of small particles are not consumed; however, such small particles are a good food source for shrimps. Moreover, clams in polyculture systems inhabit the bottom of ponds and feed on phytoplankton and detritus (Xu et al., 1987; Zhang et al., 2016). Polyculture of these three organisms at suitable farming densities may thus constitute an effective use of food and space resources in *P. trituberculatus* polyculture pond systems.

Aquaculture ponds are simple yet fragile ecosystems (Zhai, 2012; Feng et al., 2017), and system stability is susceptible to organic pollution, eutrophication, and emergence of diseases (Cai, 2006). Culture management and associated technologies (e.g., water and sediment quality regulation, feed management, and co-cultured species) are important for avoiding such threats, however, high-level culture management depends on a profound understanding of the respective aquaculture ecosystem. Various aspects of the *P. trituberculatus* pond ecosystem during the whole culture period have been investigated, including variations in biomass structure of phytoplankton (Mao et al., 2013), zooplankton (Mao et al., 2014), microbial communities (Zhang et al., 2015), variations in size-fractioned chlorophyll a concentration (Sun et al., 2012), and water quality (Zhang et al., 2015; Ban et al., 2015). Most of these studies concentrated on individual processes, which helped understand the *P. trituberculatus* pond ecosystems at population level. However, as ecosystems comprise numerous interacting factors, examination of any such factor in isolation cannot comprehensively explain ecosystem functioning (Likens, 1985; Allen, 1988; Patrício and Marques, 2006). The food web in a *P. trituberculatus* polyculture pond ecosystem was reconstructed in a previous study (Feng et al., 2018b), and energy flow and trophic structures of different *P. trituberculatus* pond culture ecosystems at ecosystem level were previously analyzed (Feng et al., 2017, Feng et al., 2018a). These studies improved our understanding of the structural and functional characteristics of *P. trituberculatus* pond culture ecosystems. However, the temporal dynamics of energy fluxes and trophic structures of *P. trituberculatus* pond ecosystems throughout the culture period remained unclear. This may hamper efforts to determine the impact of *P. trituberculatus* culture activities on pond ecosystems, and this lack precludes science-based management of *P. trituberculatus* pond culturing at ecosystem level.

Here, Ecopath modeling in combination with ecological network analyses was used to investigate temporal dynamics of energy fluxes and the trophic structure of a *P. trituberculatus* polyculture pond ecosystem. Ecopath is a mass-balanced ecosystem model, which presents a "snapshot" of the trophic flows between species in an ecosystem (Christensen et al., 2005). Ecological network analysis is a useful and efficient tool for quantifying the condition of an ecosystem, and to systematically analyze each defined ecosystem period (Dubois et al., 2012; Akoglu et al., 2014; Tecchio et al., 2015). The Ecopath model can be used in combination with ecological network analysis to analyze energy flow and trophic structures of aquaculture ecosystems (Ulanowicz, 2004; Zhou et al., 2015; Kluger et al., 2016b; Feng et al., 2017; Aubin et al., 2021; Dong et al., 2021a; Mayekar et al., 2022). Ecopath models also facilitate comparisons between different aquaculture ecosystems or within an ecosystem during different periods (Christensen and Pauly, 1992a; Días López et al., 2008; Feng et al., 2018a). In addition, this model was used to evaluate the carrying capacity of cultured species in aquaculture ecosystems (Jiang and Gibbs, 2005; Byron et al., 2011a; Byron et al., 2011b; Xu et al., 2011; Kluger et al., 2016a; Dong et al., 2021b). In this study, we used Ecopath to model energy flow in a *P. trituberculatus* polyculture pond ecosystem (co-cultured species included *L. vannamei* and *R. philippinarum*) during various culture periods (early, middle, and late). The produced insights may improve our understanding of temporal dynamics of biomass structure and system functioning in *P. trituberculatus* polyculture ecosystems.

2 MATERIAL AND METHODS

2.1 Pond and Enclosures

The *P. trituberculatus* polyculture experiment was conducted using the land-based experimental enclosures in a pond. Enclosure is an efficient tool to investigate aquatic ecosystem, which establishes a relatively closed ecosystem by enclosing the

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Abbreviations: Pot, P. trituberculatus; Liv, Litopenaeus vannamei; Rup, Ruditapes philippinarum; Mab, macrobenthos; Mib, microbenthos; Maz, macrozooplankton; Miz, microzooplankton; Beb, benthic bacteria; Bap, bacterioplankton; Mip, micro-phytoplankton; Nap, nano-phytoplankton; Pip, pico-phytoplankton; Pep, periphyton; All, Aloidis laevis; Shf, shrimp feeds; Des, detritus in sediment; Dew, detritus in water.

water body, and there is no water exchange with the surrounding water (Sun et al., 2011). Land-based enclosure in this study means the enclosure built on the bottom of the pond to simulate pond ecosystem (Li et al., 1998b), which was different from the floating enclosure (Li et al., 1993). The experimental pond located in Ganyu County, Jiangsu Province, China (34°58′17.30″ N, 119°11′53.70″ E), covered 2 ha, with a water column depth of 1.6–1.7 m at the study site. Four land-based enclosures, representing four replicates of the same size (length × width × depth = 5 × 5 × 2 m), were constructed in the pond, which were lined with polyethylene (water-proof) and supported with wood poles. The structure of the enclosures was previously described in detail (Wang et al., 1998; Tian et al., 2001). At the bottom of the enclosure, the walls were covered with mud from the same pond, and supported by posts at 2.5-m intervals. An aeration system consisting of a blower, PVC tubes, gas tubes, and air stones was used for aeration and water circulation. Five air stones connected by a gas tube were suspended approximately 20 cm from the bottom in each enclosure. The land-based enclosure can satisfactorily simulate a pond and be suitably used for aquacultural and ecological experiments (Li et al., 1998). The entire experiment was carried out for 90 days from July 13 to October 13, 2014. Detailed descriptions of the enclosure are provided in the **Supplementary Material** (part 1. Location and layout of the experiment).

2.2 Cultured Animals and Aquaculture Management

Juvenile swimming crabs (*P. trituberculatus*) were cultured with white shrimp *L. vannamei* and short-necked clam *R. philippinarum* in the four enclosures in the pond. All animals were purchased from Ganyu Jiaxin Aquatic Food Co., Ltd. (Ganyu, Jiangsu, China). The stocking densities of swimming crabs, white shrimps, and short-necked clams used in the four enclosures were 6 individuals/m^2, 45 individuals/m^2, and 30 individuals/m^2, respectively. Swimming crabs, white shrimps, and short-necked clams were stocked with an initial individual wet weight of 0.58 ± 0.10 g, 0.05 ± 0.01 g, and 0.91 ± 0.10 g, respectively.

Crabs cultured in the four enclosures were fed with chilled blue clam (*Aloidis laevis*) twice per day (06:00 and 18:00). The amounts of *A. laevis* supplied to the crabs were adjusted according to Zhou et al. (2010). Shrimps were fed a commercial pellet diet (Lianyungang Chia Tai Feed Co., Ltd., Lianyungang, Jiangsu, China), the amount of which was determined according to the product specifications. To assess growth and health condition of crabs and shrimps, 15–20 individuals per enclosure were sampled every ten days using a cage net (0.60 × 0.40 × 0.15 m) designed to trap crabs and shrimps in each enclosure; individuals were returned to the enclosure when sampling was completed.

2.3 Model Construction

The early, middle, and late periods of *P. trituberculatus* polyculturing were at 0–30, 31–60, and 61–90 d of the 90-d culture period, respectively. Three mass-balanced Ecopath models representing early, middle, and late culture periods of the *P. trituberculatus* polyculture ecosystem were constructed.

Ecopath models were constructed according to two master equations (Christensen and Pauly, 1992a; Christensen and Pauly, 1992b). The first equation represents the production of each functional group utilized in the ecosystem. Production is divided into predation, migration, biomass accumulation, exports, and other mortality, and is expressed as follows (Christensen et al., 2005):

$$B_i \times \left(\frac{P}{B}\right)_i \times EE_i - \sum_j \left(B_j \times \left(\frac{Q}{B}\right)_j \times DC_{ij}\right) - Y_i - BA_i - E_i = 0$$

where B_i represents prey biomass (i), B_j represents predator biomass (j), $(P/B)_i$ represents the ratio of production to prey biomass (i), EE_i is ecotrophic efficiency, Y_i is catch biomass, $(Q/B)_j$ is the ratio of food consumption to predator biomass (j), DC_{ij} is the ratio of biomass (i) being consumed by (j) to the total consumption of (j), BA_i is the prey biomass accumulation rate (i), and E_i is the difference between immigration and emigration of prey (i).

The second equation describes the consumption of a consumer group which is constituted by production, respiration, and unassimilated food:

$$B_i \times \left(\frac{Q}{B}\right)_i = B_i \times \left(\frac{P}{B}\right)_i + R_i + U_i$$

where R_i is respiration and U_i is food which cannot be assimilated by consumers. The Ecopath model requires that at least three of the four parameters *B*, *P/B*, *Q/B* and *EE* are imported for each functional group. Because *EE* is difficult to obtain for each functional group, the *EE* value is typically estimated through the input of the other three parameters. Detailed descriptions of limitations, applicability, and methods of this model were published previously (Christensen and Walters, 2004).

We choose to simulate the dynamics of the polyculture ecosystem by constructing Ecopath steady-state models, rather than using dynamic simulation models such as an Ecosim model (Christensen et al., 2005). This is because aquaculture pond ecosystems are vulnerable to external disturbances such as weather changes. External disturbances may prevent Ecosims from accurate and comprehensive simulation of dynamic processes. With Ecopath, most of the model inputs can be obtained by field experiments directly. The modeled results may thus be more reliable than those of Ecosim models.

2.3.1 Functional Groups

According to the definition of the functional group and setting principles of the Ecopath model, 17 functional groups were used in the three Ecopath models. Phytoplankton was divided into three groups: pico-phytoplankton (< 10 μm), nano-phytoplankton (10–38 μm), and micro-phytoplankton (> 38 μm).

Zooplankton were divided into macrozooplankton (copepods and planktonic mollusks, > 150 μm) and microzooplankton (copepodites and rotifers,< 150 μm). The zoobenthos was divided into macrobenthos (polychetes, > 500 μm) and microbenthos (polychetes and nematodes,< 500 μm). Bacteria were divided into benthic bacteria and bacterioplankton. Detritus was divided into detritus in sediment and detritus in water. The imported artificial food of *A. laevis* and shrimp feed were also set as the functional group, both were categorized as detritus groups. The other functional groups included *P. trituberculatus*, *L. vannamei*, *R. philippinarum*, and periphyton (**Table 1**).

2.3.2 Field Data

During the experiment, data on parameters for the models were determined and collected. Input data for each model were the average values of sampling results collected during each culture period. These data were collected as follows:

The input data of biomass, P/B ratio, Q/B ratio, and diet composition of each group were estimated using data derived from field experiments and literature (**Table 2**). The biomass of each functional group, expressed as kJ m^{-2}. The energy content of *P. trituberculatus*, *L. vannamei*, *R. philippinarum*, periphyton, macrobenthos, shrimp feed, blue clam *A. laevis*, and detritus of sediment were measured by an oxygen bomb calorimeter (PARR-1281, America). The energy content of microbenthos, micro-, nano-, and pico- phytoplankton, macro- and micro-zooplankton, bacterioplankton, benthic bacteria, and detritus in water were determined by measuring the organic carbon content first, then converted to energy content. The relation between organic carbon (g) and energy (kJ) was obtained from Salonen et al. (1976) (1g C = 46 kJ).

The unassimilated ratio of consumption of macrozooplankton, and microzooplankton were set at 0.40 (Winberg, 1960), those of macrobenthos and microbenthos were set at 0.4 and 0.30, respectively (Bradford-Grieve et al., 2003), and all other consumers were set at 0.20 (Winberg, 1960). Biomass accumulation of *P. trituberculatus*, *L. vannamei*, and *R. philippinarum* were obtained through field experiments. The detritus input of artificial feeds of shrimp feed and blue clam *A. laevis* were recorded every day by the pond manager.

Detritus fate: according to Heymans et al. (2016), if more than one detritus group is defined, the detritus fate must be described in the model. In this study, it was set that the production, which was not used by the ecosystem (energy of flow to detritus) from the groups of *P. trituberculatus*, *R. philippinarum*, macrobenthos, microbenthos, benthic bacteria, *A. laevis*, and shrimp feed flowed into the detritus group of detritus in sediment; energy from detritus of the groups of macrozooplankton, microzooplankton, bacterioplankton, micro-phytoplankton, nano-phytoplankton, pico-phytoplankton, and periphyton flowed into the detritus group of detritus in water. Moreover, half of the energy of flow to detritus from the group of *L. vannamei* was set to flow into the detritus groups of detritus in sediment and detritus in water, respectively. The energy of detritus in water, which was not used by recycling, ultimately flowed to detritus in the sediment. This assumption was implemented because our field observations showed almost no biomass accumulation for detritus in water at the end of the experiment in the four ecosystems.

The methods of obtaining data of biomass, P/B ratios, Q/B ratios, unassimilated consumption rates, biomass accumulation, and detritus imports of the related functional groups are described in detail in the **Supplementary Material** (part 2. Input data of B, P/B, Q/B, unassimilated consumption rate, biomass accumulation, detritus import, and detritus fate); the diet matrix of the polyculture ecosystem in the early, middle, and late periods is also provided in the **Supplementary Material** (part 3. Diet composition).

TABLE 1 | Functional groups and main species included in models of polyculture ecosystem.

Functional groups	Main species or organic material
Portunus trituberculatus	*P. trituberculatus*
Litopenaeus vannamei	*L. vannamei*
Ruditapes philippinarum	*R. philippinarum*
Macrobenthos	Polychaetes, particle size > 500 μm
Microbenthos	Polychetes and nematodes, particle size< 500 μm
Macrozooplankton	Copepods and planktonic mollusks, particle size > 150 μm
Microzooplankton	Copepod larvae and rotifers, particle size< 150 μm
Benthic bacteria	Heterotrophic bacteria in sediment
Bacterioplankton	Heterotrophic bacteria in water column
Micro-phytoplankton	Phytoplankton, particle size > 38.00 μm
Nano-phytoplankton	Phytoplankton, particle size 10–38 μm
Pico-phytoplankton	Phytoplankton, particle size< 10 μm
Periphyton	Hydrophyte, grown on the polyvinyl plastic of the experimental enclosure
Aloidis laevis	Chilled blue clam *A. laevis*
Shrimp feeds	Shrimp feeds
Detritus in sediment	Detritus in sediment
Detritus in water	Detritus in water column

2.3.3 Model Balancing and Uncertainties

We used EE values< 1 as the first criterion to balance the model; EE values > 1 indicated that the consumed biomass was larger than the produced biomass. Where this occurred, we modified the diet composition of each consumer group, which was expressed as a proportion; such changes in diet proportions never exceeded 0.05. This method was chosen because the diet composition of each consumer was the input data, which was associated with high uncertainty. Some other criteria were also considered, including that the respiration:assimilation ratios should be< 1, and the ratio of respiration:biomass should be higher for mobile consumer groups than for sedentary consumer groups. Moreover, we also ensured that the P/Q values of most of the functional groups were in the range of 0.10–0.30 (Christensen et al., 2005).

2.3.4 Ecological Network Analysis Indicators

Ecological network analysis is a system-oriented method for analyses of within-system interactions used for identifying holistic properties of ecosystems (Fath et al., 2007), which is considered an effective tool for assessing the attributes of system structure and function and to compare ecosystems. Ecopath models use a series of ecological network analysis indicators to assess ecosystem attributes (Christensen et al., 2005). Holistic

TABLE 2 | Data sources of B, P/B, Q/B, and diet composition of each group in the *Portunus trituberculatus* polyculture ecosystem.

Groups	Biomass	P/B	Q/B	Diet composition
Pot	Calculated according to field survey and mortality estimation[1]	Field experiment[1]	Yang et al. (2010)	Feng et al. (2018b)
Liv	Calculated according to field survey and mortality estimation[1]	Field experiment[1]	Qi et al. (2010)	Feng et al. (2018b)
Rup	Calculated according to field survey and mortality estimation[1]	Field experiment[1]	Zhang and Yan (2010)	Wetzel (1983); Feng et al. (2018b), and Zhang et al. (2005)
Mab	Field survey[1]	Zhou and Xie (1995)	Lin (2012)	Wang and Zhang et al. (1998); Feng et al. (2017), and Tsuchiya and Kuhihara (1979)
Mib	Field survey[1]	Schwinghamer et al. (1986)	Lin (2012)	Jin (2010)
Maz	Field survey[1]	calculated according to: P/B=Q/B*(P/Q). The P/Q was obtained from Straile (1997)	by measuring the respiration[5] first, then the Q/B was calculated according to: Q/B = P/B + R/B + U/B. U = 0.4Q (Winberg, 1960), U/B = 0.4Q/B.	Feng et al. (2018b) and Li and Lin (1995)
Miz	Field survey[1]	calculated according to: P/B=Q/B*(P/Q). The P/Q was obtained from Straile (1997)	by measuring the respiration[5] first, then the Q/B was calculated according to: Q/B = P/B + R/B + U/B. U = 0.4Q (Winberg, 1960), U/B = 0.4Q/B.	Feng et al. (2018b) and Li and Lin (1995)
Beb	Field survey[1]	calculated according to: P/B=Q/B*(P/Q). The P/Q was obtained from Moriarty (1986)	by measuring the sediment respiration[6] first, assuming the respiration of benthic bacteria accounts for 0.64 of sediment respiration (Hargrave, 1972), then the Q/B was calculated according to: Q/B = Q/B*(P/Q) + R/B + U/B. The P/Q was obtained from Moriarty (1986), U = 0.2Q (Winberg, 1960), U/B=0.2Q/B.	Zhou (2015)
Bap	Field survey[1]	Field experiment[2]	by measuring the respiration[7] first, then the Q/B was calculated according to: Q/B = P/B + R/B + U/B. U = 0.4Q (Winberg, 1960), U/B = 0.4Q/B.	Zhou (2015)
Mip	Field survey[1]	Field experiment[3]	/	
Nap	Field survey[1]	Field experiment[3]	/	
Pip	Field survey[1]	Field experiment[3]	/	
Pep	Field survey[1]	Field experiment[4]	/	
All	Daily record[1]	/	/	
Shf	Daily record[1]	/	/	
Des	Field survey[1]	/	/	
Dew	Field survey[1]	/	/	

Pot, P. trituberculatus; Liv, Litopenaeus vannamei; Rup, Ruditapes philippinarum; Mab, macrobenthos; Mib, microbenthos; Maz, macrozooplankton; Miz, microzooplankton; Beb, benthic bacteria; Bap, bacterioplankton; Mip, micro-phytoplankton; Nap, nano-phytoplankton; Pip, pico-phytoplankton; Pep, periphyton; All, Aloidis laevis; Shf, shrimp feeds; Des, detritus in sediment; Dew, detritus in water. B, biomass; U, unassimilated consumption; Q, consumption; P/B, production/biomass; Q/B, consumption/biomass; P/Q, production/consumption; R/B, respiration/biomass; U/B, unassimilated consumption/biomass. [1]: the detailed method was described in the ***Supplementary Material****; [2]: method obtained from Schwaerter et al. (1988); [3]: method obtained from Diana et al. (1991); [4]: method obtained from Zhang (2011); [5]: respiration was measured according to Williams (1981); [6]: respiration was measured according to Li et. al., (1998a); [7]: respiration was measured according to Schwaerter et al. (1988).*

ecological network analysis indices typically include total system throughput (TST), EE, Finn's cycling index (FCI), Finn's mean path length (FML), connectance index (CI), system omnivory index (SOI), average mutual information (AMI), flow diversity (H), relative ascendancy(A/C), etc. (Heymans et al., 2007).

TST is the sum of all flows in an ecosystem. It is estimated as the sum of four flow components: total consumption, total export, total respiration, and total flows to detritus. This index represents the size of the entire ecosystem in terms of flow (Ulanowicz, 1986). The *EE* value is calculated as the part of production that is used within or exported out of the system. Transfer efficiency is calculated as the ratio between the sum of exports from a given trophic level (TL) plus the flow that is transferred from one TL to the next and the throughput on the TL (Christensen et al., 2005). The FCI is the fraction of an ecosystem's throughput that is recycled. FML represents the average number of compartments that a unit of energy passes through from its entry into the ecosystem until its leave (Christensen et al., 2005). The CI is the ratio of the number of actual links between functional groups to the number of theoretically possible links. The SOI is defined as the average omnivory index of all consumers weighted by the logarithm of each consumer's food intake, which measures the diversity of consumer–prey relationships in an ecosystem (Christensen et al., 2005).

AMI, H, ascendancy, total development capacity (C), and overhead (O) are indicators built on the foundation of information-theory concepts in ecological networks (Ulanowicz, 1986). H measures both the number of interactions and the evenness of flows in the food web, and the AMI measures the degree of specialization of flows in the food web (Ulanowicz, 1986). Ascendancy is a measure of the average mutual information in an ecosystem, scaled by TST (Ulanowicz and Norden, 1990) (ascendancy (A) = AMI × TST). C measures the potential for a system to develop and is the natural upper limit of ascendancy (C = H × TST) (Ulanowicz, 1986). O measures the uncertainty of energy flow of the network (Ulanowicz and Norden, 1990), and represents the unorganized part of C in an ecosystem (Bodini and Bondavalli, 2002) (O = C-A). The ascendency, O, and C can be split into contributions from imports, internal flow, exports and respiration. The relative overhead (O/C) is calculated as O to C. The A/C is calculated as ascendancy to C. A/C is the fraction of a potential food-web organization that is actually realized, and the O/C is the fraction of a potential food-web organization that is not realized (Ulanowicz, 1986).

3 RESULTS

3.1 Parameters of Each Group of the Three Models

The basic input data and the estimated EE values of the three models are presented in **Tables 3–5**. The biomass of *P. trituberculatus*, *L. vannamei*, *R. philippinarum*, and benthic bacteria increased from the early to the middle and late period, while macro- and micro-benthos biomass showed a decreasing trend over these periods. The biomass of macro- and micro-zooplankton, bacterioplankton, and micro- and nano-phytoplankton increased from the early to the middle period, and then decreased from the middle to the late period. The biomass of pico-phytoplankton showed a marked increase from the middle to the late period. The groups of microbenthos and benthic bacteria had the lowest EE values in models of all three periods owing to the lack of consumers. The EE values of these two groups were all< 0.10. The periphyton also had low EE values in models of the early, middle, and late periods, with the values of 0.12, 0.11, and 0.04, respectively. Moreover, the EE value of pico-phytoplankton was also low in the model of the late period (0.24). The groups of *P. trituberculatus*, *L. vannamei* and *R. philippinarum* all had high EE values in the three models, and the EE values were higher than 0.90 in models of the middle and late period. The groups of blue clam *A. laevis* and shrimp feed also had high EE values (> 0.80, each) in models of all three periods. The detritus in sediment had high EE values in the early and middle periods (0.95 and 0.99, respectively). Other groups including microzooplankton and bacterioplankton had high EE values in the model of the late period (0.86 and 0.85, respectively).

Consumption by various groups in models of the three periods is presented in **Figure 1**. The total consumption increased from the early to the middle and late period. Benthic bacteria were the dominant consumers in all three culture periods, followed by *L. vannamei*. Bacterioplankton and macrozooplankton had the third and fourth largest consumption, respectively, in the early period, whereas bacterioplankton and *P. trituberculatus* had the third- and fourth-largest consumption, respectively, in both middle and late periods. Macrobenthos had the least consumption in all three culture periods, followed by microbenthos and microzooplankton.

3.2 Energy Flow Structure of the Modeled Ecosystem

Aggregating the three modeled ecosystems into discrete TLs led to the identification of five integer trophic levels in the early, middle, and late periods (**Tables 6–8**). TL I in the three modeled ecosystems was composed of primary producers, including micro-, nano-, and pico-phytoplankton; periphyton; and the detritus groups of *A. laevis*, shrimp feed, detritus in sediment, and detritus in water. 75.76%, 82.17%, and 76.77% of the energy on TL I originated from detritus groups in the early, middle and late periods, respectively, whereas the remaining 24.24%, 17.83%, and 23.23% originated from the primary producers in the early, middle and late periods, respectively. Energy flows at TL II encompassed almost all the consumer groups in the early, middle and late periods in this ecosystem, except microbenthos, which was more relevant at TL III in this ecosystem during all three periods. Most of the energy flow of the 17 groups in the early, middle, and late periods in this ecosystem was distributed at TLs I and II. Energy flow at TLs IV and V during the three culture periods was negligible. Benthic bacteria had the highest energy of flow to detritus in the early and middle periods (1493.45 and 1703.20 kJ m^{-2}, respectively), whereas in the late period, the highest energy flow to detritus was pico-phytoplankton (1950.37 kJ m^{-2}). The second-highest energy flow to detritus was pico-phytoplankton (532.80 kJ m^{-2}), macrozooplankton (638.61 kJ m^{-2}), and benthic bacteria (1914.31 kJ m^{-2}) in the early, middle, and late periods, respectively.

A Lindeman spine plot was constructed to represent flow and biomass for the models of the early, middle, and late culture periods (**Figure 2**). Primary production was calculated as 40.66, 60.38, and 54.84 kJ m^{-2} for the models of the early, middle, and late periods, respectively, which contributed approximately 11.92%, 8.78%, and 11.13%, respectively to the TST. In the early, middles, and late periods, 891.60, 1001.00, and 1180.00 kJ m^{-2} 30 $days^{-1}$, respectively, of primary production were consumed by its consumers, whereas 1156.00, 1213.00 and 2582.00 kJ m^{-2} 30 $days^{-1}$, respectively, of primary production flowed to the detritus groups. In the early, middle, and late periods, 5,683.00, 8,857.00, and 10,843.00 kJ m^{-2} 30 $days^{-1}$, respectively, of total energy flow to detritus was consumed by primary consumers, whereas 150.55, 28.63, and 750.11 kJ m^{-2} 30 $days^{-1}$, respectively, of the total energy flow to detritus was accumulated in the group of detritus in sediment. The energy transfer efficiencies from TL II to III in the early, middle, and late periods was 4%, 4%, and 2%, respectively. The transfer efficiencies were lower than the 10% energy transfer efficiency of typical natural ecosystems (Lindeman, 1942). Most of the TST was distributed at TLs I and II in all three models.

3.3 System Attributes of the Modeled Ecosystem

The system attributes of the three models are described in **Table 9**. The TST in this ecosystem comprised total respiration, total consumption, and total energy flow to detritus; TST increased

TABLE 3 | Ecopath inputs and outputs for a *Portunus trituberculatus* polyculture ecosystem model during the early period.

Groups	Biomass (kJ m^{-2})	*P/B* (30 d^{-1})	*Q/B* (30 d^{-1})	*EE*	*P/Q*	Biomass accumulation (kJ m^{-2} 30 d^{-1})	Detritus import (kJ m^{-2} 30 d^{-1})
Pot	106.80	1.94	4.73	**0.81**	**0.41**	166.85	
Liv	159.07	1.85	7.70	**0.85**	**0.24**	250.03	
Rup	51.46	0.62	10.16	**0.92**	**0.06**	29.42	
Mab	0.73	2.15	7.16	**0.32**	**0.30**		
Mib	2.39	3.08	10.25	**0.06**	**0.30**		
Maz	12.30	13.86	47.81	**0.41**	0.29		
Miz	0.67	90.05	310.50	**0.73**	0.29		
Beb	35.59	25.50	84.60	**0.02**	0.30		
Bap	10.59	22.73	69.78	**0.51**	**0.33**		
Mip	5.24	53.98		**0.67**			
Nap	12.24	62.56		**0.42**			
Pip	13.05	69.09		**0.41**			
Pep	10.13	9.63		**0.12**			
All	36.94			**0.83**			1108.16
Shf	27.31			**0.84**			819.36
Des	4159.63			**0.95**		150.55	
Dew	287.24			**0.57**			

Values in bold are the parameters estimated by the model. B, biomass; P/B, production/biomass; Q/B, consumption/biomass; EE, ecotrophic efficiency; P/Q, production/consumption. P/B, Q/B, and detritus import were calculated for the 30 d experimental period.

from the early to the middle and late period, with the values of 17,176.73, 25,211.79, and 33,803.86 kJ m^{-2} 30 days^{-1}, respectively. Total primary production, total production, and total biomass also showed an increasing trend from the early to the middle and late period. The ratio of total primary production to total respiration (TPP/TR) was 0.61, 0.43, and 0.50 in the early, middle, and late periods, respectively. On account of the high energy import *via* the addition of *A. laevis* and shrimp feed to support the growth of the cultured organisms, net system production had values of -1330.67, -2950.29, and -3752.76 kJ m^{-2} 30 days^{-1} in the early, middle, and late periods, respectively. FCI was calculated as 20.38%, 17.90%, and 12.28% for the early, middle, and late periods, respectively. FML decreased from the early to the middle and late period, with values of 5.08, 4.89, and 4.50, respectively. Values of ascendancy showed an increasing trend from the early to the middle and late period. The A/C in the early, middle, and late periods was 41.51%, 42.41%, and 45.05%, respectively, and a pronounced increase was noted in late period. The O/C in the early, middle, and late periods was 58.49%, 57.59%, and 54.95%, respectively, and a marked decrease was noted in the late period.

TABLE 4 | Ecopath inputs and outputs for a *Portunus trituberculatus* polyculture ecosystem model during the middle period.

Groups	Biomass(kJ m^{-2})	*P/B*(30 d^{-1})	*Q/B*(30 d^{-1})	*EE*	*P/Q*	Biomass accumulation (kJ m^{-2} 30 d^{-1})	Detritus import (kJ m^{-2} 30 d^{-1})
Pot	350.76	1.05	3.18	**0.93**	**0.33**	343.28	
Liv	464.93	0.96	5.05	**0.97**	**0.19**	431.28	
Rup	79.44	0.51	9.27	**0.94**	**0.06**	37.92	
Mab	0.58	2.15	7.16	**0.89**	**0.30**		
Mib	2.15	3.08	10.25	**0.06**	**0.30**		
Maz	14.94	20.54	70.84	**0.30**	0.29		
Miz	1.56	55.74	192.21	**0.79**	0.29		
Beb	41.76	24.10	79.15	**0.01**	0.30		
Bap	16.94	27.32	81.38	**0.51**	**0.34**		
Mip	10.49	44.26		**0.70**			
Nap	20.51	48.68		**0.37**			
Pip	9.13	61.32		**0.50**			
Pep	20.25	9.42		**0.11**			
All	75.76			**0.95**			2272.80
Shf	50.63			**0.90**			1518.60
Des	4284.26			**0.99**		28.63	
Dew	382.99			**0.75**			

*Values in bold are the parameters estimated by the model. Group abbreviations as in **Table 2**. B, biomass; P/B, production/biomass; Q/B, consumption/biomass; EE, ecotrophic efficiency; P/Q, production/consumption.*

TABLE 5 | Ecopath inputs and outputs for a *Portunus trituberculatus* polyculture ecosystem model during the late period.

Groups	Biomass (kJ m^{-2})	*P/B* (30 d^{-1})	*Q/B* (30 d^{-1})	*EE*	*P/Q*	Biomass accumulation (kJ m^{-2} 30 d^{-1})	Detritus import (kJ m^{-2} 30 d^{-1})
Pot	550.99	0.58	2.32	**0.98**	**0.25**	313.59	
Liv	795.55	0.63	4.88	**0.99**	**0.13**	494.24	
Rup	101.51	0.40	8.16	**0.95**	**0.05**	38.61	
Mab	0.36	2.15	7.16	**0.82**	**0.30**		
Mib	1.91	3.08	10.25	**0.04**	**0.30**		
Maz	11.42	13.61	46.93	**0.78**	0.29		
Miz	0.87	53.30	182.75	**0.86**	0.29		
Beb	51.53	19.20	91.00	**0.01**	0.21		
Bap	7.41	18.21	123.79	**0.85**	**0.15**		
Mip	3.72	76.79		**0.81**			
Nap	8.54	80.28		**0.49**			
Pip	18.27	139.68		**0.24**			
Pep	24.30	9.81		**0.04**			
All	99.90			**0.88**			2997.00
Shf	78.41			**0.91**			2352.30
Des	5450.97			**0.86**		750.11	
Dew	239.37			**0.43**			

Values in bold are the parameters estimated by the model. Group abbreviations as in ***Table 2***.

4 DISCUSSION

4.1 Biomass Dynamics of Phytoplankton With Different Particle Sizes

During the 90-day culture period in the present study, the biomass of micro-, nano-, and pico-phytoplankton expressed varying fluctuating trends. The biomass of both micro- and nano-phytoplankton increased from the early to the middle period, and then decreased from the middle to the late period. In contrast, the biomass of pico-phytoplankton showed the opposite trend: it decreased from the early to the middle period, and then increased from the middle to the late period. The total phytoplankton biomass and primary production increased from early to middle and late periods. In line with Ban et al. (2015), inorganic nutrients (e.g., NH$_4^+$, NO$_3^-$, and NO$_2^-$) increased remarkably from the early to the middle period in this *P. trituberculatus* polyculture ecosystem, considering the input of artificial foods. It was speculated that the increase in inorganic nutrients may have led to the increase in total phytoplankton biomass and primary production from the early to the middle period (Ramírez et al., 2005). The dominant phytoplankton community changed from nano-phytoplankton to pico-phytoplankton from the middle to the late period. This phenomenon may be correlated with the polyculture of *R. philippinarum*. The filter-feeding *R. philippinarum* efficiently transfers particulate matter present in the water column to the sediments by filtration coupled with biodeposition (Kautsky and Evans, 1987). Prins et al. (1998) and Souchu et al. (2001) reported that the consumption of phytoplankton by filter feeders could induce a shift in the phytoplankton composition towards phytoplankton with smaller cells and faster growth rates. *R. philippinarum* exhibits the highest phytoplankton consumption in the late period compared to the early and middle periods; thus, phytoplankton consumption by *R. philippinarum* may induce a change in the phytoplankton composition, mainly comprising pico-phytoplankton, which has a higher turnover rate. However, the low *EE* value (0.24) of pico-phytoplankton in the late period indicates that phytoplankton consumption by *R. philippinarum* could not suppress the growth of pico-phytoplankton, and that *R. philippinarum* was not food limited in this ecosystem.

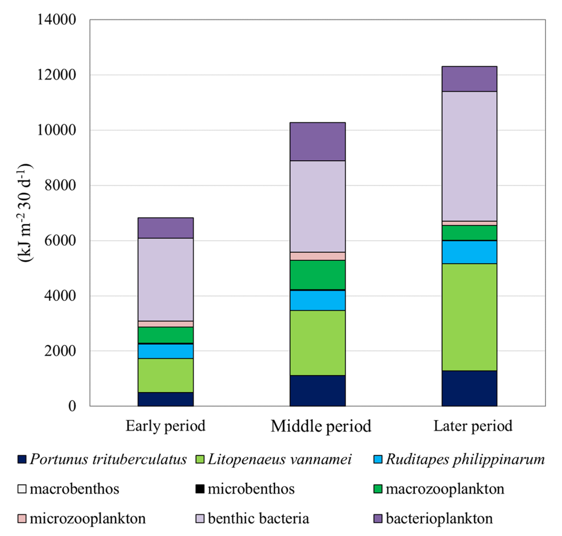

FIGURE 1 | Allocations of total consumption (kJ m^{-2} 30 d^{-1}) of each consumer for a *Portunus trituberculatus* polyculture ecosystem during the early, middle, and late periods, respectively.

4.2 Analysis of Dynamics of Energy Flow Structure From Early to Late Period

The energy input in aquaculture pond ecosystems mainly comprises primary production and imported foods (Feng et al.,

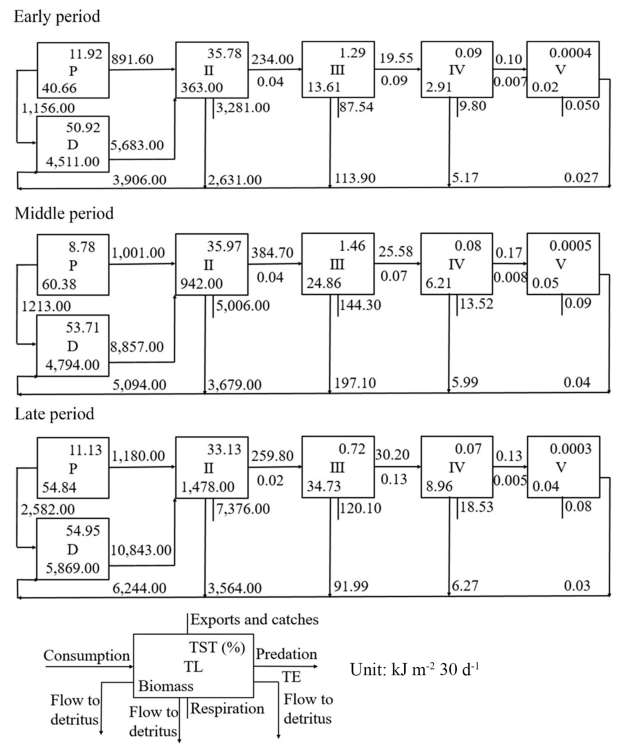

FIGURE 2 | Aggregation of flows and biomass (kJ m^{-2} 30 d^{-1}) of a *Portunus trituberculatus* polyculture ecosystem during the early, middle, and late periods, respectively (D, detritus; P, primary production; TL, trophic level; TE, transfer efficiency; TST, total system throughput).

2015; Dong et al., 2017). Primary production and imported foods (blue clam *A. laevis* and shrimp feed) increased from early to late period in this ecosystem. Primary production accounted for 50.29%, 34.79%, and 39.71% of total energy input in the early, middle, and late periods, respectively. However, approximately 59.28%, 59.97%, and 73.29% of primary production was not utilized by consumers and flowed to detritus groups. Energy originating from detritus accounted for 83.00%, 87.00%, and 85.00% of total system throughput in early, middle, and late period, respectively. As a result, the polyculture ecosystem remained a detritus-dominated ecosystem throughout, which was in accordance with the results of Feng et al. (2017) and Feng et al. (2018a), showing that the detritus food chain is predominant in *P. trituberculatus* polyculture systems.

The group of benthic bacteria was the largest consumer over the entire culture period, thus the associated energy pathway of detritus in sediment to benthic bacteria was the main energy flow pathway. However, the energy flow structure showed some changing trends associated with organisms as total consumption by *P. trituberculatus*, *L. vannamei*, and *R. philippinarum* to total system consumption showed an increasing trend from early to middle and late periods, which accounted for 32.99%, 40.90%, and 48.64% of the total system consumption, respectively. Increased consumptions of these three functional groups led to increased energy flow to detritus during the entire culture period; moreover, pico-phytoplankton showed the highest energy flow to detritus in the late period. The total energy flow to detritus groups thus showed an increasing trend from the early to the middle and late period (4,221.29, 5,366.55, and 6,810.27 kJ m^{-2} 30 $days^{-1}$, respectively), and large amounts of energy were accumulated in the detritus group of detritus in sediment. Increased detritus accumulation was considered a potential risk to the ecosystem, which may cause deterioration of the bottom environment in a pond ecosystem (Lin et al., 2005). Management practices such as increasing bottom oxygen supply should be implemented to reduce this risk.

TABLE 6 | Absolute flows of each group by trophic level decomposition of a *Portunus trituberculatus* polyculture ecosystem during the early period.

Groups	**Trophic levels**					**FD (kJ m^{-2} 30 d^{-1})**	**ETL**
	I	**II**	**III**	**IV**	**V**		
Pot		324.40	10.24	3.60	0.03	141.37	2.05
Liv		934.80	29.38	10.55	0.04	289.22	2.05
Rup		486.50	6.91	0.00		107.05	2.01
Mab		4.46	0.42	0.30		3.13	2.20
Mib		8.56	15.90	0.00		14.23	2.65
Maz		432.30	155.20	0.62		335.42	2.27
Miz		205.30	2.92			99.57	2.01
Beb		3011.00				1493.45	2.00
Bap		738.90				266.48	2.00
Mip	283.10					92.55	1.00
Nap	765.80					445.28	1.00
Pip	901.60					532.80	1.00
Pep	97.52					85.76	1.00
All	1108.00					184.69	1.00
Shf	819.40					130.29	1.00
Des	2993.00					0.00	1.00
Dew	1078.20					820.80	1.00
Total value	8046.62	6146.23	220.97	15.07	0.08		

*Group abbreviations of each functional group as in **Table 2**. FD, flow to detritus; ETL, effective trophic level.*

TABLE 7 | Absolute flows of each group by trophic level decomposition of a *Portunus trituberculatus* polyculture ecosystem during the middle period.

Groups	Trophic levels					FD (kJ m^{-2} 30 d^{-1})	ETL
	I	II	III	IV	V		
Pot		740.50	23.40	8.18	0.08	248.10	2.05
Liv		1878.00	28.21	10.08	0.04	484.64	2.03
Rup		681.00	17.46			149.88	2.03
Mab		3.57	0.34	0.24		1.79	2.20
Mib		7.71	14.32	0.00		12.86	2.65
Maz		778.90	278.20	1.17		638.61	2.27
Miz		294.80	5.10			138.11	2.02
Beb		3306.00				1703.20	2.00
Bap		1379.00				504.53	2.00
Mip	464.20					139.17	1.00
Nap	998.60					624.64	1.00
Pip	559.90					279.07	1.00
Pep	190.80					169.62	1.00
All	2273.00					123.78	1.00
Shf	1519.00					148.55	1.00
Des	3424.00					0.00	1.00
Dew	1914.22					621.78	1.00
Total value	11343.72	9069.48	367.03	19.67	0.12		

*Group abbreviations of each functional group as in **Table 2**. FD, flow to detritus; ETL, effective trophic level.*

4.3 Analysis of Ecosystem Attribute Dynamics Using Ecological Network Analysis

Biomass change of each functional group during the entire culture period could lead to changes in ecosystem structure, which in turn could lead to changes in ecosystem function. The indices of TST, AMI, A/C, and O/C are assigned to a number of groups that describe ecosystem properties in terms of ecosystem structure (Tomczak et al., 2013). In the polyculture ecosystem of the present study, TST, AMI, and A/C all increased from the early to the late period. The increase in TST indicated that the ecosystem size increased from the early to the late period. The increase in AMI indicated that the inherent organization, i.e. the degree of specialization of flow in the network of this polyculture ecosystem, increased from the early to the late period, which represents a trend of simplification of the food web structure (Latham and Scully, 2002). The A/C, which is the ratio of AMI to H, showed a marked increase from the middle to the late period, because of the decrease in H and increase in AMI, and this indicated that the ecosystem had the highest level of system organization in the late period. The system overhead represents

TABLE 8 | Absolute flows of each group by trophic level decomposition of a *Portunus trituberculatus* polyculture ecosystem during the late period.

Groups	Trophic levels					FD (kJ m^{-2} 30 d^{-1})	ETL
	I	II	III	IV	V		
Pot		925.20	29.95	9.54	0.06	261.65	2.05
Liv		3327.00	46.10	14.83	0.04	783.41	2.02
Rup		768.40	21.32			167.66	2.03
Mab		2.23	0.21	0.15		1.18	2.20
Mib		6.85	12.72	0.00		11.52	2.65
Maz		405.30	130.40	0.40		248.26	2.24
Miz		158.10	1.60			70.22	2.01
Beb		4689.00				1914.31	2.00
Bap		917.60				203.93	2.00
Mip	285.80					53.86	1.00
Nap	685.90					349.84	1.00
Pip	2552.00					1950.37	1.00
Pep	238.40					227.70	1.00
All	2997.00					356.51	1.00
Shf	2352.00					209.85	1.00
Des	4687.00					0.00	1.00
Dew	1373.00					1816.00	1.00
Total value	15171.10	11199.68	242.30	24.93	0.10		

*Group abbreviations of each functional group as in **Table 2**. FD, flow to detritus; ETL, effective trophic level.*

TABLE 9 | System attributes for a *Portunus trituberculatus* polyculture ecosystem during the early, middle, and late period, respectively.

System attribute parameters	Early period	Middle period	Late period	Units
Total consumption	6828.50	10268.38	12313.51	kJ m^{-2}
Total respiration	3378.62	5163.70	7514.78	kJ m^{-2}
Total flow to detritus	6969.61	9779.72	13975.57	kJ m^{-2}
Total system throughput	17176.73	25211.79	33803.86	kJ m^{-2}
Total production	9751.87	16364.67	22005.13	kJ m^{-2}
Total primary production	2047.95	2213.41	3762.02	
TPP/TR	0.61	0.43	0.50	
Total net production	-1330.67	-2950.29	-3752.76	
TPP/TB	4.87	2.14	2.39	
Total biomass (no detritus)	420.25	1033.45	1576.40	kJ m^{-2}
Connectance index	27.20	27.20	27.20	%
System omnivory index	0.06	0.05	0.05	
Finn's cycling index (FCI)	20.38	17.90	12.28	%
Finn's mean path length (FML)	5.08	4.89	4.50	
Total development capacity (C)	81781.80	122382.40	154362.50	Bits m^{-2}
Overhead (O)	47837.00	70479.30	84822.50	Bits m^{-2}
Redundancy (R)	34880.80	49135.20	58158.70	Bits m^{-2}
Ascendancy (A)	33944.80	51903.10	69540.00	Bits m^{-2}
Relative ascendancy (A/C)	41.51	42.41	45.05	%
Relative overhead (O/C)	58.49	57.59	54.95	%
Average mutual information (AMI)	1.98	2.06	2.06	
Flow diversity (H)	4.76	4.85	4.57	
Proportion of total flow originating from detritus	83.00	87.00	85.00	%
Pedigree	0.86	0.86	0.86	

Units were calculated over each 30d experimental period. TPP/TR, Total primary production/total respiration; TPP/TB, Total primary production/total biomass.

the resilience of an ecosystem against external perturbations (Ulanowicz and Norden, 1990; Christensen, 1995). The O/C in the late period was lower than that in the early and middle periods, indicating that the ecosystem was less resilient in late period than in the early and middle periods.

FCI and FML are indices that describe the system attributes of energy flow (Tomczak et al., 2013). The values of FCI and FML decreased from the early to the middle and late periods. The fluctuating trends of FCI was contrary to that of A/C. which was in accordance with Baird et al. (1991), who demonstrated an inverse relationship between FCI value and A/C. The decreased FCI value indicated that the proportion of the TST that is recycled decreased from the early to the late period. Considering that the food composition of each consumer was almost unchanged during culturing, the decreased FCI may explain the decreased FML during the entire culture period. The decreased FML also indicated a simplification of the food web structure.

4.4 Ecosystem Optimization

The polyculture system structure changed gradually to an ecosystem with larger size and higher level of organization from the early to middle and late periods. However, the overly increased TST and AMI may jeopardize the stability of ecosystem structure (Ulanowicz, 2003). It was demonstrated that the introduction of *R. philippinarum* may improve H through increasing total energy flow pathways (Feng et al., 2018a); improved H will decrease the AMI and increase system resilience (Ulanowicz, 2004). Moreover, as *R. philippinarum* was not food-limited in the late period, increasing the biomass of *R. philippinarum* may increase the *EE* value of pico-phytoplankton, thereby increasing the energy utilization of primary production. As a result, increasing the stocking density of *R. philippinarum* to maintain higher biomass may be an effective way to optimize such polyculture ecosystems.

Large amounts of detritus energy were accumulated in the group of detritus in sediment. Increased detritus accumulation may cause deterioration of the bottom environment of the pond ecosystem, which would further affect growth of organisms, especially the cultured organisms (Lin et al., 2005). In the present study, energy flow from pico-phytoplankton contributed the largest part of the total energy flow to the detritus. As the growth of phytoplankton was mainly supported by inorganic nutrients (Ban et al., 2015), introduction of macro-algae at a suitable biomass into this ecosystem may help absorb superfluous inorganic nutrients, thereby reducing phytoplankton production, detritus accumulation, and increase ecosystem stability. Moreover, macro-algae can also be used as aquatic products.

5 CONCLUSIONS

The dynamics of biomass composition, energy flow structure, and system function of *P. trituberculatus* was investigated by using the Ecopath model. This study illustrated that the growth of cultured organisms and aquaculture activities such as imported artificial food showed large impacts on the biomass structure of the polyculture ecosystem. *R. philippinarum* was considered to have a dominant influence on the phytoplankton community, which changed from nano- to pico-phytoplankton-dominated from the middle to the late period. Because of the increased primary production, imported artificial food, and consumption of the cultured organisms, the energy flow structure showed some remarkable changes from the early to the middle and late period, and large amounts of detritus energy were accumulated in the sediment in the late period. System network analysis indicated that ecosystem size and level of system organization increased from the early to the middle and late periods, whereas food web complexity and system resilience decreased from the early to the middle and late periods, which may indicate decreasing ecosystem stability. Increasing the stocking density of *R. philippinarum* may help increase the energy flow diversity of this ecosystem, which would also increase the system's resilience and energy utilization. Moreover, introducing macro-algae to this ecosystem may help reduce superfluous inorganic nutrients, which may decrease phytoplankton production and detritus accumulation. These two measures are thus proposed to optimize such ecosystems.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

JF carried out the experiments, constructed the models and drafted the manuscript. X-LT and S-LD contributed to the design of the experiment and revised the manuscript. R-PH, KZ, and D-XZ prepared materials and carried out the experiments. D-XZ and Q-QZ revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/articles/10.3389/fmars.2022.873643/full#supplementary-material

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Effect of Aquaculture-Related Diets on the Long-Term Performance and Condition of the Rock Crab, *Cancer irroratus*

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Shellfish and salmonid aquaculture operations in Eastern Canada attract several mobile epibenthic species as a result of added structural complexity and increased food availability (bivalve fall-off and waste salmonid feed). It is not clear whether the aggregation of predators and scavengers below coastal farms contributes positively or negatively to their population dynamics, due to concerns about the quality of food items found under farms. We conducted an 18-month laboratory study to investigate the effect of diets composed of 1) mixed items, 2) mussels (*Mytilus edulis*), and 3) salmonid feed on the performance and condition of the rock crab, *Cancer irroratus*. Diet had no impact on crab survival but several negative consequences were observed in crabs fed the salmonid feed diet when compared to the mixed diet: reduced 1) moulting rates during the second growing season, 2) inter-molt growth, 3) gonad and hepatopancreas indices, 4) hemolymph dissolved compounds, 5) hepatopancreatic glycogen, and 6) shell hardness. Crabs fed the mussel diet had similar performance and condition when compared to the mixed diet. Fatty acid composition of muscle, gonad, and hepatopancreas tissues revealed that a salmonid feed diet decreased n3/n6 ratio when compared to a mixed or a mussel diet; those differences were mostly due to increases in the proportions of terrestrial (18:1n9 and 18:2n6) and decreases in proportions of marine essential (20:5n3 and 22:6n3) fatty acids. Together, these results point to a minimal impact of a mussel-only diet on crabs, whereas the salmonid feed diet resulted in negative impacts on condition. Our experimental results explored the consequences of a ‘worst-case scenario’ in which crabs were forced to feed on a single item for a long period of time; the realized impact in field settings will depend on other factors such as consumption of alternate food items underneath a farm, proportion of time spent in farms, and level of overlap between crab habitat and aquaculture facilities.

Keywords: waste salmonid feed, mussel fall-offs, anthropogenic food subsidies, fatty acid composition, shellfish aquaculture, salmonid aquaculture

INTRODUCTION

Coastal aquaculture has been developing rapidly over the past few decades with well-documented negative impacts on the biodiversity of infaunal organisms located directly below and in the immediate vicinity of farms, largely due to organic enrichment (Weise et al., 2009; Holmer, 2010; Weitzman et al., 2019). However, farms also have positive impacts by adding structural complexity to otherwise featureless bottoms and water columns, offering substrate for sessile organisms and shelter for mobile organisms (McKindsey, 2011; Theuerkauf et al., 2021). In addition, large quantities of food can fall to the bottom under and in the proximity of farms. For instance, in mussel farms, many individuals fall to the bottom through the process of self-thinning as mussels grow in size and compete for space on suspended lines (McKindsey et al., 2011; Fréchette, 2012). In finfish net farms, a non-negligible proportion of food pellets are not consumed by the farmed fish and sink to the bottom (Holmer et al., 2005; Wang et al., 2012). In both cases, several species of mobile predators and scavengers tend to be more abundant under farms compared to surrounding areas (Drouin et al., 2015; Callier et al., 2018; Barrett et al., 2019; Theuerkauf et al., 2021). Several taxa have been determined to assimilate energy coming from farms, either directly or indirectly; these include sponges (Laroche et al., 2021), polychaetes (Salvo et al., 2015; White et al., 2017a; Woodcock et al., 2019), echinoderms (White et al., 2017b; Woodcock et al., 2018), bivalves (Handå et al., 2012; Irisarri et al., 2015), and decapods (Olsen et al., 2012; Izquierdo-Gomez et al., 2015; Woodcock et al., 2018; Sardenne et al., 2019; Baltadakis et al., 2020; Sardenne et al., 2020). Therefore, these anthropogenic inputs of resources might impact natural population dynamics, including the productivity of several commercially-important species.

It remains unclear if farm-related food subsidies have overall positive or negative effects on associated wild populations. If individuals frequenting farms are able to use the energy added to the ecosystem to improve their condition and reproductive outputs, then farms could contribute positively to population dynamics. This has been suggested for the sea star *Coscinasterias muricata* in a New Zealand mussel farm, where sea star aggregations might improve their fertilization rates during broadcast spawning (Inglis and Gust, 2003). Fish associated with salmonid farms often have elevated condition indices when compared to fish collected away from farms (Fernandez-Jover et al., 2007; Dempster et al., 2011; Fernandez-Jover et al., 2011; Barrett et al., 2018) with limited documented consequences on reproductive output (Barrett et al., 2018). Alternately, if individuals aggregated in farms consume large amounts of salmonid feed or mussels, then nutritional requirements might not be met and farms may have overall negative impacts on population dynamics and stock productivity. For example, American lobsters (*Homarus americanus*) feeding exclusively on mussels in a laboratory experiment had lowered survival and condition when compared to those fed a mixed diet of fish, crustaceans and mollusks (Wang and McGaw, 2016), suggesting that a mussel diet might not meet all dietary requirements of decapod crustaceans. Similarly, salmonid feed may lack some essential dietary components for marine organisms; over the last few decades, fish oil supplies for the production of fish feed could not keep up with growing demand and therefore, sources of proteins and fatty acids in fish feed have shifted progressively from marine to terrestrial sources (Ytrestøyl et al., 2015). Nowadays, most commercial diets include substantial proportions of soy, wheat, rapeseed, linseed oil, and other terrestrial ingredients (Turchini et al., 2009; Ytrestøyl et al., 2015). Modern fish feeds are rich in terrestrial short-chained monounsaturated and omega-6 polyunsaturated fatty acids (e. g., 18:1n9 and 18:2n6) and poor in marine long-chain omega-3 polyunsaturated fatty acids (e. g., 20:5n3 and 22:6n3; Sardenne et al., 2020). This lipidic imbalance may have negative effects for marine species for which long-chained omega-3 are often considered essential fatty acids. For example, White et al. (2016; 2018) found that a diet with high concentrations of omega-6 polyunsaturated fatty acids had negative impacts on reproduction and larval development for two sea urchin species. Similar effects on other marine invertebrates are likely, with possible deleterious consequences on individuals' condition and population dynamics.

In Eastern Canada, the crabs *Cancer irroratus*, *C. borealis* and *Hyas* spp. are fished commercially with landings of 3.5-6 kt annually (2015-2019), for a market value of CAD$ 5-8 million (Fisheries and Oceans Canada, 2021). The rock crab, *C. irroratus*, inhabits coastal rocky and soft-bottom habitats where they feed on polychaetes, mussels, sea urchins, and various other animals and debris (Scarratt and Lowe, 1972). They form reproductive couples, by which a male will grasp a pre-moult female, mate shortly after her moulting, and then release her after her carapace hardens; fertilized eggs are extruded the following year and the female will carry the embryos on her abdomen until hatching (Bigford, 1979). Field studies showed that *C. irroratus* is found at higher density below both mussel and salmonid farms compared to surrounding areas (Clynick et al., 2008; D'Amours et al., 2008; Drouin et al., 2015; McKindsey, unpublished). Acoustic telemetry revealed that they remain relatively sedentary under mussel farms for periods of weeks; the same pattern is observed under salmon farms, although more movement is observed (McKindsey, unpublished data). Under mussel farms, crabs actively feed on mussel fall-off since analyses of gut content showed a greater proportion of mussels compared to those of crabs in surrounding areas (McKindsey, unpublished data). Crabs also consume salmon waste or waste feed as analyses of muscle, hepatopancreas and gonads showed terrestrial fatty acid and stable isotope fingerprints of salmonid feed in rock crabs collected under salmon farms (Sardenne et al., 2020). In addition, diet breadth in crabs collected under farms was reduced when compared to crabs collected further from farms (Sardenne et al., 2020). The relatively long residence time of rock crabs under farms and apparent dietary preference for mussels and salmonid feed make them likely to be impacted by their diet in the long term, either negatively or positively. This may be particularly relevant since rock crabs are a preferred and essential prey for American lobsters (Gendron et al., 2001), the most lucrative Canadian fishery with an annual value of CAD$ 1.5 billion (Fisheries and Oceans Canada, 2021). Lobsters also frequent mussel and salmonid farms, where they consume a mix of crab, mussels and waste salmonid feed (Sardenne et al., 2019; Sardenne et al., 2020). Therefore, crab condition, whether enhanced or decreased, may cascade down to lobster stock productivity.

In this study, we evaluated the long-term effects of feeding rock crabs with a 1) mixed diet, 2) exclusively salmonid feed, and 3) exclusively mussels to investigate potential impacts of aquaculture operations on rock crab population dynamics. We maintained individuals for 18 months in the laboratory and monitored survival, moulting, and growth. At the end of the experiment, individuals were dissected and several physical and physiological variables measured as proxies for condition and detailed lipid profiles obtained from various tissues. The results directly address concerns raised by fisherman about impacts of aquaculture on their activities, and contribute to our knowledge of the impact of aquaculture-related anthropogenic food subsidies to the benthic ecosystem.

MATERIALS AND METHODS

Animal Collection and Laboratory Setup

The study was conducted in the wetlabs at the Maurice-Lamontagne Institute (Fisheries and Oceans Canada, Mont-Joli, QC, Canada) under natural photoperiod regimes. Rock crabs were collected between April 27 and May 23 2018 using baited-traps deployed at the institute's wharf. Crabs were transported to the laboratory within one hour of being collected from traps and maintained in 1-m^3 tanks with flow-through seawater pumped from a depth of ~15 m in the Saint Lawrence Estuary, approximately 2 km from shore, through a sand-filter. Crabs were fed a mixture of blue mussels (*Mytilus edulis)*, fish (frozen Atlantic mackerel *Scomber scombrus*) and green sea urchins (*Strongylocentrotus droebachiensis)* until they were transferred to the experimental tanks on June 14th 2018. Experimental tanks consisted of 76 L plastic containers (80 × 45 × 30 cm) with the bottom covered in sand; rocks and small plastic buckets cut in half longitudinally provided shelter. Each experimental tank was supplied with flow-through seawater at ambient temperature and salinity (measured twice weekly using a Hanna multiparameter water quality meter model HI98194; range of temperature: 2-12°C and salinity: 22-30 PPT; **Figure S1**) and the flow was adjusted at 2.5 L min^{-1} using a valve and flow meter. We randomly assigned 3 females and 2 males (individually tagged using numbered plastic circles glued to the carapace; carapace width mean ± SD = 50.91 ± 4.76 mm, n = 84 for females and 68.64 ± 4.09 mm, n = 56 for males) to each of 28 experimental tanks. Those crab sizes were chosen because they represent the onset of sexual maturity in females and females tend to mate with males that are slightly larger than themselves (Bigford, 1979).

Experimental Protocol

Tanks were randomly assigned one of three diets: 1) mixed (n=10), 2) blue mussels only (n=9), and 3) salmonid feed only (n=9). The mixed diet was designed to recreate a natural diet and consisted, in random alternation, of whole blue mussels *M. edulis* (20-30 mm in shell length), thawed fish pieces (10-20 cm^3; Atlantic mackerel *S. scombrus*, rainbow smelt *Osmerus mordax* or Atlantic herring *Clupea harengus*, depending on availability), green sea urchin *S. droebachiensis* with the test broken open, and whole common periwinkles *Littorina littorea*. The mussel diet consisted of whole *M. edulis* (20-30 mm shell length) whereas the salmonid feed diet consisted of commercially available formulated pellets (5.5 mm in diameter). Tanks were maintained twice weekly during the growing season (May to November) and weekly during the rest of the year. During maintenance, food remains (e.g. uneaten flesh, shells, and tests) were removed using a fish net and weighed. Then rocks and caches were carefully moved and the substrate cleaned using an aquarium siphon and gravel cleaner; care was taken to minimize stress. New food items were weighed and distributed to each tank according to its assigned diet. Food consumption varied considerably over the course of the experiment because of natural changes in water temperature and increases in crab size; we thus developed a protocol to ensure that crabs fed to satiation while minimizing wastes. If the entire amount of food allotted to a tank of a particular diet was eaten since the last maintenance, the quantity of food given was increased by 20% for all replicates of that diet; the same logic was used to reduce the rations. Whole wet weight per tank varied as follows: blue mussels (~15 to 70 g), salmonid feed (~0.5 to 8 g), common periwinkles (~4 to 40 g), green sea urchins (~15 to 100 g) and fish (~2 to 15 g) for maintenance period.

Based on the amounts of food consumed, the caloric content of the different food items and the weight of crabs present in each tank, we estimated the overall energy consumption (Kcal/100 g of crab) for each inter-maintenance period. We estimated food consumption for each tank based on the amount given during a maintenance and the amount remaining at the following maintenance. For fish, periwinkles, and sea urchins (test broken and body fluids drained), wet weight of each ration given was measured and the uneaten prey remains (flesh, intact shells and shell fragments) carefully collected and weighed; the difference between the weights given and recovered provides a good estimation of the amount of soft tissue consumed. For mussels, the number of individuals given and total whole wet weight was recorded. Because mussels can trap water within their valves and were weighed whole, a correction factor was applied to remove contribution of water to the measurements. This was done by relating wet soft tissue weight to whole mussel weight following dissection of a total of 224 individuals (18-32 mm in shell length) on 6 different dates during summer-fall 2018. We then used the linear relationship between whole wet weight and tissue weight (tissue = 0.21 wet weight -0.01, R = 0.67), the number of individuals consumed, and the average weight of individuals given to calculate flesh consumption. For salmonid feed, we weighted the whole ration and counted the number of pellets given and the number of pellets that were still intact after an inter-maintenance period to estimate weight of pellets consumed.

Samples were prepared to measure the caloric content of each food item. Periwinkles (17-30 mm in shell length), sea urchins (45-67 mm in test diameter) and mussels (20-30 mm shell length) were dissected and soft tissues isolated; individuals were dissected until we obtained a pooled sample of ~100 g wet weight for each species (> 100 periwinkles, 6-9 sea urchins, and > 200 mussels). This was repeated 4 times for periwinkles and sea urchins and 6 times for mussels between May and November 2018. In addition, single samples (~ 100 g) of the three species of fish (mackerel, smelt, and herring) and salmonid feed pellets were prepared. All samples were homogenized and preserved frozen until further processing in a commercial laboratory, where the energy content (kcal/g) was measured.

For each inter-maintenance period, the energy consumed by crabs in a given tank was estimated by multiplying the weight of food consumed by its caloric content (average from multiple samples for periwinkles, sea urchins, and mussels). This value was then divided by the weight of crab present in a tank (taking into account the mortalities and the weight increases due to moulting) to calculate energy consumption in Kcal/100 g of crab. We then calculated averages from all inter-maintenance periods for each tank from mid-June 2018 to early-July 2019. Tanks were monitored regularly and dead individuals were noted and removed. In 2018, a tank was closed if there was not at least one male and one female still alive in the tank since we originally planned to focus on reproduction; remaining individuals were removed and the tank was no longer followed. In 2019, this practice was stopped and all individuals were kept as we approached the end of the experiment and dissection of individuals. The date at which individuals moulted was noted and newly-moulted crabs were placed on floating cages to protect them from cannibalism. After the carapace had hardened, carapace width was measured with a digital vernier caliper, individuals were re-tagged, and returned to their respective tanks. The date and duration of couple formation were also noted.

Dissections

At the end of the experiment (October 27th 2019), all remaining crabs (n = 23 [8 males and 15 females], 11 [6 males and 5 females], and 13 [7 males and 6 females] for the mixed, salmonid feed and mussel diets, respectively) were dissected in the laboratory to measure several indices of condition. Wet crab body weight was measured and a final measure of carapace width was taken. We removed gonads and hepatopancreas tissues and measured their wet weight.

We estimated concentration of hemolymph dissolved compounds using the Brix index, a measure based on refraction of hemolymph which relates well to general physiological condition of crustaceans (Wang and McGaw, 2014). We collected a 1 ml sample of hemolymph from the articulations of walking legs using a 5 ml syringe and a 21 gauge needle and placed the sample on the cell of an Atago Digital Pocket Refractometer (model 3810 PAL-1) to measure the refraction index.

We measured shell hardness using a PTC Shell Hardness Durometer Model 307HF, which provides a dimensionless comparison of breaking point of materials (Hicks and Johnson, 1999). Readings were taken at three locations on the dorsal side of the carapace: at the posterior end (on the middle line) and at the anterior end (both right and left sides).

Glycogen Analyses

Glycogen content analyses were used to evaluate levels of stored energy. During the dissection, 60 mg hepatopancreas samples were collected and preserved at -80°C. Glycogen assays were performed using a commercial Glycogen Assay Kit (Cayman Chemical) at the Centre de Recherche sur les Biotechnologies Marines (CRBM, Rimouski, QC, Canada). Hepatopancreases were homogenized using a Precellys 24 tissue homogenizer, at a concentration of 200 mg/mL with the buffer solution of the kit supplemented with protease inhibitors (Protease Inhibitor Cocktail powder, Sigma-Aldrich) in tubes containing 1.4 mm ceramic balls. The assay was performed with excitation wavelengths between 530 and 540 nm and emission wavelengths from 585 to 595 nm. The glycogen content was reported as mg/g of wet weight.

Fatty Acid Analyses

During the dissection, two 200 mg samples of hepatopancreas, gonads, and muscle (collected from the crusher claw) were collected from the 47 crabs. One sample was oven-dried at 60°C for 120 h to estimate water content; this value was subsequently used to calculate ratio of fatty acid weight to tissue dry weight. The other sample was frozen at -80°C until further processed. Fatty acids (FA) were extracted and methylated from frozen samples following a one-step extraction/methylation procedure (direct methylation; modified from Lepage and Roy, 1984). Samples were homogenized in a conical tissue grinder with 4 ml 2:1 dichloromethane:methanol solution. FA were then transesterified using 3 ml of 3:2 MeOH : Toluene and 2 ml of Hilditch reagent (2:98 H_2SO_4:MeOH). Samples were heated (100°C; 10 min), cooled (10 min) and centrifuged at 1000 rpm for 1 minute; the supernatant was collected and transferred into 2 ml amber vials prior to nitrogen blowdown. 300 µl of Hexane were added to each vial as a final step before purification.

A purification was done to eliminate the sterols that can obstruct the mass spectrometer. To form a column, a small pipette was filled with fiberglass wool and 0.5 cm of previously burned silica. Columns were conditioned with 1.5 ml of 1:1 hexane:ethyl acetate solution. Samples (300 µl) were injected into the column. When the sample was well distributed into the silica, 1.5 ml of 1:1 solution was added to rinse and collect all the lipids. Samples were blown down under nitrogen and stored at -80°C for FA analyses.

Fatty acid methyl esters (FAME) were analyzed by a gas chromatograph (Trace GC Ultra) equipped with a Omegawax 250 column (30 m x 0.25 mm) and coupled to an ion trap mass spectrometer (Trace ITQ900) (GC-MS, ThermoScientific) at the Institut des Sciences de la Mer de Rimouski (ISMER, Rimouski, QC, Canada). FA were identified and quantified by comparing retention times and mass spectra with known standards calibration curves (37 Component FAME Mix, Supelco) with Xcalibur v.2.1 software.

Statistical Analyses

Variation in estimated energy consumption was analyzed with a one-way ANOVA with the fixed factor Diet as the independent variable and the energy consumption per 100 g of live crab (average for all inter-maintenance periods) as the dependent variable. Data were log-transformed to meet parametric assumptions and multiple comparisons among diets were done using Tukey HSD *post-hoc* test.

We used a Kaplan-Meier log-rank test (survival analysis; Fox, 2001) to compare survival over the whole experiment among diets; individuals that were present in tanks that were closed were considered censored data, i.e. their status was considered as unknown after the closure date. The same test was used to analyze moulting for the 2018 and 2019 growing seasons separately with the proportion of individuals that had not moulted at a particular date as the dependent variable. The moulting analysis for 2018 only included individuals that were still alive in the fall (i.e., 150 days after the start of the experiment) and those that died during moulting; for 2019, we included individuals that were alive in June 2019 and survived until the end of the experiment in fall 2019 or that died during moulting. Multiple comparisons among diets were done using the Holm-Sidak method following a significant overall test.

Growth was assessed using inter-molt proportional increase in carapace width ($[\text{Width}_{\text{postmoult}} - \text{Width}_{\text{premoult}}]/\text{Width}_{\text{premoult}}$). Data was analyzed using a one-way ANOVA with the fixed factor Diet; the dependent variable was the mean growth of all moults recorded within a replicate experimental tank in 2018 and 2019 combined. Assumptions of normality and homoscedasticity were assessed by inspection of residual plots and no transformation was deemed required. Multiple comparisons among diets were conducted using Tukey HSD *post-hoc* tests.

Condition indices were analyzed with one-way ANOVAs with the fixed factor Diet; the variables analyzed were: 1) Gonad index (wet gonad weight/wet total weight; females that were ovigerous at the time of dissection were removed from the analysis since their gonads were very small), 2) Hepatopancreas index (wet hepatopancreas weight/wet total weight), 3) Brix index, 4) Hepatopancreatic glycogen concentration and 5) Carapace hardness, with a separate analysis for the 3 carapace positions. In all cases, the dependent variable was the average for a replicate experimental tank. Assumptions of normality of residuals and homoscedasticity were assessed by inspection of residual plots and appropriate transformations were used when necessary (see results). When a significant Diet effect was detected, multiple comparisons were conducted using Tukey HSD *post-hoc* tests.

Lipid profiles were analyzed using the software Primer v7 with PERMANOVA. Bray-Curtis similarity matrices were built using the mean proportion of each fatty acid in a given tank as dependent variables. Only fatty acids with proportion greater than 0.5% (average from all samples) were retained in the analysis. A two-way factorial PERMANOVA was conducted using Diet and Tissue as fixed factors. This analysis showed a significant interaction between the two factors; we thus conducted a one-way analysis for each tissue separately. Following a significant Diet effect, we conducted pair-wise tests to determine which pairs of treatment differed significantly and SIMPER analyses to determine which fatty acids contributed the most to significant differences among pairs of diets. Finally, we used principal component analyses (PCA) and plotted replicate values on the plane composed of the two dominant PCs and the ordination of the fatty acids that contributed the most to differences.

Proportion of each fatty acid, total proportion of saturated, monounsaturated, n-6 and n-3 polyunsaturated, total polyunsaturated fatty acids, n-3/n6 ratios and total fatty acid content were analyzed with one-way ANOVAs with the fixed factor Diet for each tissue separately. Various transformations (log or arcsin-square root) were used to meet assumptions of normality of residuals and homoscedasticity; when no transformation succeeded in meeting assumptions, we used Kruskall-Wallis tests. Detection of significant Diet effect was followed by Tukey HSD *post-hoc* comparisons (ANOVA) or multiple comparison of ranks (Kruskall-Wallis tests).

RESULTS

Estimation of Energy Consumed

Estimates of caloric intake per 100 g of live crab varied significantly among diets (ANOVA: MS = 0.43, $F_{2,25} = 50.88$, $p < 0.001$). Estimates were lowest for the mussel diet, intermediate for the mixed diet, and highest for the salmonid feed diet (**Figure 1**); all pairwise comparisons were significant.

Survival

We observed considerable mortality over the course of the experiment (34% of individuals survived to the end of the experiment) but survival was not related to Diet (**Figure 2**; Kaplan-Meier log-rank test statistic = 1.06, df = 2, p = 0.59). Most mortality occurred early in the experiment as a result of 1)

FIGURE 1 | Estimated energy consumption for rock crabs Cancer irroratus fed the different experimental diets. Replicates are the mean values for each experimental tank. Log-transformed data was analyzed, but raw data is presented. Bars show mean +SE and columns not sharing a common letter are significantly different (Tukey HSD post-hoc tests, n = 9-10).

an unidentified disease expressed as the presence of black spots on the legs and carapace (~70% of recorded mortalities) and 2) individuals being killed by their conspecifics following moulting (~20% of recorded mortalities). Occurrence of the disease greatly diminished in the fall and important outbreaks did not happen afterwards; this resulted in low mortality during the winter. Removing the period of high disease-related mortality from the statistical analysis (i.e. starting the analysis in October 2018) did not change the results (Kaplan-Meier log-rank test statistic = 0.51, df = 2, p = 0.78). A few individuals died during the 2019 growing season, mostly as a result of individuals being killed by conspecifics during moulting (**Figure 2**). At the end of the experiment, the number of tanks that still had live individuals had decreased to 9 for the mixed diet and 5 for each of the salmonid feed and mussel diets.

Moulting and Growth

In 2018, moulting was not affected by Diet (**Figure 3A**; Kaplan-Meier log-rank test statistic = 1.78, df = 2, p = 0.41). Among individuals included in the analysis, ~75% were observed moulting. Moulting activity was maximal between the end of August and the end of September and the last moult observed was on October 9th (**Figure 3A**); no moult was observed until the following summer. In 2019, the moulting pattern was significantly affected by Diet (**Figure 3B**; Kaplan-Meier log-rank test statistic = 6.32, df = 2, p = 0.04). Of the individuals included in the analysis, the proportion that moulted was highest for the mixed diet, intermediate for the mussel diet, and lowest for the salmonid feed diet; the only statistically significant multiple comparison was between the mixed and salmonid feed diets (**Figure 3B**).

Diet impacted growth over the course of the experiment (ANOVA: MS = 7.11 E-3, $F_{2,24} = 6.02$, *p* = 0.008). Carapace width following a moult increased by ~ 25% in experimental tanks fed a

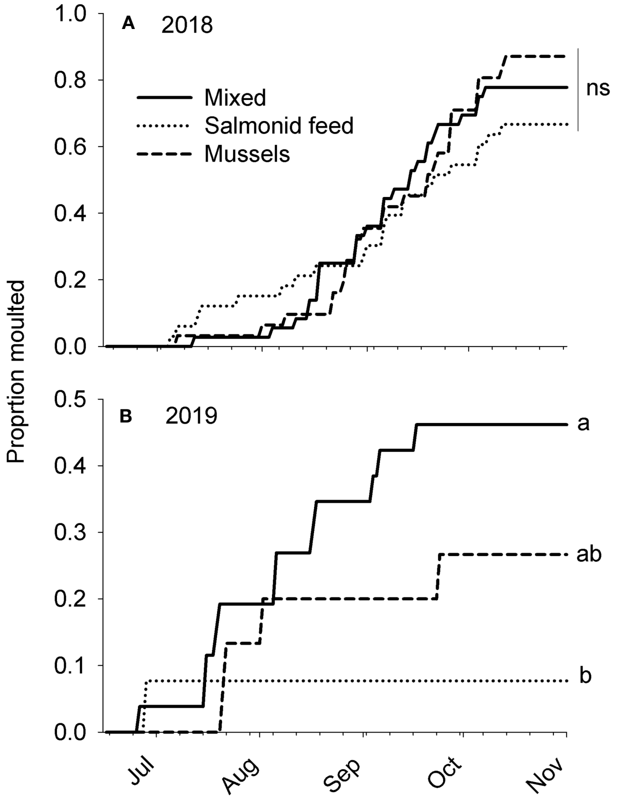

FIGURE 3 | Moulting of rock crabs Cancer irroratus fed different aquaculture-related diets in a laboratory experiment in **(A)** 2018 and **(B)** 2019. Proportion not moulted was analyzed through survival analyses, but proportion moulted is presented. Lines not sharing a common letter are significantly different (Holm-Sidak post-hoc test), ns = not significant. The number of individuals included in the analysis in 2018 was 36, 33, and 31 for the mixed, salmonid feed and mussel diets, respectively and, in the same order, 26, 13, and 15 in 2019.

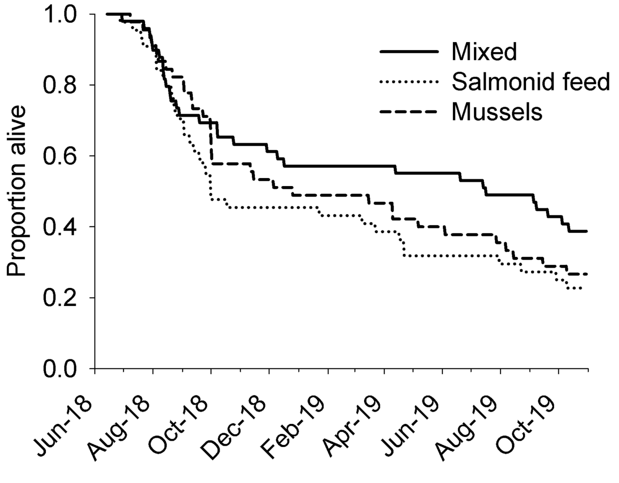

FIGURE 2 | Survival of rock crabs Cancer irroratus fed different aquaculture-related diets in a laboratory experiment. Survival was not significantly influenced by diet (Kaplan-Maier log-rank survival analysis, n = 45-50 for each treatment).

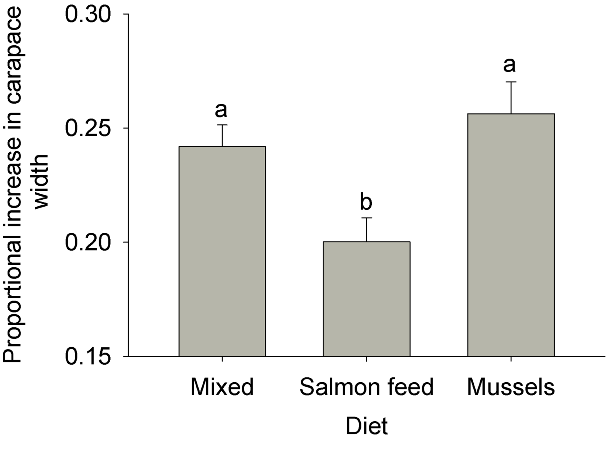

FIGURE 4 | Impact of experimental diets on the growth of rock crabs Cancer irroratus. Replicate values are the average proportional increases in carapace width for each experimental tank. Bars show mean + SE and columns not sharing a common letter are significantly different (Tukey HSD post-hoc tests, n = 5-9). The total number of moults for which growth was recorded was 37, 17, and 29 for the mixed, salmonid feed and mussel diets, respectively.

mixed or a mussel diet but significantly less (~ 20%) in those fed the salmonid feed diet (**Figure 4**).

Condition Indices

Both gonad and hepatopancreas indices were significantly affected by Diet (ANOVAs: log-transformed gonad index, MS = 0.28, $F_{2,18} = 5.05$, $p = 0.02$; hepatopancreas index, MS = 1.80E-3, $F_{2,18} = 11.18$, $p < 0.001$). Gonad index was greatest for individuals fed the mixed diet, intermediate for the mussel diet, and lowest for the salmonid feed diet; only the mixed and salmonid feed diets differed significantly (**Figure 5**). Hepatopancreas index was significantly lower for individuals from the salmonid feed diet compared to other treatments (**Figure 5**). Concentration of hemolymph dissolved compounds (Brix index) and hepatopancreatic glycogen were both affected by diet (ANOVAs: Brix index, MS = 18.50, $F_{2,18} = 16.14$, $p < 0.001$, log-transformed glycogen, MS = 1.29, $F_{2,18} = 11.41$, $p < 0.001$). Brix index was significantly lower in the salmonid feed diet when compared to other treatments (**Figure 6**). Hepatopancreatic glycogen concentration was highest in the mussel diet, intermediate in the mixed diet, and lowest in the salmonid feed diet; all treatments differed significantly from each other (**Figure 6**). Finally, carapace hardness was significantly affected by diet for the 3 positions evaluated (ANOVAs, MS = 184.60, 520.39, and 327.36, $F_{2,18}$ = 3.64, 4.08, and 5.18, p = 0.05, 0.04, and 0.02 for the posterior, anterior left and anterior right positions, respectively). *Post-hoc* comparisons found no differences for the posterior position. Anterior carapace hardness was highest for the mixed diet, intermediate for the mussel diet and lowest for the salmonid feed diet; only the mixed and salmonid feed diets differed significantly (**Figure 7**).

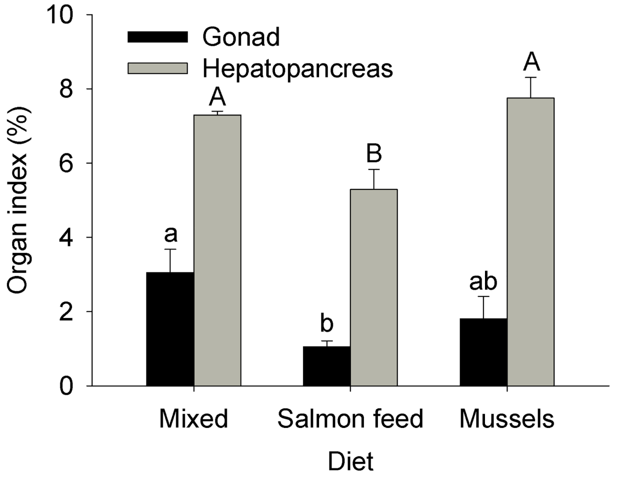

FIGURE 5 | Impact of experimental diets on gonadal and hepatopancreatic indices in the rock crab *Cancer irroratus*. Replicates are the mean values for each experimental tank. Log-transformed gonad index was analyzed, but raw data is presented. Bars show mean +SE and columns not sharing a common letter are significantly different (Tukey HSD *post-hoc* tests, n = 5-9). The total number of individuals included in the analyses was 23, 11, and 13 for the mixed, salmonid feed and mussel diets, respectively.

FIGURE 6 | Impact of experimental diets on concentration of hemolymph dissolved compounds (Brix index) and hepatopancreatic glycogen in the rock crab *Cancer irroratus*. Replicates are the mean values for each experimental tank. Log-transformed glycogen content was analyzed, but raw data is presented. Bars show mean +SE and columns not sharing a common letter are significantly different (Tukey HSD *post-hoc* tests, n = 5-9). The total number of individuals included in the analyses was 23, 11, and 13 for the mixed, salmonid feed and mussel diets, respectively.

Lipid Content

Fatty acid profiles were significantly affected by diet in all three tissues (PERMANOVA, MS = 923.54, 1774.30, and 3014.60, pseudo-$F_{2,16}$ = 42.44, 5.41, and 42.12 for muscles, gonads, and hepatopancreas, respectively; p < 0.003 for all tissues). Pairwise tests were all significant with the exception of the Mixed and Mussel comparison for gonads (**Figures 8A–C**). Individual fatty

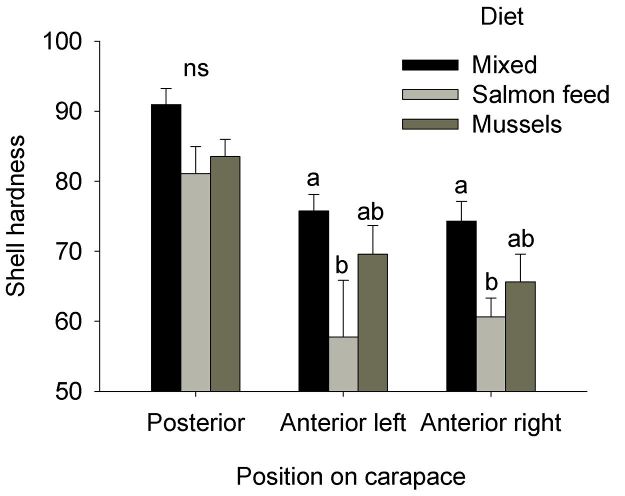

FIGURE 7 | Impact of experimental diets on carapace hardness measured at three positions on rock crabs *Cancer irroratus*. Replicates are the mean values for each experimental tank. Bars show mean +SE and columns not sharing a common letter are significantly different, ns = not significant (Tukey HSD *post-hoc* tests, n = 5-9). The total number of individuals included in the analyses was 23, 11, and 13 for the mixed, salmonid feed and mussel diets, respectively.

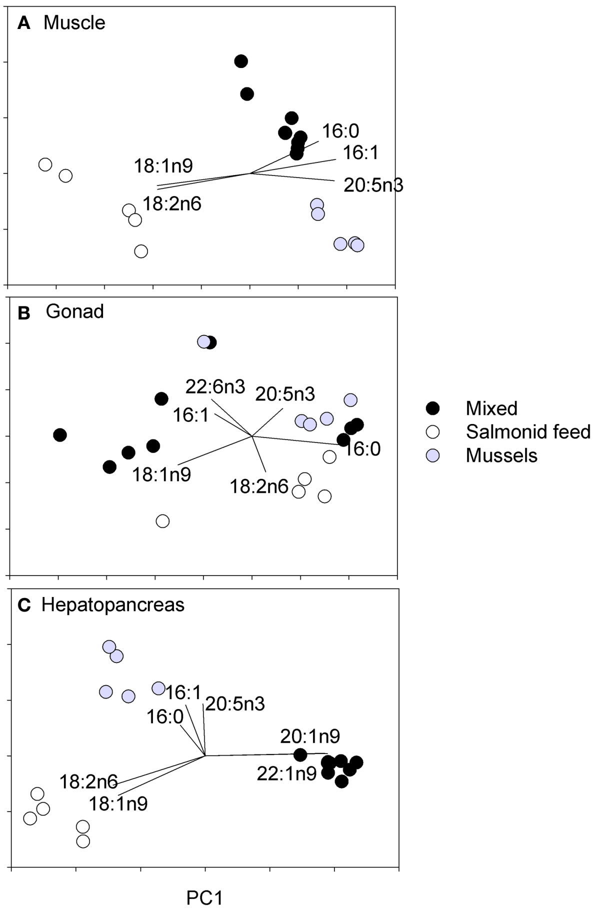

FIGURE 8 | Principle component (PCA) analysis of the lipidic profiles in **(A)** muscle, **(B)** gonad, and **(C)** hepatopancreas of rock crabs, Cancer irroratus fed different experimental diets. Dots show means for each experimental tank (n = 5-9 for the different diets) and lines show loading of the fatty acids that contributed the most to diet differences (SIMPER analysis) within each tissue. The total number of individuals included in the analyses was 23, 11, and 13 for the mixed, salmonid feed and mussel diets, respectively.

acids that contributed most consistently to differences among diets were terrestrial markers (18:1n9 and 18:2n6) that were found in higher proportions, and marine essential fatty acids (20:5n3 and 22:6n3) in lower proportion in the salmonid feed diet than the mixed and mussel diets for all organs (**Figures 8A–C**; **Table 1**). Bacterial markers (16:0 and 16:1; Kelly and Scheibling, 2012) were generally found in higher proportion in the mussel diet than the other two diets (**Figures 8A–C**; **Table 1**). There was a greater proportion of zooplankton markers (20:1n9 and 22:1n9; George and Parrish, 2015) in the muscle and hepatopancreas of crabs fed the mixed diet when compared to those fed the other two diets (**Figure 8C**; **Table 1**).

Total proportions of saturated and monounsaturated fatty acids often varied significantly as a function of diet, but the diet effect was inconsistent among the three tissues. For all three tissues, proportions of n-3 and n-6 and their ratio varied with diet with more n-6, less n-3 and a smaller n-3 to n-6 ratio for the salmonid feed diet when compared to the mixed and mussel diets (**Table 1**). Total fatty acid content was marginally affected by Diet (p = 0.041, 0.085, and 0.049 for muscle, gonad, and hepatopancreas respectively) but no multiple comparison was significant; overall, fatty acid tended to be more abundant in the salmonid feed diet (**Table 1**)

DISCUSSION

Recent reviews have highlighted that many wild species are more abundant within coastal aquaculture facilities when compared to the surrounding areas and that farms act as anthropogenic food subsidies to natural populations (Callier et al., 2018; Barrett et al., 2019; Theuerkauf et al., 2021). However, it is not clear if farms have a positive (acting as source), neutral, or negative (acting as a sink) effect on individuals, populations, and fisheries, and the factors determining whether farms act as population sources or sinks. The present study is part of a larger research program designed to address concerns raised by fisherman on long-term impacts of aquaculture on decapod fisheries in Eastern Canada. One concern was that farms may function as ecological sinks (Fernandez-Jover et al., 2011), attracting individuals from the surrounding area, where these could consume highly available mussels or salmonid feed, resulting in detrimental effects, because of poor nutritional quality of these readily available food items. Our study was designed to experimentally investigate the 'worst-case scenarios' in terms of individual performance when they consumed exclusively mussels or salmonid feed.

An important aspect of our work was to make sure that crabs had a comparable energy intake among diets to avoid potential effects of partial starvation. This was done by adjusting the amounts of each food item based on previous consumption (i.e. increase the amount when all the food was consumed in a previous period) to ensure crabs were fed to satiation. A good indication that starvation did not occur is the amount of food left; on average for all feedings, the amounts of edible food left (total weight of fish left, soft tissue left for shelled organisms, and pellets left untouched for salmonid feed) corresponded to 24.7, 22.1, and 16.2% of the amount given for the mixed, salmonid feed and mussel diets, respectively. In addition, our estimates of energy consumption did not relate to overall condition; the two diets for which estimated energy intake was lowest (mixed and mussel diets) were the diets for which indices of condition were better. In contrast, condition was poorest in the salmonid feed diet for which estimated energy consumption was highest. It is clear that these estimates are biased because it was not possible to recover all the small fragments of shells and small pieces of soft tissue (for fish and shelled organisms). The largest bias, probably leading to an over-estimation of energy intake, was for the salmonid feed diet; when crabs handled pellets, they tended to disintegrate into fine particles that were too small to be handled by the mouth pieces of crabs. However, based on visual observations, we are confident that the proportion of the pellets that disintegrated corresponded to less than 50% of the pellets that were consumed (estimates of energy consumed in the salmonid feed treatment was ~ twice that of the other diets). Although it cannot be formally excluded, it is unlikely that the differences observed in

TABLE 1 | Fatty acid profiles of muscle, gonad and hepatopancreas of rock crabs, *Cancer irroratus*, fed different experimental diets.

	Muscle			Gonad			Hepatopancreas		
Lipid	**Mixed**	**Salmonid feed**	**Mussel**	**Mixed**	**Salmonid feed**	**Mussel**	**Mixed**	**Salmonid feed**	**Mussel**
14:0	0.5 (0.2)	0.2 (0.1)	0.2 (0.05)	1.6 (0.5)	0.1 (0.1)	0.1 (0.1)	2.5 (0.1)^{c}	0.6 (0.1)^{a}	1.5 (0.2) ^{b}
16:0	18.3 (0.2)^{c}	14.5 (0.4)^{a}	16.8 (0.5)^{b}	4.9 (2.1)	8.8 (2.3)	10.0 (2.6)	10.7 (0.6)	10.4 (1.0)	13.6 (1.6)
18:0	5.7 (0.2)^{a}	6.7 (0.5)^{b}	6.4 (0.2)^{ab}	1.4 (0.7)^{a}	4.4 (1.3)^{ab}	5.1 (13.2)^{b}	4.9 (0.3)	4.8 (0.6)	4.8 (0.9)
∑Sat	24.4 (0.4)^{b}	21.4 (0.8)^{a}	23.4 (0.6)^{ab}	7.8 (2.4)	13.3 (3.5)	15.2 (3.9)	18.1 (0.6)^{ab}	15.8 (0.8)^{a}	19.9 (1.5)^{b}
16:1	5.4 (0.3)^{b}	1.1 (0.2)^{a}	6.1 (0.3)^{b}	8.7 (0.5)^{b}	2.4 (0.7)^{a}	8.0 (1.0)^{b}	8.0 (0.2)^{a}	5.0 (1.1)^{a}	17.0 (2.1)^{b}
17:1	0.6 (0.1)^{b}	0.5 (0.2)^{b}	1.3 (0.3)^{a}	0.4 (0.05)^{ab}	0.3 (0.1)^{a}	0.6 (0.05)^{b}	0.3 (0.02)^{b}	0.1 (0.02)^{a}	0.6 (0.05)^{c}
18:1n9	15.3 (0.4)^{a}	25.6 (1.1)^{b}	14.8 (0.4)^{a}	28.8 (3.6)	31.2 (3.9)	17.6 (1.2)	23.1 (0.4)^{b}	51.6 (2.4)^{a}	23.8 (2.9)^{b}
20:1n9	3.0 (0.2)^{a}	1.1 (0.2)^{b}	1.5 (0.1)^{b}	2.1 (0.9)	2.1 (0.6)	1.9 (0.5)	14.6 (0.8)^{b}	3.7 (0.4)^{a}	7.0 (1.2)^{a}
22:1n9	1.4 (0.2)^{b}	0.3 (0.1)^{a}	0.1 (0.03)^{a}	2.2 (1.0)	1.1 (0.4)	0.8 (0.5)	17.2 (1.1)^{c}	3.5 (0.4)^{a}	7.5 (0.6)^{b}
24:1n9	0.7 (0.3)	1.8 (0.7)	1.6 (1.0)	2.3 (1.0)	2.9 (0.9)	3.3 (0.7)	0.8 (0.03)^{c}	0.2 (0.03)^{b}	0.06 (0.02)^{a}
∑MUFA	26.4 (0.5)^{a}	30.3 (0.01)^{b}	25.3 (0.6)^{a}	44.5 (2.4)^{b}	40.0 (3.5)^{ab}	32.1 (1.2)^{a}	64.0 (0.9)^{b}	64.0 (2.4)^{b}	55.9 (1.7)^{a}
18:2n6	1.5 (0.1)^{a}	10.2 (0.5)^{b}	1.2 (0.1)^{a}	0.8 (0.2)^{a}	11.7 (1.8)^{b}	0.8 (0.2)^{a}	1.1 (0.1)^{a}	8.2 (1.6)^{b}	1.7 (0.4)^{ab}
20:2n6	1.1 (0.04)^{a}	2.0 (0.2)^{b}	1.0 (0.1)^{a}	0.6 (0.2)^{a}	3.5 (0.8)^{b}	0.9 (0.3)^{a}	0.7 (0.1)^{a}	1.9 (0.4)^{b}	0.8 (0.1)^{a}
20:4n6	7.8 (0.3)^{b}	5.6 (0.6)^{a}	7.1 (0.3)^{ab}	9.9 (0.8)	7.6 (0.7)	10.4 (1.0)	3.1 (0.1)^{a}	2.7 (0.6)^{a}	4.8 (0.3)^{b}
∑n6	10.5 (0.4)^{a}	17.8 (1.1)^{b}	9.3 (0.4)^{a}	11.3 (0.8)^{a}	22.8 (1.0)^{b}	12.1 (0.9)^{a}	4.9 (0.2)^{a}	12.7 (1.8)^{b}	7.2 (0.4)^{ab}
18:3n3	0.4 (0.03)	0.6 (0.04)	0.5 (0.02)	0.5 (0.1)	0.6 (0.1)	0.5 (0.1)	0.7 (0.1)	1.0 (0.2)	0.9 (0.1)
20:3n3	0.4 (0.02)^{b}	0.1 (0.01)^{a}	0.1 (0.02)^{a}	3.7 (1.3)	0.03 (0.01)	2.1 (2.1)	0.3 (0.03)^{a}	0.1 (0.03)^{b}	0.01 (0.01)^{b}
20:5n3	23.0 (0.6)^{b}	18.9 (0.6)^{a}	25.7 (0.5)^{c}	16.0 (2.2)^{ab}	13.9 (1.1)^{a}	22.0 (1.1)^{b}	4.8 (0.2)^{b}	3.3 (0.7)^{a}	7.4 (0.4)^{c}
22:6n3	13.9 (0.5)^{b}	10.5 (0.3)^{a}	14.4 (0.3)^{b}	16.0 (0.8)^{b}	9.2 (1.0)^{a}	15.0 (1.5)^{b}	6.6 (0.2)^{b}	3.0 (0.5)^{a}	6.9 (0.4)^{b}
∑n3	37.83 (0.59)^{b}	30.1 (0.8)^{a}	40.7 (0.5)^{c}	36.2 (2.7)^{b}	23.7 (1.8)^{a}	39.6 (4.4)^{b}	12.5 (0.3)^{b}	7.3 (1.0)^{a}	15.2 (0.7)^{c}
∑PUFA	48.3 (0.6)	47.9 (0.9)	50.0 (0.8)	47.4 (2.9)	46.5 (1.4)	51.8 (4.8)	17.3 (0.4)^{a}	20.0 (2.8)^{ab}	22.4 (0.8)^{b}
n3/n6	3.6 (0.2)^{b}	1.7 (0.1)^{a}	4.4 (0.2)^{c}	3.3 (0.2)^{a}	1.1 (0.1)^{b}	3.3 (0.3)^{a}	2.6 (0.1)^{c}	0.6 (0.02)^{a}	2.1 (0.1)^{b}
Total FA	2.8 (0.3)	4.5 (0.8)	2.6 (0.4)	5.7 (0.8)	8.2 (0.5)	5.5 (1.6)	23.7 (2.0)	23.5 (4.7)	14.0 (1.6)

Values show mean percentage (out of total fatty acids) of a fatty acid or lipid class for a diet (SE) or mean value (n3/n6, Total FA [ug/g tissue dry weight]). Diets not sharing a common letter within a tissue are significantly different for a particular variable (Tukey post-hoc test or Kruskall-Wallis rank test). Sat, Saturated fatty acids, MUFA, Monounsaturated fatty acid, PUFA, Polyunsaturated fatty acid, FA, fatty acid.

this study resulted from differential energy consumption as crabs in all treatments probably fed to satiation.

Overall, rock crabs fed exclusively mussels had performance and condition that were comparable to those fed a mixed diet designed to mimic a natural one that could be expected outside of farm areas. Differences were found in the lipidic profiles between the two diets for somatic tissues; the main fatty acids contributing to the differences (increased proportions of 16:0, 16:1, and 20:5n3 in the mussel diet) are found in high concentration in mytilid mussels (e.g. Alkani et al., 2007; Dernekbasi et al., 2015). A mussel diet thus appears to provide crabs all their required essential fatty acids, which is not surprising given that phytoplankton (the main producer of marine n-3 polyunsaturated fatty acids; Jónasdóttir, 2019) is an important part of mussel diets (e.g. Rouillon et al., 2005).

In contrast to the mussel diet, crabs feeding solely on salmonid feed had hindered performance and condition; with the exception of survival, all variables measured were negatively affected when compared to the mixed diet. These effects on condition and performance were accompanied by important switches in fatty acid profiles of somatic tissues (muscle and hepatopancreas) typical of wild organisms frequenting finfish farms, i.e., high lipid content, and low n3-n6 ratios driven by increases in proportions of 18:1n9 and 18:2n6 and decreases in 20:5n3 and 22:6n3 (reviewed in White et al., 2019). Specific dietary fatty acid requirements are not available for adult *C. irroratus*, but several studies have investigated essential fatty acids in larval and post-larval decapod crustaceans. Substituting fish oils for terrestrial plant oils had detrimental impacts (Noordin et al., 2015; Zhu et al., 2019) and polyunsaturated fatty acids, including 20:5n3, 20:4n6, and 22:6n3, are essential for proper development and various physiological processes (Harrison, 1991; Xu et al., 1994; Sheen and Wu, 2002; Thériault and Pernet, 2007; van der Meeren et al., 2009). It is possible that the observed deficiencies in essential fatty acids, especially 20:5n3 and 22:6n3, related to consumption of salmonid feed could be responsible for the whole suit of negative effects observed in adult *C. irroratus*. Other unmeasured dietary components may also have contributed to these effects. Essential minerals, such as calcium, may be assimilated when crabs ingest fragments of shells when they feed on natural prey items; low levels of minerals in salmonid feed may lead to deficiencies. Our results contrast with those of previous studies based in the field, where somatic condition of fish and invertebrates frequenting finfish farms was improved when compared to reference areas (Fernandez-Jover et al., 2007; Fernandez-Jover et al., 2011; Dempster et al., 2011; Izquierdo-Gomez et al., 2015), and invertebrate condition and/or growth was unaffected or positively affected by salmon farm wastes in trials pertaining to integrated multi-trophic aquaculture (Sarà et al., 2009; Lander et al., 2012; Baltadakis et al., 2020); only two studies found negative impacts on some variables measured in mussels (Handå et al., 2012; Irisarri et al., 2015).

One of our main objective was to evaluate the effect of diets on the reproductive output of crabs, i.e., clutch size, embryo development, larval development, and survival. Unfortunately, we were unable to get enough embryos to conduct this work. We were, however, able to gather information on other aspects of reproduction; we found no difference in the proportion of females that were observed forming a couple among diets (data not shown), suggesting the absence of diet-related behavioral changes. However, rock crabs fed the salmonid feed diet accumulated terrestrial fatty acids in gonads and had smaller gonads compared to crabs fed the mixed diet. Relatively few studies have investigated the impacts of a finfish feed diet on reproductive processes. Barrett et al. (2018) found no accumulation of terrestrial fatty acids in gonads of Atlantic cod (*Gadus morhua*) and minimal difference in the condition of eggs, embryos, and larvae in wild Atlantic cod caught in heavily-farmed areas compared to reference ones. In contrast, Gonzalez-Silvera et al. (2020) found accumulation of terrestrial fatty acids in four species of fish and accelerated ovarian development for individuals collected close to farms when compared to more distant areas. In a laboratory experiment, White et al. (2016) found no differences in lipid profiles in gonads or eggs in the sea urchin *Heliocidaris erythrogramma* fed a natural (mixed), current feed (n3-n6 ratios currently used by the industry), and future feed diets (very low n3-n6 ratios that could be used if the proportion of terrestrial fatty acids keep increasing in feed used by the industry); current feed did not influence reproductive processes, but future feed had serious consequences on egg diameter, fertilization, and larval development and survival. Finally, in the sea urchin *Echinus acutus*, a laboratory diet of fish feed increased gonad size, gonad, and egg terrestrial fatty acid content when compared to a natural diet (White et al., 2017b), resulting in reduced egg and larval quality (White et al., 2018).

While we observed negative impacts of feeding rock crabs solely with salmonid feed, it is important to remember that the experiment was designed to investigate the ‘worst-case scenarios’ where individuals are obliged to feed on a single item. The realized net impact in a field situation will depend on the contribution of salmonid feed in the overall diet. This in turn will depend on food selection when crabs are inside a farm and amount of time spent in that farm. Future studies should investigate dietary choices made by individuals in presence of several food items. It would also be useful to design laboratory experiments in which individuals receive various proportions of their total energy from salmonid feed and evaluate how this relates to lipidic composition and performance on a continuous scale. From there it would be possible to sample crabs from farm areas and estimate how much salmonid feed they consume in the wild. Preliminary comparisons are possible using data from Sardenne et al. (2020) in which detailed profiles were obtained from crabs collected in farms and surrounding areas. They found n3-n6 ratios slightly higher than our mixed diet in crabs collected outside farms (average from muscle, gonads, and hepatopancreas: Sardenne et al. 3.93 compared to 3.17 in this study) and also slightly higher under farms compared with that of crabs fed the salmonid feed diet (Sardenne et al. 1.60 compared to 1.13 in this study). While preliminary, this comparison suggests that crabs consume a good proportion of salmonid feed in farms as they have ratios similar to laboratory individuals feeding solely on fish food for 1.5 years. Even if crabs eat a great proportion of salmonid feed while below farms, the effect at the individual level will depend on the proportion of time spent there. Crabs could consume only salmonid feed when under a farm, but the negative effects might not be manifest if they only spend a few days or weeks under farms and return to unaffected areas where they resume a natural diet. Hydroacoustic telemetry data is currently being analyzed and this will inform on farm frequentation habits (McKindsey, unpublished data). These two factors (i.e. food selection and time spent under farms) may explain why field studies typically find positive or neutral effects of farms on somatic condition and reproductive processes. While fish feed can be a dominant component of stomach content of fish caught near fish farms, other natural prey items are also found (Fernandez-Jover et al., 2007; Fernandez-Jover et al., 2011); thus fish either still prey on other items or feed outside of farms. Similarly, suspension-feeding invertebrates ingest farm wastes (Sarà et al., 2009; Handå et al., 2012; Lander et al., 2012; Irisarri et al., 2015) but still have access to natural suspended matter and plankton communities. In contrast, White et al. (2016; 2017b; 2018) found negative impacts in sea urchins fed solely of specific pelletized food in the laboratory, a design similar to the present study. There likely exists a balance between essential fatty acid and/or minerals requirements and overall energy consumption. If an individual can fulfill its essential dietary requirements by feeding on a minimum amount of natural items, then the energy subsidy coming from consumption of salmonid feed could improve condition and reproduction. In contrast, if a specific combination of natural items and salmonid feed consumed does not meet essential requirements, then deleterious impacts might occur. Future studies should verify this idea and try to identify potential thresholds which are likely to vary according to specific composition of finfish feed used and taxon-specific dietary requirements.

Even if individuals spend significant time under farms and consume predominantly finfish feed, the effects at the individual level will translate into changes at the population level based on the proportion of a population that frequents farms (White et al., 2018). In turn, this value will depend on the overall proportion of the habitat where farms are present and farm attraction. Given the current extent of farming in Eastern Canada, the overall effect at the population level for *C. irroratus* is likely small, but could increase if aquaculture operations end up covering an important proportion of rock crab habitat.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

DD, CR, RE, SR, BG and CM contributed to the design and concept of the study. CR was in charge of the experimental setup, with active contributions from RE and SR. DD and BG performed the statistical analyses. DD and CM obtained funding. DD was in charge of writing the original draft of the manuscript, together with CR, RE and SR. All authors reviewed and edited the manuscript contributing to the final version. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.865390/full#supplementary-material

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Effects of large-scale *Sargassum fusiforme* culture on phytoplankton community structure and water quality

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The large-scale cultivation of *S. fusiforme* was hypothesized to effectively improve water quality and environmental characteristics. Specifically, such cultivation could promote habitat restoration effects by increasing DO and pH levels in addition to decreasing nitrogen and phosphorus levels within cultivation area waters. Phytoplankton diversity could also reflect the quality and health of waters. This study explored the effects of large-scale cultivation of *Sargassum fusiforme* on the biodiversity and the ecosystem of a phytoplankton community in the coastal waters of Dongtou island, China. *S. fusiforme* cultivation increased DO and pH values within the area but decreased N contents of the cultivation area waters. A total of 75 phytoplankton species were identified based on morphological identification, while a parallel survey of phytoplankton communities using high-throughput cultivation-independent sequencing identified 601 phytoplankton OTUs. Large scale cultivation of *S. fusiforme* was associated with increased phytoplankton diversity as reflected by multiple alpha and beta diversity metrics. These data suggested that *S. fusiforme* cultivation could affect phytoplankton community structure when the *S. fusiforme* biomass reached maximum levels. Redundancy analysis (RDA) indicated that *S. fusiforme* played an important role in controlling the dynamics of phytoplankton communities in coastal ecosystems. Thus, the results suggested that *S. fusiforme* cultivation could enhance phytoplankton community biodiversity. Overall, this study demonstrated that large-scale cultivation of *S. fusiforme* in coastal regions was an effective approach for mitigating environmental problems in marine ecosystems.

KEYWORDS

***Sargassum fusiforme*, harmful algal blooms, phytoplankton biodiversity, high-throughput sequencing, bioremediation**

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1 Introduction

Phytoplankton are important primary producers in marine ecosystems (Behrenfeld et al., 2006; Jardillier et al., 2010), as they assimilate nutrients, fix inorganic carbon, and produce organic matter (Falkowski et al., 2000; Emerson, 2014). Biodiversity is essential for enhancing ecosystem productivity but is also critical for maintaining stability at the community and ecosystem levels in addition to maximizing the resource use and ecological efficiency that underpin ecosystem services (Chai et al., 2018). Species composition and phytoplankton community diversity are highly sensitive to changing environmental conditions and are affected by physical, chemical, biological, and climatic factor (Leblad et al.,2020; Liu et al., 2022). Thus, phytoplankton community diversity serves as an indicator of the influences from environmental factors and processes (Peng et al., 2021; Parsons et al., 2021). Consequently, these community characteristics can be used as ecological indicators to evaluate aquatic ecosystem health (Chai et al., 2018; Shan et al., 2019; Navas-Parejoa et al., 2020; Zhou et al., 2021).

Marine phytoplankton biodiversity research has primarily focused on microscopic morphological observations (Lee et al., 2010). However, morphological approaches have limitations for identifying species (Massana, 2011), (Manoylov, 2014) for example in picophytoplankton that exhibit very small cell sizes or organisms that have no clear defining morphological characteristics in addition to those species that have not yet been described or characterized (Egge et al, 2015; de Vargas et al., 2015; Malviya et al., 2016; Liu S et al., 2020). Furthermore, high levels of impurities such as sediments in samples can lead to the inability to observe species. Moreover, morphological observation methods generally take considerable amounts of time and require specialist training for morphological identification. The rapid development of molecular biology tools has led to breakthroughs in understanding microalgae diversity (Lefebvre et al., 2005). Handelsman et al. (1998) formally proposed the field of metagenomics in 1998, focusing on understanding the diversity of microorganisms *via* environmental genomics techniques. Subsequent studies have demonstrated that high-throughput metagenomic sequencing methods are much more effective than morphological approaches for exploring the spatio-temporal dynamics of marine phytoplankton communities (Egge et al, 2015; de Vargas et al., 2015; Chai et al., 2018; Qiao et al., 2020). The 18S rDNA genes represent small ribosomal subunit genes that exist in all eukaryotes and that have both informational and functional roles within cells (Liu et al., 2017). The genes comprise nine variable regions, among which the V4 and V9 regions are widely used in eukaryotic biodiversity studies (Neefs et al., 1990). For example, Stoeck et al. (2010) demonstrated that the V4 region better distinguishes species with close genetic relationships. Moreover, diversity results based on the V4 region are more closely aligned with those from full-length 18S rDNA sequences compared to the results using the V9 region (Pawlowski et al., 2011; Dunthorn et al., 2012), thereby rendering the V4 region of 18S rDNA genes an appropriate tool for phytoplankton identification (Liu et al., 2017).

Sargassum fusiforme is a species endemic to the temperate coastal areas of the northwestern Pacific region and is particularly abundant in coastal waters of China, Korea, and Japan (Li et al., 2019). *S. fusiforme* is an edible alga in China and is widely cultivated in southeastern coastal areas, the Yellow Sea, and the Bohai Sea (Hu et al., 2016; Zhang et al., 2021). Among these areas, the yield and quality are highest near the coast of Zhejiang province, and especially the coastal area of Dongtou Island (Zhang et al., 2002). The alga has become one of the most important species for seaweed mariculture in China in recent years due to its high economic value and increasing consumer demand (Chen et al., 2018). In addition to providing food for human consumption (Zhang et al., 2020), the algae have also been used as a traditional Chinese medicinal plant to treat various diseases (Zhang et al., 2006; Liu J et al., 2020).

Local farmers culturing *S. fusiforme* have observed that during *S. fusiforme* culture, and especially in April when *S. fusiforme* biomass is highest, red tides rarely occur in cultivation areas, while red tides frequently occur in the cultivation areas after the *S. fusiforme* harvest (local fisherman's expertis). Previous studies have shown that *S. fusiforme* can also inhibit *Karenia mikimotoi* growth (Ma et al., 2017). Interestingly, identification of microscopically observable microalgae has indicated that the abundance of one of the red tide microalgae, *Skeletonema costatum*, was significantly lower in the *S. fusiforme* cultivation areas compared to areas without *S. fusiforme* (unpublished data). These observations prompted the present study to evaluate whether a positive impact on phytoplankton community diversity occurs due to *S. fusiforme* cultivation, and also whether the latter influences the cell abundances of red tide microalgae. Moreover, these studies were used to evaluate whether additional red tide microalgae could be inhibited by *S. fusiforme*. To investigate these hypotheses, we analyzed the phytoplankton biodiversity associated with *S. fusiforme* cultivation *via* high-throughput sequencing of 18S rRNA genes using newly designed PCR primers for eukaryotes. In addition, water quality parameters in *S. fusiforme* cultivation and non-cultivation areas were analyzed in tandem. These results are of significance for comprehensively understanding the ecological value of large-scale *S. fusiforme* culture.

2 Materials and methods

2.1 Sampling site descriptions

The study area is located at the Banpingshan cultivation zone in southeastern Dongtou Island (27°41′19″–28°01′10″ E, 120°59′45″–121°15′58″ N), one of the largest *S. fusiforme* mariculture bases in the Dongtou district (Figure 1). The cultivation area was only 57.2 ha in 1989 and is currently maintained at approximately 1,000 ha per year, with an annual seaweed dry-weight production of about 7,000–9,000 tons.

In total, 54 samples were collected in April and June 2019. Nine sampling sites were selected in areas with and without *S. fusiforme* cultivation. The sampling sites and samples were collected as follows: (1) A: *S. fusiforme* cultivation areas, comprising three sites (A1-A3), (2) B: 200–500 m away from the "A" cultivation areas, with three sites (B1-B3), and C: three sites in the open sea about 2 km away from the cultivation area (C1-C3). The average depths at sites A, B, and C were 9.0 m, 8.7 m, and 9.1 m, respectively. A total of 54 samples comprising three replicates for each site were collected from surface waters (0.5 m below the surface), with samples labeled as Ban Pingshan, BS, and CS. Samples were also identified by the time of sampling, with Apr-A, Apr-B, and Apr-C for April samples, and Jun-A, Jun-B and Jun-C for June samples.

2.2 Sample collection and water quality

Water samples (4 L) from the surface and bottom of the sea were collected with organic glass hydrophores. A subsample (1 L) of water from each sample was then used for microbial community investigation by filtering with a sterile microfiltration membrane (47 mm, 0.22 μm filter). After filtering, microfiltration membranes were placed in 5 mL sterile tubes and immediately placed on dry ice.

The remaining water from each sample was stored at −80°C and transported to the laboratory. Dissolved oxygen (DO), salinity (Sal), temperature (T), and pH were measured for each sample using 550A, EC300A, and PH100A YSI instruments, respectively (YSI Inc., Yellow Springs, OH, USA). Ammonia-nitrogen (NH_4^+-N), nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), active phosphate (PO_4^{3-}-P), silicate (SiO_3^{2-}), total nitrogen (TN), and total phosphorus (TP) were all measured using previously described methods (Zhang et al., 2021).

2.3 Phytoplankton morphological analysis

Unfiltered water samples (1 L) were fixed with 5 mL of Lugol's solution, and each sample was subsequently concentrated to 10 mL. Phytoplankton species were identified in these samples, and cell numbers were counted using a phytoplankton enumeration chamber with an inverted microscope (Olympus CKX41, Olympus Corporation, Tokyo, Japan).

2.4 High-throughput sequencing of phytoplankton and bioinformatics analyses

2.4.1 DNA extraction, PCR amplification, and sequencing

Total genomic DNA was extracted from samples using the FastDNA spin kit for soils (MP Biomedicals, OH, USA), following the manufacturer's instructions. DNA quality and concentration were measured by gel electrophoresis and a Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), respectively. PCR was performed using the 18S rDNA gene primer pair 18sV4F (5′-CCAGCASCYGCGGTAATTCC-3′) and 18sV4R (5′-

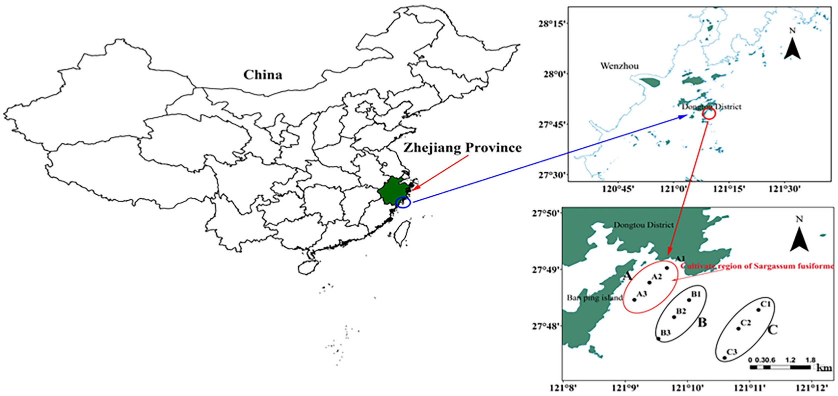

FIGURE 1
Location of sampling sites in Dongtou Island, China. **(A)** *Sargassion fusiforme* cultivation area with three sites (A1-A3); **(B)** adjacent area (without *S. fusiforme* cultivation) with three sites (B1-B3); **(C)** open sea area (without *S. fusiforme* cultivation) with three sites (C1-C3). The depth of each site was showed (A1, A2, A3, B1, B2, B3, C1, C2 and C3:9.6, 9.0.9.8, 9.2, 9.1, 9.4, 9.4, 9.8 and 10.0 m respectively.

ACTTTCGTTCTTGATYRA-3′ (Stoeck etal., 2010). PCR products were extracted from a 2% agarose gel and further purified using the AxyPrepDNA Gel Extraction Kit (Axygen Biosciences, USA) according to the manufacturer's instructions, and then quantified using the QuantiFluor^{TM}-ST system (Promega, USA). Purified amplicons were pooled in equimolar concentrations and subjected to paired-end (PE300) read sequencing on an Illumina MiSeq platform (Illumina, San Diego, USA) at Majorbio Bio-Pharm Technology Co. Ltd. using standard protocols (Shanghai, China). Sequence data were deposited in GenBank under the accession number PRJNA761497.

2.4.2 Bioinformatic analysis

The pretreatment of raw data was performed by QIIME software (Caporaso et al., 2010). The raw tags were merged by FLASH using an overlap length > 10 bp and a maximum mismatch rate < 0.2. The quality filtration of raw tags was executed by Trimmomatic software, and raw tags with a quality level < 75% were discarded. The UCHIME software was applied to identify and discard the chimeric sequences. The valid tags were clustered into operational taxonomic units (OTUs) at a 97% identify threshold. The OTUs were annotated and classified by QIIME software based on the Silva database. Taxonomic assignments were determined for representative sequences of each OTU using the Basic Local Alignment Search Tool (BLAST) in the NCBI database (http://www.ncbi.nlm.nih.gov). Following the exclusion of sequences annotated as bacterial and those that were unclassified, phytoplankton sequences were selected for community compositional analyses based on taxonomic information.

2.4.3 Ecological network analysis of the microbial community

To illuminate phytoplankton interactions and differences between groups, phylogenetic molecular ecological networks (pMENs) were constructed *via* a random matrix theory-based interface approach in the molecular ecological network analysis pipeline (MENA, http://ieg2.ou.edu/MENA/)

2.5 Phytoplankton community analysis

The dominance of abundant species identified by morphological analysis (> 10% of communities) was defined as the species cell abundance/total cell abundance ratio. The dominance of abundant genera revealed by high-throughput sequencing (>10% of sequence reads) was defined as the percentage of sequences for the taxa among the total sequences. Phytoplankton were further classified as picophytoplankton (≤ 2 μm), small nanophytoplankton (> 2 μm and ≤ 10 μm), large nanophytoplankton (> 10 μm and < 20 μm), and microphytoplankton (> 20 μm and < 200 μm) based on cell sizes (Qiao et al., 2020).

Three α-diversity metrics were calculated for communities, the Shannon–Wiener, Simpson, and Ace indices, using the QIIME software program. Differences in α-diversity metric values were analyzed by one-way ANOVA, and statistical analyses were performed with the SPSS software program (v. 22.0). Prior to statistical analyses, data were checked for normality of distributions and homogeneity of variance by the Kolmogorov-Smirnov and Levene's tests, respectively. Statistical significance was identified at the $p < 0.05$ level. β-diversity was also calculated as a comparative indicator of community structures among samples. Histograms of phyla and classes were constructed using the Origin 9.0 software program. In addition, linear model-based redundancy analysis (RDA) was used based on correlations among phytoplankton community composition and water quality parameters with the Canoco 5.0 software package.

3 Results

3.1 Water quality parameters

S. fusiforme cultivation increased DO and pH values but decreased N contents of the cultivation area waters. The DO, NO_3-N, NO_2-N, and TN values of the cultivation area in April 2019 were significantly higher than in the adjacent and control areas ($p < 0.05$). In June 2019, NO_3-N was significantly higher in the cultivation area than in the adjacent and control areas. NO_2-N and NH_4-N were also significantly higher in the cultivation area than in the control area ($p < 0.05$) (Table 1).

3.2 Phytoplankton community composition

Five phyla 75 species of phytoplankton were observed based on morphological observations: Bacillariophyta, Dinophyta, Chrysophyta, Cyanophyta, and Chlorophyta, with Bacillariophyta and Dinophyta being the most dominant phyla. The abundances of each phylum among different survey areas/times were as follows: Bacillariophyta (Apr-A=93.87%; Apr-B=94.15%; Apr-C=96.59%; Jun_A=94.94%; Jun_B=88.71%; Jun_C=88.58%), Dinophyta (Apr-A=1.82%; Apr-B=2.85%; Apr-C=1.01%; Jun_A=4.16%; Jun_B=9.72%; Jun_C=9.72%), Chrysophyta (Apr-A=1.82%; Apr-B=1.00%; Apr-C=0.71%; Jun_A=0.00%; Jun_B=0.52%; Jun_C=0.33%), Cyanophyta (Apr-A=1.21%; Apr-B=1.00%; Apr-C=0.74%; Jun_A=0.44%; Jun_B=0.52%; Jun_C=0.33%), and Chlorophyta (Apr-A=1.28%; Apr-B=1.00%; Apr-C=1.01%; Jun_A=0.46%; Jun_B=0.52%; Jun_C=0.33%) (Figure 2A).

TABLE 1 Temporal and spatial variation in environmental parameters of waters measured in 2019.

Parameter	April			June		
	AS	BS	CS	AS	BS	CS
Depth (m)	10.0±0.24	9.9±0.27	9.9±0.15	10.0±0.18	9.8±0.21	10.0±0.13
Temperature (°C)	14.6±0.2	14.7±0.2	14.5±0.2	22.0±0.4	22.1±0.1	22.3±0.1
Salinity	26.5±0.2	26.5±0.1	26.5±0.1	28.2±0.2	28.4±0.1	28.3±0.0
pH	8.32±0.05	8.21±0.02	8.13±0.03*	8.23±0.0	8.25±0.0	8.27±0.0
DO (mg/L)	10.4±0.2	10.1±0.1*	10.1±0.1*	9.3±0.1	9.3±0.1	9.3±0.1
NO_3-N (mg/L)	0.474±0.025	0.467±0.011	0.507±0.032*	0.317±0.109	0.287±0.008*	0.290±0.015*
NO_2-N (mg/L)	0.008±0.008	0.009±0.008	0.026±0.017**	0.013±0.002	0.010±0.004	0.009±0.003*
NH_4-N (mg/L)	0.149±0.019	0.133±0.018	0.121±0.024	0.102±0.027	0.095±0.003	0.093±0.003*
TN (mg/L)	3.12±0.35	3.07±0.26	3.33±0.36*	4.84±1.40	4.81±0.57	4.75±1.41
PO_4-P (mg/L)	0.039±0.008	0.036±0.002	0.037±0.003	0.048±0.003	0.042±0.010	0.046±0.003
TP (mg/L)	0.05±0.01	0.05±0.01	0.05±0.01	0.041±0.01	0.042±0.01	0.040±0.01
SiO_3-Si (mg/L)	0.452±0.027	0.445±0.445	0.447±0.036	0.720±0.026	0.717±0.004	0.720±0.031

Values are means ± standard deviations. *: $p < 0.05$ indicating that BS or CS was significantly different from AS, based on one-way ANOVA t test.
**$p < 0.01$.

After removing macroalgal, zooplankton, protozoan, fungal, and unclassified OTUs from the sequence dataset *via* comparisons against the NCBI database and literature searches, a total of 601 phytoplankton OTUs were identified comprising the seven phyla Dinophyta, Bacillariophyta, Chlorophyta, Cryptophyta, Chrysophyta, Ochrophyta, and Rhodophyta. Among these, Bacillariophyta, Dinophyta, Chlorophyta, and Cryptophyta were the dominant phyla. The relative abundances of each phylum among the survey areas based on molecular analysis were as follows: Bacillariophyta (Apr-A=11.3%; Apr-B=13.6%; Apr-C=9.2%; Jun_A=47.0%; Jun_B=44.5%; Jun_C=41.1%), Dinophyta (Apr-A=29.5%; Apr-B=32.0%; Apr-C=29.7%; Jun_A=18.8%; Jun_B=22.5%; Jun_C=26.9%), Chlorophyta (Apr-A=32.8%; Apr-B=28.9%; Apr-C=31.2%; Jun_A=23.0%; Jun_B=19.3%; Jun_C=18.8%), Cryptophyta (Apr-A=24.0%; Apr-B=22.7%; Apr-C=27.9%; Jun_A=10.3%; Jun_B=12.9%; Jun_C=10.3%), Chrysophyta (Apr-A=1.0%; Apr-B=0.9%; Apr-C=0.8%; Jun_A=0.7%; Jun_B=0.3%; Jun_C=0.5%), Ochrophyta (Apr-A=1.2%; Apr-B=1.4%; Apr-C=1.1%; Jun_A=0.2%; Jun_B=0.4%; Jun_C=0.4%), and Rhodophyta (Apr-A=0.2%; Apr-B=0.4%; Apr-C=0.1%; Jun_A=0.0%; Jun_B=0.0%; Jun_C=0.0%) (Figure 2B).

Significant differences were observed for phytoplankton composition, specifically the abundances of Bacillariophyta, Ochlorophyta, Chlorophyta, Cryptophyta, and Rhodophyta between April and June communities ($p < 0.05$) (Figure 3). No significant differences were observed between Dinophyta and Chrysophyta abundances across the two time periods ($p > 0.05$). Bacillariophyta abundances in April were significantly higher than in June, while the abundances of Ochlorophyta, Chlorophyta, Cryptophyta, and Rhodophyta were significantly lower in April than in June.

A total of 23 genera/species of picophytoplankton and 17 genera/species of small nanophytoplankton were detected *via* high-throughput sequencing that were not observed with

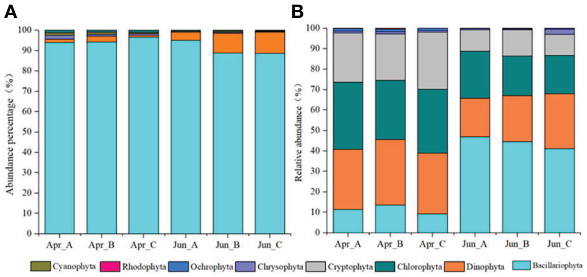

FIGURE 2
Temporal variation in the relative abundances of phytoplankton phyla based on morphological observations **(A)** and high-throughput sequencing **(B)** approaches.

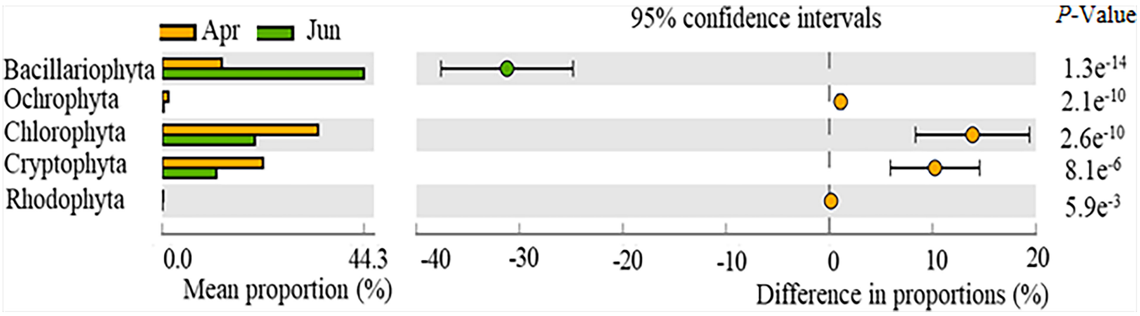

FIGURE 3
Temporal variation in the relative abundances of phytoplankton phyla, as observed with high-throughput sequencing of community compositions.

morphological analyses (Table 2), accounting for 22.21% and 13.18% of the abundance percentage. The dominant Chlorophyta species that were observed included *Bathycoccus prasinos*, *Micromonas pusilla*, *Pyramimonas australis*, and *Nannochloris* sp., all of which exhibited cell sizes less than 2 µm; all are considered to be photosynthetic picoeukaryotes (PPEs) and are widely distributed in marine environments (Mayer and Taylo, 1979; Luis et al., 1992; Rashid et al., 2018). Thus, the overall diversity and community structure of phytoplankton were not comprehensively evaluated by traditional morphological observations alone.

A total of 75 phytoplankton species were identified based on morphological characters, while a parallel survey of phytoplankton communities using high-throughput cultivation-independent sequencing identified 601 phytoplankton OTUs. Thus, the number of OTUs in this study was 3 to 7.6 times higher than the number of morphospecies identified from the same communities. Therefore, the high-throughput sequencing results were selected to analyze the dominant species and diversity of phytoplankton.

3.3 Dominant phytoplankton species or genera based on high-throughput sequencing

Dominant phytoplankton genera/species (dominance ≥ 0.01) were calculated based on OTU compositions. The dominant phytoplankton species were primarily distributed among the phyla Chlorophyta (6 OTUs), Cryptophyta (7 OTUs), Dinophyta (3 OTUs), and Bacillariophyta (10 OTUs). Among April communities, *Heterocapsa* sp. (Dinophyta) were dominant, with values in the cultivation, adjacent, and control areas of 0.165, 0.159, and 0.171, respectively. The second most dominant species was *Plagioselmis prolonga* (Cryptophylum) that exhibited dominance values of 0.109, 0.102, and 0.114, respectively, in the above areas. The third most abundant species was *B. prasinos* (Chlorophyta), with dominance values of 0.086, 0.087, and 0.092, respectively. Among June communities, the most abundant species was *Heterocapsa* sp. (Dinophyta), with dominance values in the culture, adjacent, and control areas of 0.089, 0.080, and 0.076, respectively. The second most abundant species in June was *S. costatum* (Bacillariophyta), with dominance values of 0.092, 0.081, and 0.070 respectively. The third most abundant species was *Chaetoceros tenuissimus* (Bacillariophyta) that exhibited dominance values of 0.086, 0.074, and 0.080 respectively (Table 3).

Several dominant microalgae in the April communities, including *B. prasinos*, *Geminigera cryophila*, *Heterocapsa* sp., *S. marinoi*, and *Thalassiosira tenera*, exhibited lower dominance values in the adjacent and control areas. In June, *B. prasinos*, *G. cryophila*, *Heterocapsa* sp., *S. costatum*, and *T. tenera* exhibited higher dominance values in the cultivation area waters than in the adjacent or control areas. We consequently speculated that *S. fusiforme* exhibited an inhibitory effect on the above dominant species and that *S. fusiforme* selectively prevents the growth of some microalgae (Table 3).

3.4 Phytoplankton community structure based on high-throughput sequencing

3.4.1 Phytoplankton community alpha diversity

Large scale cultivation of *S. fusiforme* was associated with increased phytoplankton diversity. Specifically, the Shannon-Wiener index of April communities in the culture area (3.29 ± 0.15) was significantly higher than in the control area (2.69 ± 0.24) ($p < 0.05$). In contrast, no significant differences were observed among diversity values in June in the culture, adjacent, or control areas. The Simpson index values of the cultivation area communities in April (0.069 ± 0.016) were significantly lower than in the control area (0.088 ± 0.005) ($p < 0.05$). In contrast, June culture area community Simpson index values (0.0980 ± 0.026) were significantly higher than in the adjacent (0. 067 ± 0.004) ($p < 0.05$) and control (0.063 ± 0.006) ($p < 0.01$)

TABLE 2 List of described picophytoplankton and small nanophytoplankton species revealed by high-throughput sequencing.

Phylum	Genus	Closet known species	NCBI accession(similarity)	Relative abundance (%)	Cell size(μm)	Reference
Picophytoplankton(Minimum size≤2μm)						
Bacillariophyta	*Skeletonema*	*S. menzellii*	AB948147.1(99.74%)	0.72	2-12	(Pfannkuchen et al, 2018)
	Skeletonema	*S. marinoi*	KY364698.1(100%)	1.31	2-12	(Pfannkuchen et al, 2018)
	Skeletonema	*S. costatum*	KY290715 (94.50%)	4.78	2-12	(Pfannkuchen et al, 2018)
	Skeletonema	*S. potamos*	MW541631(91.88%)	0.00	2-12	(Pfannkuchen et al, 2018)
	Skeletonema	*S. subsalsum*	AY684967.1 (91.36%)	0.00	2-12	(Pfannkuchen et al, 2018)
	Arcocellulus	*A. cornucervi*	KU561153.1(100%)	0.21	1.0-17.0	(Vaulot et al., 2008)
	Triparma	*T. strigata*	KR998402.1(100%)	0.00	2.0-3.0	(Ichinomiya et al., 2019)
	Brockmanniella	*B.brockmannii*	KY979974 (100%)	0.02	2×10	(Samanta et al., 2017)
	Chaetoceros	*C.socialis*	KY852277.1(100%)	0.00	2×10	(Mahadik et al., 2017; Antoni et al., 2020)
Chlorophyta	*Micromonas*	*M. pusilla*	KY980039.1(100%)	4.83	0.2-0.3	(Song et al., 2020)
	Bathycoccus	*B. prasinos*	MT571465.1(100%)	5.35	1-3	(Simon et al., 2017)
	Ostreococcus	*Ostreococcus* sp.	MT117941(100%)	2.02	1-3	(Chai et al., 2018)
	Micromonas	*M.commoda*	KU612123.1(100%)	0.00	1.0-2.0	(Simon et al., 2017)
	Pycnococcus	*P.provasolii*	MK541772.1(100%)	0.00	≤2μm	(Moro et al., 2002)
	Pyramimonas	*P.tetrarhynchus*	FN562441.1(99.74%)	0.00	≤2μm	(Zhang et al., 2015)
	Ostreococcus	*Ostreococcus* sp.	JN862919.1(99.73%)	0.00	0.8	(Liu et al., 2017)
	Nannochloris	*Nannochloris* sp.	AB058312.1(100%)	2.84	2-4	(Qiao et al., 2020)
	prasinophyte	*prasinophyte* sp.	AB058375.1(100%)	0.00	2-5	(Jouenne et al., 2011)
	Nannochloropsis	*N.oculata*	KY054986.1(99.21%)	0.02	2-4	(Kagan and Matulka 2015)
	Picochlorum	*P.maculatum*	KM055115.1(99.21%)	0.00	1-2	(Augustine et al., 2017)
	Stichococcus	*S.bacillaris*	MT053478.1(100%)	0.01	2-3	(Sivakumar et al., 2014)
	Tetrastrum	*T.staurogeniiforme*	LC425390.1(99.74%)	0.00	2-5	(Bock et al., 2013)
Ochrophyta	*Aureococcus*	*A.anophagefferen*	KY980028.1(100%)	0.00	1.5-2	(Sieburth et al., 1985)
small nanophytoplankton (≤10μm)						
Bacillariophyta	*Micrometopion*	*M.nutans*	HQ121441.1(92.03%)	0.00	5-7	(Howe et al., 2011)
	Cyclotella	*C.choctawhatcheeana*	JQ217341.1(100%)	1.06	10	(Christiane, 2003)
	Chaetoceros	*C.muellerii*	JF790991.1(98.67%)	0.03	3.5-9.2	(Song et al., 2020)
	Chaetoceros	*C.calcitrans*	AY625894.1(100%)	0.10	2.8-3.0	(Song et al., 2020)
	Minidiscus	*M. comicus*	MW722948.1(100%)	0.05	2.0-7.5	(Leblanc et al., 2018)
	Chaetoceros	*C.tenuissimus*	KC309487.1(99.74%)	0.04	5.0	(Tomaru et al., 2018)
Cryptophyta	*Teleaulax*	*T.amphioxeia*	KY980249 (100%)	8.31	5.0-6.8	(Song et al., 2020)
	Goniomonas	*G.avonlea*	JQ434475.1(90.31%)	0.02	9.7	(Song et al., 2020)
	Plagioselmis	*prolonga*	MK956143.1(100%)	0.11	(5-7)×(3-3.7)	(Xing et al., 2008)
Chlorophyta	*Dolichomastix*	*Dolichomastix sp.*	FN562449.1(96.32%)	0.02	3.0-5.0	(Vaulot et al., 2008)
	Prasinoderma	*Prasinoderma sp.*	AB058379.1(94.01%)	0.00	2.2-5.5	(Vaulot et al., 2008)
Ochrophyta	*Florenciella*	*Florenciella sp.*	AB518483.1(99.74%)	0.01	3.0-6.0	(Vaulot et al., 2008)
	Pseudochattonella	*P.verruculosa*	AB217629.1(99.74%)	0.03	5×10	(Lincoln et al., 2011)
	Ciliophrys	*C.infusionum*	AB081641.1(89.41%)	0.00	5	(Hiroshi et al., 2002)
Chlorophyta	*Tetraselmis*	*Tetraselmis sp.*	MH055454 (98.95%)	0.73	(8-16)×(7-9)×(3-5)	(Nozaki, 2003)
	Pyramimona	*P.australis*	KY980350.1(100%)	2.71	8	(Moro et al., 2002)
	Mamiella	*M.gilva*	FN562450.1(99.47)	0.12	(5-8)×(4-7)	(Alonso-González et al., 2014)

TABLE 3 Dominant genera and species based on high throughput sequencing, in addition to their dominance values and sequence similarity to database accessions.

Phylum	Taxa	OTU ID	April				June				NCBIAccession (similarity)
			AS	BS	CS	All	AS	BS	CS	All	
Chlorophyta	s: *Bathycoccus prasinos*	OTU1583	0.086	0.087	0.092	0.088	0.019	0.014	0.015	0.016	MT571465.1 (100%)
	g:*Ostreococcus* sp.	OTU99	0.043	0.046	0.033	0.041	/	/	/		MT117941 (100%)
	s:*Micromonas pusilla*	OTU2003	0.036	0.035	0.044	0.038	0.076	0.049	0.054	0.060	KT860879.1 (100%)
	g:*Micromonas* sp.	OTU557	0.034	0.033	0.032	0.033	/	/	/		MT117944.1 (100%)
	s:*Pyramimonas australis*	OTU1951	0.030	0.027	0.027	0.028	0.034	0.025	0.021	0.027	KY980350.1 (100%)
	g:*Picochlorum* sp.	OTU3226	0.041	0.022	0.021	0.028	0.027	0.030	0.025	0.027	KT860896.1 (100%)
Cryptophyta	g:*Plagioselmis prolonga*	OTU350	0.109	0.102	0.114	0.108	0.020	0.020	0.020	0.020	MK956143.1 (100%)
	g:*Teleaulax gracilis*	OTU1747	0.030	0.036	0.033	0.033	0.016	0.024	0.022	0.021	JQ966995 (98.93%)
	s:*Storeatula major*	OTU232	0.018	0.015	0.017	0.016	0.020	0.020	/	0.020	KY980340.1 (100%)
	s:*Teleaulax minuta*	OTU3028	/	/	/		0.014	0.015	0.016	0.015	JQ966996.1 (99.73%)
	s:*Geminigera cryophila*	OTU489	0.015	0.017	0.015	0.016	0.023	0.024	0.022	0.023	MK956818.1 (98.93)
Dinophyta	g:*Heterocapsa* sp.	OTU1048	0.165	0.159	0.171	0.165	0.089	0.080	0.076	0.082	MH071708.1 (99.74)
	s:*Heterocapsa rotundata*	OTU2153	0.040	0.046	0.049	0.045	0.027	0.039	0.045	0.037	KY980288 (100%)
	g:*Warnowia* sp.	OTU667	0.057	0.072	0.063	0.064	0.018	0.022	0.031	0.024	KY980035.1 (100%)
Bacillariophyta	s:*Skeletonema marinoi*	OTU1132	0.016	0.023	0.014	0.018	/	/	/	/	KY364698.1 (100%)
	s:*Skeletonema costatum*	OTU1820	/	/	/	/	0.092	0.081	0.070		KY290715 (94.50%)
	s:*Thalassiosira rotula*	OTU2063	0.010	0.010	0.010	0.010	/	/	/	/	KT860983.1 (99.22%)
	s:*Thalassiosira tenera*	OTU601	0.048	0.054	0.027	0.043	0.041	0.048	0.041	0.043	MW722948.1 (100%)
	g:*Minidiscus comicus*	OTU1983	0.010	/	0.068	0.039	0.076	0.072	0.023	0.057	MN528623.1 (100%)
	s:*Cerataulina pelagica*	OTU2032	/	/	/	/	0.020	/	0.030	0.025	KC309487.1 (99.74%)
	g:*Chaetoceros* sp.	OTU2669	/	/	/	/	0.086	0.074	0.080	0.080	MG972315.1 (100%)
	s:*Cyclotella choctawhatcheeana*	OTU2226	/	/	/	/	0.020	0.023	0.018	0.020	JQ217341.1 (99.74%)
	s:unclassified-dinoflagellate	OTU2935	/	/	/	/	0.030	0.022	/	0.026	KY290715.1 (95.01%)

"/" indicates that the taxon was not a dominant species for a given sampling season. "g:" indicates a genus classification, while "s:" indicates a species-level classification.

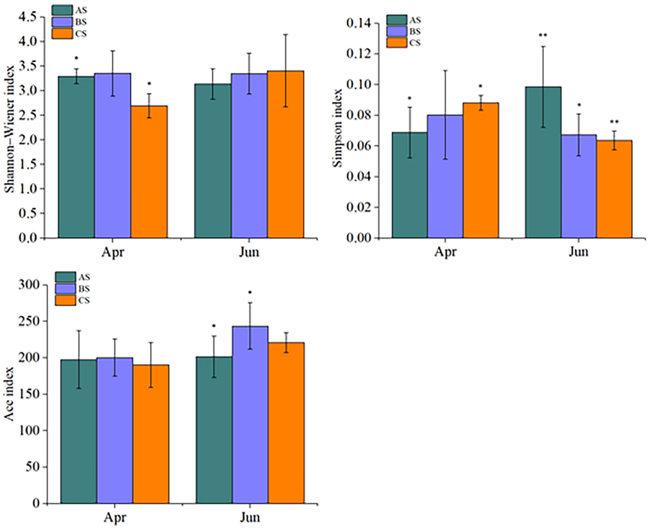

FIGURE 4
Shannon-Wiener, Simpson, and Ace alpha diversity index values for AS, BS, and CS communities. Note AS: surface samples from culture area BS: surface samples from Adjacent area CS: surface samples from control area,"*" and "**" indicates that BS or CS have significant differences compared with AS (*p <0.05, **p<0.01, one-way ANOVA).

areas (Figure 4). The Ace richness index did not significantly differ among areas in April, while the June culture area Ace index (201.4 ± 18.4) was significantly lower than that of the adjacent area (243.5 ± 31.8) ($p < 0.05$).

3.4.2 Phytoplankton community beta diversity

Principal component analysis (PCA) was used to evaluate differences in phytoplankton community structure among all surface samples (Figure 5). Significant differences in phytoplankton community structure were observed among sampling areas in April ($p = 0.010$), although significant differences were not observed in June ($p = 0.088$) (Figure 5).

3.4.3 Correlation between dominant phytoplankton species and environmental factors

RDA analysis indicated that most dominant species abundances were positively correlated with metrics of N, P, and Si (except NO_2-N) in April. NO_2-N, DO, and PO_4^{3-}P were the primary environmental factors associated with phytoplankton community structure in April. In addition, the abundances of dominant species were negatively correlated with nutrient values (except TN) in June, while TN and TP were the primary environmental factors associated with phytoplankton community structure (Figure 6).

The correlations between environmental factors (NH_4^+-N, NO_2-N, NO_3-N, PO_4^{3-}P, SiO_3^{2-}Si, TN, TP, DO PH, and Sal) and dominant phytoplankton species abundances in April were analyzed with phylogenetic molecular ecological networks (pMENs) (Figure 7). NH_4^+-N concentrations were significantly correlated with *Teleaulax amphioxeia* abundances ($p < 0.05$), while NO_2-N concentrations were significantly correlated with *Ostreococcus* sp., *P. australis*, and *Minidiscus comicus* ($p < 0.05$) in addition to *B. prasinos*, *M. pusilla*, and *Micromonas* sp. ($p < 0.01$). In addition, NO_3-N concentrations were significantly correlated with *Storeatula major*, *M. comicus*, and *Thalassiosira rotula* abundances ($p < 0.05$), while TP was significantly correlated with *M. comicus* abundances ($p < 0.05$). DO was significantly correlated with *Nannochloris* sp. abundances ($p < 0.05$), while pH was significantly correlated with *Ostreococcus* sp., *Cryptomonas* sp., *Heterocapsa* sp., unclassified Dinophyta, *S. marinoi*, *T. tenera*, and *M. comicus* abundances ($p < 0.05$).

The same analyses were conducted for environmental factors and dominant phytoplankton species in June communities (Figure 7). Again, TP levels were significantly correlated with unclassified Dinophyta abundances ($p < 0.01$). NO_3-N concentrations were significantly correlated with *Cryptomonas* sp. and *Heterocapsa* sp. abundances ($p < 0.05$), while DO concentrations were significantly correlated with unclassified Dinophyta abundances ($p < 0.01$) in addition to those of *Cyclotella choctahatcheeana*, *S. costatum*, *Minidiscus comicus*, and *T. tenera* ($p < 0.05$). Lastly, SiO_3^{2-}Si concentrations were significantly correlated with unclassified Dinophyta abundances ($p < 0.05$).

4 Discussion

4.1 Large-scale *S. fusiforme* cultivation could effectively increase marine carbon sequestration and helped mitigate coastal environmental problems

Large-scale *S. fusiforme* cultivation was associated with increased DO and pH in addition to decrease N within the cultivation area waters. Thus, *S. fusiforme* took up carbon (C), N, and P during photosynthesis and reduced N and P concentrations in seawater in addition to atmospheric carbon dioxide. These activities led to increase marine carbon sink capacity and DO levels that then increased water pH and helped preventing ocean acidification while also preventing eutrophication *via* efficient absorption of N and P along with other nutrients. Therefore, large-scale *S. fusiforme* cultivation

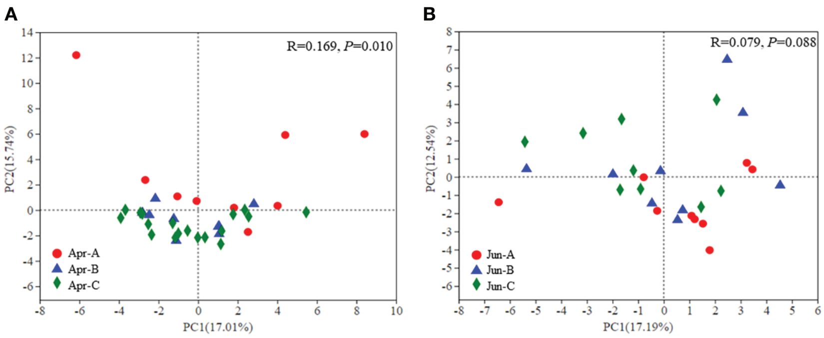

FIGURE 5
β-diversity of phytoplankton community composition in April **(A)** and June **(B)** based on PCA ordinations.

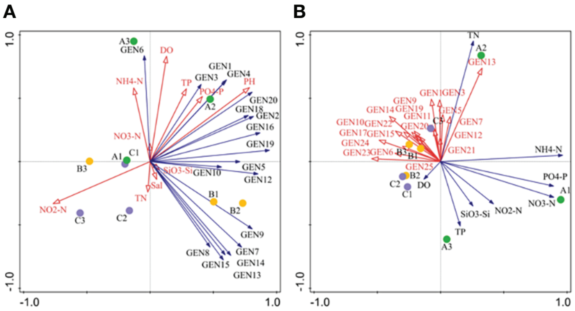

FIGURE 6
Redundancy anaysis (RDA) of phytoplankton community composition based on high-throughput sequencing for April **(A)** and June **(B)** communities. Abbreviations are as follows: GEN1: *Bathycoccus prasinos*, GEN2: *Ostreococcus* sp., GEN3: *Micromonas pusilla*, GEN4: *Micromonas* sp., GEN5: *Pyramimonas australis*, GEN6: *Nannochloris* sp., GEN7: *Plagioselmis prolonga*, GEN8: *Teleaulaxamphioxeia*, GEN9: *Cryptomonadales* sp., GEN10: *Storeatula major*, GEN11 *Teleaulax minuta*, GEN12: *Geminigera cryophila*, GEN13: *Heterocapsa* sp., GEN14: unclassified dinoflagellate, GEN15: *Gymnodinium* sp., GEN16: *Skeletonema marinoi*, GEN17: *Skeletonema costatum*, GEN18: *Thalasssiosira rotula*, GEN19: *Thalassiosira tenera* GEN20: *Minidiscus comicus*, GEN21: *Cerataulina pelagica*, GEN22: *Chaetocerus tenuissimus*, GEN23: *Chaetoceros* sp., GEN24: *Cyclotella choctawcheeana*, and GEN25: unclassified dinoflagellate.

could effectively improve water quality and environmental characteristics, leading to beneficial habitat restoration effects. *S. fusiforme* might inhibit phytoplankton growth by reducing nutrient availability. The inhibited growth of phytoplankton living in shaded areas might be due to light shading effects by expanded seaweed thalli (Chai et al., 2018).

The dominant microalgae in the non-cultivation area waters in April were *B. prasinos*, *Geminigera cryophila*, *Heterocapsa* sp, *S. marinoi*, and *Thalassiosira tenera*, but *B. prasinos*, *G. cryophila*, *H.* sp., *S. costatum*, and *T. tenera* in June. We speculate that *S. fusiforme* inhibited the growth of the above species, leading to their dominance in non-cultivation waters. Consistent with this interpretation, seawater extracts of hijiki dry powder contains substances that inhibit *S. costatum*, wherein the inhibitory effect increases with extract concentrations, suggesting that inhibitory substances in the extracts were presumably secondary metabolites with allelopathic effects (Zhang et al., 2021).

In this study there was no significant difference in the relative abundance of the dominant species *Heterocapsa* sp., *P. prolonga*, and *B. prasinos* among the sampling areas in April. Therefore, the dominant phyla of phytoplankton in the coastal ocean would not change due to the cultivation of *S. fusiforme*. The observed differences in community structure between April and June indicated a seasonal influence on communities that was consistent with other studies showing such trends due to seasonal variation of environmental factors such as temperature and nutrients (Chen et al., 2016; Lamont et al., 2018).

Significant differences between areas with and without *S. fusiforme* cultivation were observed when comparing several estimates of phytoplankton α and β biodiversity based on high-throughput sequencing approaches. Thus, these results pointed to a critical ecological significance of *S. fusiforme* cultivation, since biodiversity is one of the most important indicators of coastal ecosystem health and forms the basis of ecosystem productivity (Cardinale et al., 2012; Fraser et al., 2015; Isbell et al., 2015). Specifically, the results of this study indicated that large-scale cultivation of *S. fusiforme* maintained or enhanced phytoplankton community biodiversity *via* nutrient and light competition between seaweeds and phytoplankton. Therefore, these results demonstrated that large-scale cultivation of *S. fusiforme* could provide raw materials with high edible, medicinal, and industrial

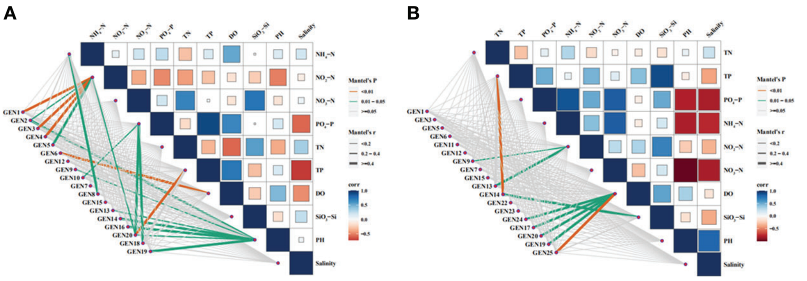

FIGURE 7
Relationships between environmental factors and the abundances of dominant phytoplankton species based on phylogenetic molecular ecological network analysis. Abbreviations are as follows: GEN1: *Bathycoccus prasinos*, GEN2: *Ostreococcus* sp., GEN3 *Micromonas pusilla*, GEN4: *Micromonas* sp., GEN5: *Pyramimonas australis*, GEN6: *Nannochloris* sp., GEN7: *Plagioselmis prolonga*, GEN8: *Teleaulax amphioxeia*, GEN9: *Cryptomonadales* sp., GEN10: *Storeatula major*, GEN11: *Teleaulax minuta*, GEN12: *Geminigera cryophila*, GEN13: *Heterocapsa* sp., GEN14: unclassified dinoflagellate, GEN15: *Gymnodinium* sp.,GEN16: *Skeletonema marinoi*, GEN17: *Skeletonema costatum*, GEN18: *Thalassiosira rotula*, GEN19: *Thalassiosira tenera* GEN20: *Minidiscus comicus*, GEN21: *Cerataulina pelagica*, GEN22: *Chaetoceros tenuissimus*, GEN23: *Chaetocerus* sp., GEN24: *Cyclotella choctawhatcheeana*, and GEN 25: unclassified dinoflagellate.

value and thus represented a promising strategy for controlling harmful algal blooms and eutrophication.

4.2 High-throughput sequencing provided more accurate microplankton identification

A total of 75 phytoplankton species were identified based on morphology, while a parallel survey of phytoplankton communities using high-throughput cultivation-independent sequencing identified 601 phytoplankton OTUs. Thus, the number of OTUs in this study was 3 to 7.6 times higher than the number of morphospecies identified from the same communities. This difference was likely attributable the high morphological similarity of species based on current taxonomy. For example, *Skeletonema* was one of the most abundant genera of phytoplankton observed in this study, comprising the five species *S. marinoi, S. costatum, S. potamos, S. menzellii*, and *S. subsalsum* based on high-throughput sequencing. In contrast, only *S. costatum* was identified based on traditional morphological observations due to high similarity in morphological appearance. In addition, the smallest known eukaryotes (~ 1 mm diameter cell sizes) (Chai et al., 2018) were observed for the first time in the study area. The actual number of phytoplankton species should be lower than those indicated by DNA sequencing approaches (Qiao et al., 2020). However, these higher OTU numbers likely reflected the genetic diversity among many morphospecies, and possibly the presence of some cryptic species or species complexes (Chai et al., 2018).

Phytoplankton communities detected with high-throughput sequencing are strikingly more diverse than those determined by microscopic detection, as many species exhibit relatively small sizes or are morphologically simple or similar to others (Liu et al., 2022). Furthermore, dormant cells, dead cells, or even cell fragments can be identified and lead to spurious diversity or abundance estimates (Santoferrara et al., 2020; Eliáš, 2021; Burki et al., 2021; Liu et al., 2022). Previous investigations of phytoplankton composition and diversity have primarily relied on traditional optical microscopy (Soares et al., 2011) that relies on cell morphological identification, rendering it difficult to recognize and distinguish similar taxa (Qiao et al., 2020). In this study, 21 species of nanophytoplankton accounted for 22.21% of the total sequences, while 17 species of picophytoplankton accounted for 13.18% of the total sequences from high-throughput sequencing, and these taxa were not observed using morphological analysis. Dominant Chlorophyta species such as *B. prasinos*, *M. pusill*a, *P. australis*, and *Picochlorum* sp. that are less than 2 μm in diameter are PPEs that are widely distributed in marine environments (Mayer and Taylo, 1979; Luis et al., 1992; Rashid et al., 2018). However, it was impossible to observe the above species by morphological observation; consequently, the use of high-throughput sequencing was necessary to comprehensively assess the diversity and community structure of phytoplankton. This will generally lead to higher biodiversity estimates.

4.3 The effects of *S. fusiforme* on phytoplankton community structure

RDA analysis indicated that DO, PO_4^{3-}-P, and NH_4^+-N were the main environmental factors affecting phytoplankton community structure in April among the factors measured, while DO levels in the culture area were significantly higher than in adjacent and control areas. The latter was primarily due to the photosynthetic oxygen release of *S. fusiforme*, and thus the effect of DO on phytoplankton community structure could be attributed to the effects of *S. fusiforme* on the phytoplankton community structure. A positive correlation was observed between phytoplankton community structure and nutrient concentrations, suggesting that seawater nutrients were consumed by *S. fusiforme*, thereby limiting phytoplankton growth to a certain extent. Consequently, *S. fusiforme* appeared to play a key role in regulating phytoplankton growth.

5 Conclusions

In summary, the results of the present study indicated that large-scale cultivation of *S. fusiforme* could reduce N and P concentrations in seawater, thereby helping to prevent eutrophication. Moreover, cultivation could increase water DO levels that could also help reduce the adverse effects of hypoxia while increasing water pH to prevent ocean acidification. In addition, large-scale cultivation of *S. fusiforme* played an important role in stabilizing phytoplankton community structure by improving phytoplankton diversity *via* the inhibition of harmful algal growth, potentially through allelopathy, nutrient competition and shading. Consequently, this study demonstrated that large-scale cultivation of *S. fusiforme* in coastal regions was an effective approach for increasing marine carbon sink capacities and helping to solve environmental problems in marine ecosystems.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA761497

Author contributions

YZ: Conceptualization, Methodology, Software, Writing-original draft, Funding acquisition. DX: Supervision, Project

administration, Validation, Writing - review and editing. TL: Investigation, Methodology, Validation. LQ: Investigation, Methodology, Validation. NX: Resources, Validation, Supervision, Writing - review and editing, Funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Publisher's note

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Coastal aquaculture farms for the sea cucumber *Apostichopus japonicus* provide spawning and first-year nursery grounds for wild black rockfish, *Sebastes schlegelii*: A case study from the Luanhe River estuary, Bohai bay, the Bohai Sea, China

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Aquaculture farms are known to attract wild organisms from nearby areas. Sea cucumber *Apostichopus japonicus* aquaculture farms in Bohai Bay, the Bohai Sea, China, might provide spawning and nursery grounds for wild black rockfish *Sebastes schlegelii* populations. To identify the above, we studied the variation in the numbers of *S. schlegelii* larvae (and length-categories as a percentage of the natural population) *via* ichthyoplankton surveys by months from January to October 2020, and assessed the food web structure and energy flow distribution of the farm and trophic links of *S. schlegelii* natural stock by using Ecopath modeling in July 2016 to August 2017. Larvae with a length between 3.90-5.80 mm were observed in the farm in May 2020, the juveniles with an estimated trophic level of 4.31. Juveniles used the farms as first-year nursery ground, and then they swam into deeper waters in August of the following year. MTI analysis showed increasing biomass of *S. schlegelii* might result in a decrease in the biomass of crustacea, and had positive and negative impacts on mollusk and *Hexagrammos otakii* biomass. We argued that aquacultural infrastructures constructed by artificial reefs could be used as a fisheries management tool to enhance *S. schlegelii* stocks and that monitoring of other marine organisms, such as oysters, *Rapana venosa*, *Charybdis japonica*, and Asterias spp. etc., was necessary to maintain sustainable exploitations of aquacultural farm ecosystems. A paucity of knowledge

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surrounding the interactions between aquaculture farms and wild organisms needs furthermore researches.

KEYWORDS

Apostichopus japonicus, *Sebastes schlegelii*, aquaculture farm, ichthyoplankton, Ecopath modelling, spawn

Highlights:

1. Spawning and first-year nursery grounds of *S. schlegelii* being in the farms.
2. Aquacultural infrastructures constructed by artificial reefs might enhance *S. schlegelii* populations.

Introduction

Aquaculture farms (for fishes, shellfishes, crustaceans, seaweeds, etc.) in coastal areas attract invertebrates (e.g. *Pandalus borealis*, Olsen et al., 2012; *Homarus americanus*, Drouin et al., 2015), various fishes (Callier et al., 2017), marine mammals (e.g. grey seals *Halichoerus grypus* on the Atlantic coast, Nash et al., 2000; monk seals *Monachus monachus* in the Turkish Aegean Sea, Güçlüsoy and Savas, 2003; sea lions *Otavia flavescens* in Chile, Sepulveda and Oliva, 2005; bottlenose dolphins *Tursiops truncatus* in the Mediterranean Sea and along the coast of Italy, Díaz-López and Bernal-Shirai, 2007, Bearzi et al., 2009) and various birds (e.g. *Nycticorax nycticorax* and *Phalacrocorax auritus*, Bradley et al., 2000) at both the individual and population levels. The distribution of organisms attracted to aquaculture farms varies over various temporal (day and season) and spatial scales (horizontal and vertical) (Valle et al., 2007; Fernandez-Jover et al., 2008; Sudirman et al., 2009; Uglem et al., 2009; Dempster et al., 2010; Goodbrand et al., 2013; Arechavala-Lopez et al., 2015; Bacher et al., 2015). Coastal aquacultural infrastructures such as fish cages, cement blocks, ropes, anchors, buoys, nets, stones, and other man-made submerged structures are thought to artificially aggregate fish by providing food and habitats (Bayle-Sempere et al., 2013; Kluger et al., 2017). Many studies have shown that the total biomass and abundance of wild fishes in aquaculture farms were dozens of times greater than in the surrounding area, e.g. coastal Atlantic salmon farms in Norway (Dempster et al., 2010), oyster aquaculture in Rhode Island (Tallman and Forrester, 2007). Thus, Aquacultural infrastructures might be a fisheries management tool to add fisheries resources and coastal aquaculture farms might be considered as artificial ecosystems.

In China, industrial aquaculture production of the sea cucumber *Apostichopus japonicus* was worth $ 46.38 billion USD in 2020. The area under aquaculture in China and its production reached 242,813 km^2 and 196,564 t in 2020, respectively (Fisheries and Fisheries administration et al., 2021). Of this, 1,000 km^2 (China Fisheries Association, 2021) of coastal aquaculture farms have been constructed using the deployment of artificial hard substrates, such as stones, to create so-called “sea ranching” (Yang, 2016). In 2013, a local community in the study area established a 2 km^2 sea cucumber aquaculture farm by deploying stones and artificial reefs (Xu et al., 2021). These hard substrates were gradually colonized naturally by native pacific oysters (*Crassostrea gigas*), to create artificial marine oyster reefs. We observed wild *Sebastes schlegelii* juveniles between 30-60 mm long inside the farm in July 2016. However, sea cucumber aquaculture farms in China have been suggested to only attract these wild *S. schlegelii* individuals without increasing their production *via* enhancing natural stocks.

On the other hand, *S. schlegelii*, this species called ‘*Kurosoi*’ in Japanese, is an ovoviviparous fish that is widely distributed in the coastal waters of the western North Pacific, including the northern coastlines of China and the coastal areas of Japan (except for the Ryukyu islands and the Korean Peninsula) (Yamada et al., 2007). Owing to its high commercial value, fast growth, and limited migration, it is a promising species for aquaculture and recreational fisheries in northeastern Asia, including China, Japan, and Korea (Lee, 2002). In Japan, their adults mate in winter, after which the females migrate to shallow coastal waters to give birth to larvae in the spring (Takahashi et al., 1994). Juveniles use shallow *Sargassum* and eelgrass beds in the innermost part of the bay as a nursery area during their first year, and then start to migrate to deeper waters in around August of the following year, as water temperature decreases (Sasaki et al., 2002, Nakagawa, 2008). In China, whether juveniles of this species also use aquaculture farms as first-year nursery grounds is unclear.

In this study, we aim to: (1) verify whether sea cucumber aquaculture farms provide spawning and first-year nursery grounds for wild *S. schlegelii* populations by identifying larvae and the relative occurrence of length-categories of populations by month; (2) understand the food web structure and energy flow distribution of the farm and the trophic links of wild *S. schlegelii* by constructing a trophic model using Ecopath. The results bring benefits to understand ecosystem-based aquaculture farm management and the dynamic relationships between aquaculture farms and wild organisms. Also, we argue that it is possible to use aquacultural infrastructures constructed by artificial reefs as a fisheries management tool to enhance fishery resources.

Material and methods

Ethical statement

Samples, including larval collections in the study area, were authorized by the local fisheries community. All procedures were performed following the American Fisheries Society guidelines for the use of fishes and crustaceans in research (Jenkins et al., 2014). The study was approved by the ethics committee of the East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The study did not involve any endangered or protected species as listed in the China Red Data Book of Endangered Animals.

Study site and sampling

The survey area (39°7'-39°11'N, 118°58.55'-119°2.55'E) is adjacent to Xiangyun Island, located inside the estuary of the Luanhe River, at the northeast part of Bohai Bay, the Bohai Sea (Figure 1). The Luanhe River is 1,200 km long and arises at the foot of the Yanshan Mountains and flows into Bohai Bay. In 2013, local fishermen deployed stones and artificial reefs (ARs) in the sea to create a ca. 2 km^2 sea cucumber aquaculture farm, surrounded by a 4 km long, 8 m height breakwater to protect against wave damage (Figure 1).

To identify whether aquaculture farms provide spawning grounds for the wild *S. schlegelii* population, we operated six stations to collect larval samples from January to October 2020 (Figure 1). We used a 0.505 mm mesh ichthyoplankton net (0.80 m diameter, 3.50 m length) to collect *S. schlegelii* larval samples. We sampled horizontally and vertically (raising the nets from the bottom to the surface) in 10 min sweeps from a vessel travelling at a speed of 1-2 knots. The samples were immediately preserved in seawater with 5% formalin *in situ* and identified to the lowest possible taxonomic level in the laboratory. The larvae of *S. schlegelii* were measured to the nearest 0.1 mm from the front of the snout to the terminal end of the crest bone using a light microscope (Sterm 2000, Karl Zeiss, Germany) (Pattrick and Strydom, 2014).

To describe the food web structure and energy flow distribution of the farm and trophic links among wild *S. schlegelii* adult stock, between July 2016 and August 2017 (sampled in July 2016, September 2016, November 2016, December 2016, January 2017, March 2017, April 2017, May 2017, June 2017, July 2017, and August 2017), we established nine sampling stations to collect biomass data for our Ecopath model precisely and from August 2016 to the present established one environmental monitoring station to measure water temperature (°C) and dissolved oxygen (DO) (mg L^{-1}) in the farm (Xu et al., 2021). We used crab pots, gill nets, and trawl nets to collect samples and estimate the biomass of functional groups (measured in t km^{-2}) in the farm. The samples were identified to the lowest possible taxonomic level, and species were counted and measured to an accuracy of ±1 mm using a 40 cm ruler, and weighed to an accuracy of ±0.1 g using a balance (JJ-Y series, SSWA).

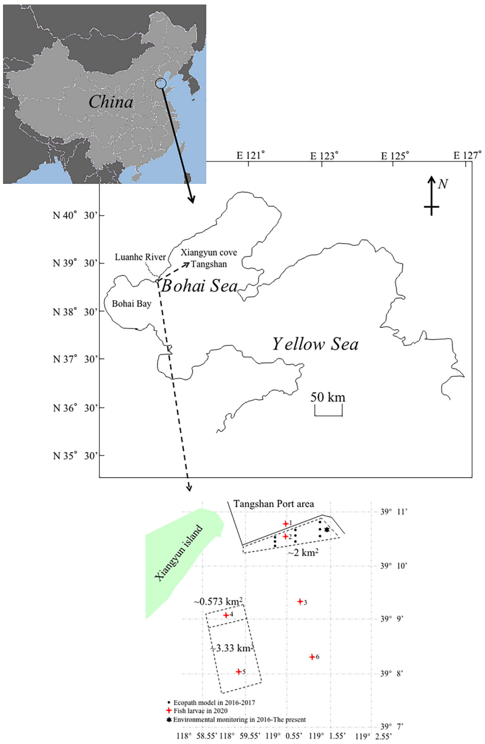

FIGURE 1
Schematic map showing the survey area in the northernmost part of Bohai Bay, the Bohai Sea, China (39°7'-39°11'N, 118° 58.55'-119°2.55'E). Showing: 16 stations, including 12 sites inside the area of artificial reefs (ARs); 3 control sites (Nos. 1, 3 and 6); and 1 environmental monitoring station in Xiangyun cove, adjacent to Xiangyun Island and the Tangshan Port area. The white trapezoid and rectangles surrounded by black dashed lines represent: 3,000 mu (~2 km^2) of ARs created in 2013; 860 mu (~0.573 km^2) of ARs created in 2019; and 5,000 mu (~3.333 km^2) of ARs created in 2020. One mu is approximately 666.667 m^2. We established one environmental monitoring station (denoted by a black hexagon), operated from 2016 to the present, and nine sampling stations (denoted by black dots) in 2016 to 2017 to create the Ecopath model, and six sampling stations (denoted by red square stars) created in 2020 to sample *Sebastes schlegelii* larvae.

Inputs, adjustments, and calibrations of the Ecopath model

The Ecopath approach and modeling software (Ecopath with Ecosim Version 6.5) (Christensen and Pauly, 1992; Christensen and Walters, 2004) were used in the study to assess food web structure and energy flow distribution of the farm and the trophic links of wild *S. schlegelii*. It is expressed by the mass-balance equation:

$$P_i - B_iM2_i - P_i(1 - EE_i) - EX_i - BA_i = 0 \tag{1}$$

where P_i represents the production of group i, B_i represents biomass in tons (wet weight), and $M2_i$ represents mortality by predation. EE_i represents ecotrophic efficiency and EX_i represents export, and BA_i represents the biomass accumulation of i during the study period.

The MTI was calculated by constructing an n x n matrix, where (Xu et al., 2019):

1. the i, j^{th} element represents the interaction between the impacting group i and the impacted group j where $MTI_{i,j}=DC_{i,j}-FC_{j,i}$;
2. $DC_{i,j}$ is the diet composition term denoting how much j contributes to the diet of i;
3. $FC_{j,i}$ is the host composition term giving the proportion of the predation on j that is due to i as a predator.

The composition of the functional groups of marine organisms were separated based on dietary similarity, preferred physical habitat, and other ecological similarities and were described in Table 1. The functional groups had to span all of the trophic system components and all the fishing targets in the study area. Marine fishes and crustaceans of high economic value (e.g. *S. schlegelii*, *Pholis fangii*, *Hexagrammos otakii*, *Oratosquilla oratoria*, *Charybdis japonica*, *Rapana venosa*, and *A. japonicus*) and large biomass oysters were assigned to separate distinct groups. We determined the input values of Biomass (B), Production/Biomass (P/B), Consumption/Biomass (Q/B), Ecotrophic efficiency (EE), and landing data for all functional groups using the Fishbase platform (Table 2). The P/B and Q/B values of fishes were determined using empirical regression of Pauly (1980) with the Fishbase platform. Input values were set equal to the averages of the parameters for the individual group members, weighted by their relative biomass. The Ecopath model generated the missing parameter values. The dietary composition of each functional group was then determined using data obtained from the literature and this study (Table 3). These data showed that each consumer group had a diet consisting of one or more other groups. Thus, each prey item was entered as a proportion of the consumer's diet, and adjustments were made to obtain a balanced model. Most

TABLE 1 Description of species composition of the function groups on the sea cucumber aquaculture farm (~2 km^2) adjacent to the artificial breakwaters in Xiangyun cove, Bohai Bay, the Bohai Sea, China, between July 2016 and August 2017.

Group name	Species composition
Octopodidae	*Octopus variabilis* and *Octopus ocellatus*
Sebastes schlegelii	Sebastes schlegelii
Pholis fangii	Pholis fangii
Hexagrammos otakii	Hexagrammos otakii
Gobiidae	*Chaeturichthys stigmatias* and *Parachaeturichthys polynema*
Oratosquilla oratoria	Oratosquilla oratoria
Charybdis japonica	Charybdis japonica
Pelagic fishes	*Hyporhamphus gernaerti*, *Thrissa kammalensis*, *Konosirus punctatus*, *Strongylura anastomella*, *Stolephorus indicus* and *Sardinella zunasi*
Benthopelagic fishes	*Chirolophis japonicus* and *Trachidermus fasciatus*
Reef-associated fishes	*Agrammus agrammus*, *Lateolabrax japonicus*, *Platycephalus indicus*, *Cociella crocodilus* and *Sillago sihama*
Demersal fishes	*Acanthopagrus schlegeli*, *Ernogrammus hexagrammus*, *Cynoglossus semilaevis*, *Cynoglossus joyneri*, *Platichthys bicoloratus*, *Paralichthys olivaceus*, *Tridentiger bifasciatus*, *Tridentiger trigonocephalus*, *Tridentiger obscurus* and *Takifugu niphobles*
Crustacean	*Eucrate crenata*, *Dorippe japonica*, *Xenophthalmus pinnotheroides*, *Pisidia serratifrons*, *Pugettia nipponensis*, *Matuta planipes*, *Penaees chinensis*, *Penaeus japonicus*, *Acetes chinensis*, *Lysmata vittata*, *Palaemon gravieri*, *Leptochela gracilis*, *Alpheus japonicus*, *Crangon affinis*, *Diogenes edwardsii*
Rapana venosa	Rapana venosa
Oyster	*Crassostrea gigas*, *Ostrea denselamellosa*
Mollusk	*Loligo japonica*, *Neverita didyma*, *Chlorostoma rustica*, *Nassarius siquijorensis*, *Nassarius variciferus* and *Scapharca inflate*
Apostichopus japonicus	Apostichopus japonicus
Asterias rollestoni	Asterias rollestoni
Temnopleurus hardwickii	Temnopleurus hardwickii
Echinodermata	*Amphiura vadicola* and *Caudina similis*
Aurelia aurita	Aurelia aurita
Aphrodita talpa	Aphrodita talpa
Microzoobenthos	Polychaete, Copepods, Nematodes, Bivalvia, Gastropods, Ostracoda, Amphipoda
Macrozoobenthos	Paguridae and other benthos except for microzoobenthos
Zooplankton	*Labidocera euchaeta*, *Sagitta crassa*, *Calanus sinicus*, *Centropages abdominalis*, *Sagitta crassa*, *Gastropods larvae*, *Macrura larvae*, *Brachyura zoea larva*, *Polychaeta larva*, *lamellibranchia larva*, *Calanus sinicus*, *Labidocera euchaeta*, *Sagitta crassa*

(Continued)

TABLE 1 Continued

Group name	Species composition
Heterotrophic bacteria	Heterotrophic bacteria
Phytoplankton	*Melosira sulcata*, *Coscinodiscus asteromphalus*, *Coscinodiscus jonesianus*, *Coscinodiscus thorii*, *Coscinodiscus spinosus*, *Thalassiothrix frauenfeldii*, *Coscinodiscus asteromphalus*, *Skeletonema costatum*, *Coscinodiscus granii*, *Chaetoceros curvisetus*
Macroalgae	*Sargassum miyabei*, *Sargassum thunbergii*, *Ulva lactuca*
Detritus	DOC, suspension POC, oyster excrements

resident species fed both inside and outside of the aquaculture farm and could therefore be regarded as importing food items in their dietary composition (Xu et al., 2021). Flows were expressed in t wet weight km^{-2} year^{-1}, and biomasses were expressed in t wet weight km^{-2}. When a balanced model was established, Ecopath was used to calculate a trophic level (TL) for each group on the basis of its diet. Adjustments and calibrations of the parameters in the model followed the procedures of Xu et al., 2019 and Xu et al., 2021.

Results

The variation in length of *S. schlegelii* larvae

Larvae of *S. schlegelii* with lengths range of 3.90-5.80 mm were found in May 2020 (Figure 2). Most were found inside the farm in early May at a water temperature of 12.73°C, but larger individuals swam outside the farm in late May (Figure 2). More individuals were found at the bottom of the water column than

TABLE 2 The parameter values of each functional group in a sea cucumber farm (~2 km^2) in Xiangyun cove, Bohai Bay, the Bohai Sea, China, from an Ecopath model from July 2016 to August 2017.

Group name	TL	B	P/B	Q/B	Res	AS	EE	Fishing	OI
1. Octopodidae	4.70	0.364[a]	3.3[b]	12[b]	2.2932	3.4944	0.8021	1.2807[a]	0.5702
2. Sebastes schlegelii	4.31	0.6879[a]	0.94[b]	18.3[b]	9.4242	10.0709	0.3881	0.1517[a]	1.4231
3. Pholis fangii	3.63	0.13[a]	4[b]	13.6[b]	0.8944	1.4144	0.9257	0.4014[a]	0.7064
4. Hexagrammos otakii	4.13	0.1722[a]	1.11[b]	4.3[b]	0.4012	0.5924	0.5856	0.6061[a]	1.5931
5. Gobiidae	3.84	1.5[a]	2.323[b]	8[b]	6.1155	9.6000	0.9169	0.2316[a]	0.9898
6. Oratosquilla oratoria	3.28	0.6347[a]	8[b]	30[b]	10.1552	15.2328	0.0513	0.0016[a]	0.0301
7. Charybdis japonica	3.43	1.4804[a]	8[b]	27[b]	20.1334	31.9766	0.2539	1.1920[a]	0.8049
8. Pelagic fishes	3.26	0.5006[a]	3[b]	9.925[b]	2.4730	3.9748	0.7381	0.1042[a]	0.3949
9. Benthopelagic fishes	4.32	0.061[a]	2[b]	7.025[b]	0.2208	0.3428	0.8212	0.8555[a]	1.1806
10. Reef-associated fishes	4.32	0.171[a]	0.6078[b]	4.314[b]	0.4862	0.5902	0.0000	–	0.9254
11. Demersal fishes	4.54	0.3242[a]	1.4658[b]	6[b]	1.0809	1.5562	0.3123	0.1610[a]	0.4819
12. Crustacean	3.33	1.416[a]	9[b]	30[b]	21.2400	33.9840	0.9822	–	0.4204
13. Rapana venosa	3.05	80.159[a]	0.26[b]	2.82[b]	159.9974	180.8387	0.0492	0.0123[a]	–
14. Oyster	2.05	216.733[a]	1.097875[b]	10.5[b]	1127.4720	1365.4180	0.9500	–	0.0475
15. Mollusk	2.65	1.435[a]	5.19[b]	17.2[b]	12.2980	19.7456	0.7189	–	0.4855
16. Apostichopus japonicus	2.30	6.344[a]	0.6[b]	3.36[b]	13.2463	17.0527	0.0000	–	0.2121
17. Asterias rollestoni	3.37	0.3731[a]	0.49[b]	3.24[b]	0.7843	0.9671	0.0000	–	0.0042
18. Temnopleurus hardwickii	2.17	0.0114[a]	1.4[b]	5[b]	0.0296	0.0456	0.0000	–	0.2462
19. Echinodermata	2.53	0.00035[a]	1.21[b]	4.7[b]	0.0009	0.0013	0.0000	–	0.2898
20. Aurelia aurita	3.11	0.08681[a]	5.011[b]	25.05[b]	1.3047	1.7397	0.4999	–	0.1111
21. Aphrodita talpa	2.54	0.0255[a]	2.41[b]	15[b]	0.1680	0.2295	0.0000	–	0.7282
22. Microzoobenthos	2.30	3[a]	26.25[b]	75[b]	101.2500	180.0000	0.7965	–	0.2121
23. Macrozoobenthos	2.43	32.155[b]	2.41[b]	15[b]	308.3664	385.8600	0.5065	–	0.2861
24. Zooplankton	2.00	3.9764[a]	57[b]	151.775[b]	256.1597	482.8145	0.5996	–	–
25. Heterotrophic bacteria	2.01	3[b]	84.06[b]	171.55[b]	159.5400	411.7200	0.8770	–	0.0050
26. Phytoplankton	1.00	22.66[a]	119.31[b]	–	–	–	0.4203	–	–
27. Macroalgae	1.00	15[a]	9.447[b]	–	–	–	0.6841	–	–
28. Detritus	1.00	43[b]	–	–	–	–	0.5715	–	0.4219
Detritus inputs	–	1193.2[b]	–	–	–	–	–	–	–

Note:[a] Shows that the value was measured in this study. [b]Shows that the value was taken from the referenced literature. The other values were estimated by the model.
TL, Trophic level, B, Biomass (t km^{-2}), P/B, Production-to-Biomass ratio (yr^{-1}), Q/B, Consumption-to-Biomass ratio (yr^{-1}), Res, Respiration (t km^{-2} yr^{-1}), AS, Assimilation (t km^{-2} yr^{-1}), EE, Ecotrophic efficiency, Fishing (t km^{-2} yr^{-1}), OI, Omnivory index. '-' denotes no data available.

TABLE 3 The dietary composition of the functional groups in the sea cucumber aquaculture farm (~2 km^2) in Xiangyun cove, Bohai Bay, the Bohai Sea, China, derived from July 2016 to August 2017.

Functional group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1. Octopodidae	0.0875			0.0035					0.2625																
2. *Sebastes schlegelii*		0.0114		0.0009	0.0002																				
3. *Pholis fangii*	0.05	0.0007		0.0002						0.2736															
4. Hexagrammos otakii		0.0006																							
5. Gobiidae	0.45	0.0037		0.0001	0.017				0.025		0.319														
6. Oratosquilla oratoria	0.0375	0.0001		0.0029	0.0077																				
7. *Charybdis japonica*	0.175	0.0026		0.0048	0.0037						0.2042														
8. Pelagic fishes				0.0247	0.0244			0.15																	
9. Benthopelagic fishes					0.004																				
10. Reef-associated fishes																									
11. Demersal fishes		0.0004			0.0076																				
12. Crustacean		0.48	0.3	0.324	0.391			0.03	0.025	0.389	0.2708										0.1				
13. *Rapana venosa*									0.09																
14. Oyster													1												
15. Mollusk				0.0005								0.125						0.1			0.1				
16. *A. japonicus*																									
17. *Asterias rollestoni*																									
18. *T. hardwickii*																									
19. Echinodermata																									
20. *Aurelia aurita*																				0.1					
21. *Aphrodita talpa*																									
22. Microzoobenthos						0.3						0.1925					0.5		0.1				0.1		
23. Macrozoobenthos		0.0003	0.1	0.0043	0.1565	0.45	0.45	0.33	0.3975			0.1925					0.5				0.1				
24. Zooplankton			0.3			0.25		0.38				0.165		0.05	0.242				0.1	0.9					
25. Heterotrophic bacteria																0.3			0.3			0.3	0.3		0.005
26. Phytoplankton								0.11						0.2	0.128				0.1				0.1	1.0	0.05
27. Macroalgae		0.0002		0.0344	0.0308					0.0378	0.006							0.7	0.1				0.2		
28. Detritus					0.057									0.75		0.7		0.2	0.3		0.7	0.7	0.3		0.945
Import	0.2	0.5	0.3	0.6	0.3		0.55		0.2	0.3	0.2	0.325			0.63										

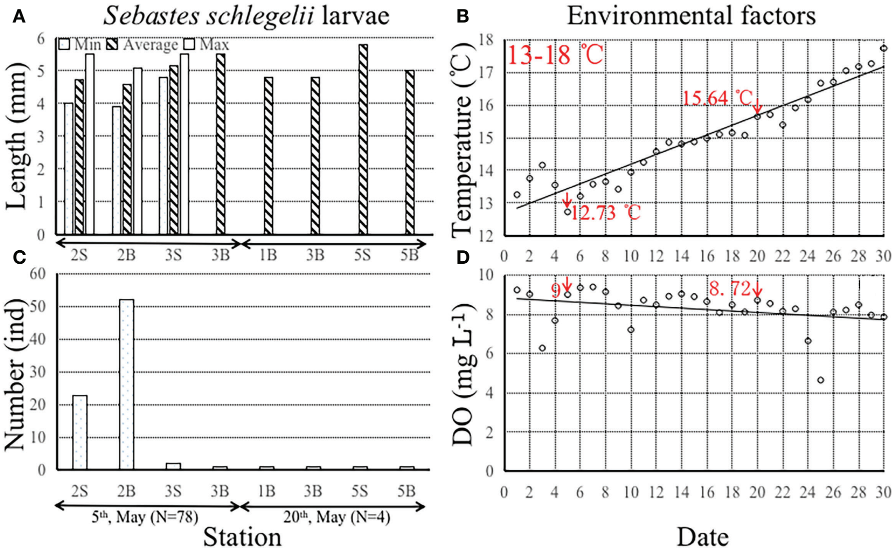

FIGURE 2
(A) The minimum, average and maximum length (mm) of *Sebastes schlegelii* larvae at the different sampling stations between 5th May and 20th May 2020 (upper-left); **(B)** The number (ind) of *Sebastes schlegelii* larvae at the different stations between 5th May (Total number=78 ind) and 20th May 2020 (Total number=4 ind) (bottom-left);**(C)** The water temperature increase (°C) from 1st May to 30th May 2020 (upper-right); **(D)** The dissolved oxygen content (DO, mg L^{-1}) decreased from 1st May to 30th May 2020 (bottom-right). Notes: "S" denotes the sea surface; "B" denotes the sea bottom. Red arrowheads point to the sampling dates. All the measurements of water temperature and DO were taken at an environmental monitoring station at 10:00 am between 1st May and 30th May 2020.

at the surface. The ranges of water temperature (increasing trend) and DO (decreasing trend) were 12.73-17.73°C and 4.65-9.40 mg L^{-1} in May 2020, respectively (Figure 2).

The monthly variation in length-categories percentage of the population

In July 2016 and 2017, the majority of *S. schlegelii* were in the 30-59, 60-89, 90-119 mm length-categories, with very few older individuals of >120 mm length. They were similar in average length (56.34 versus 61.26 mm, respectively) at similar average water temperatures (25.81 ± 0.23 versus 25.36 ± 0.71°C respectively). Between August and December, the overall average length increased from 92.40 to 102.94 mm, while the average water temperature decreased from 26.58 ± 1.00 to 5.01 ± 0.80°C. The percentage of length-categories >90 mm increased from March to May. The 0-29 mm category was also observed in May 2017 (Figure 3). The total number and average length (77.50→88.45→94.30→106.59 mm) of fish increased from January→March→April→May, as average water temperature rose from 0.06→4.84→11.34→16.98°C. In particular, we found two especially large fish (L=225.00 mm, W=297.70 g; L=203.00 mm, W=270.00 g) about to spawn in March and two even larger ones (L=235.00 mm, W=395.60 g; L=215.00 mm, W=262.80 g) which finished spawning in the farm in May.

MTI analysis and trophic links of *S. schlegelii* in the farm

We performed MTI analyses of the functional groups of organisms. The increasing biomass of detritus increased the biomass of benthic species and oysters, leading to an increasing biomass of *O. oratoria, R. venosa*, and *A. rollestoni*. The increasing biomass of oysters decreased the biomass of phytoplankton, zooplankton and detritus, which decreased the biomass of *O. oratoria*, *A. japonicus*, Echinodermata, *A. aurita*, *A. talpa*, microzoobenthos, *A. rollestoni*, and heterotrophic bacteria. The increasing biomass of *R. venosa* decreased the biomass of oysters, which led to an increase in biomass of zooplankton and detritus, and had a positive impact on *O. oratoria*, *A. japonicus*, Echinodermata, *A. aurita*, *A. talpa*, microzoobenthos, and heterotrophic bacteria. The increasing biomass of *C. japonica* decreased the biomass of macrozoobenthic species, thus increasing the biomass of *T. hardwickii*, microzoobenthos and macroalgae and decreasing the biomass of *H. otakii*, benthopelagic fishes and *R. venosa*. The increasing biomass of Gobiidae decreased the biomass of crustacea, which had a negative impact on fishes such as *P. fangii*, *H. otakii*, pelagic fishes, benthopelagic fishes, reef-associated fishes, and demersal fishes.

The trophic level of *S. schlegelii* in the farm was 4.31 compared with the Octopodidae, the highest ranked at 4.70. *S.*

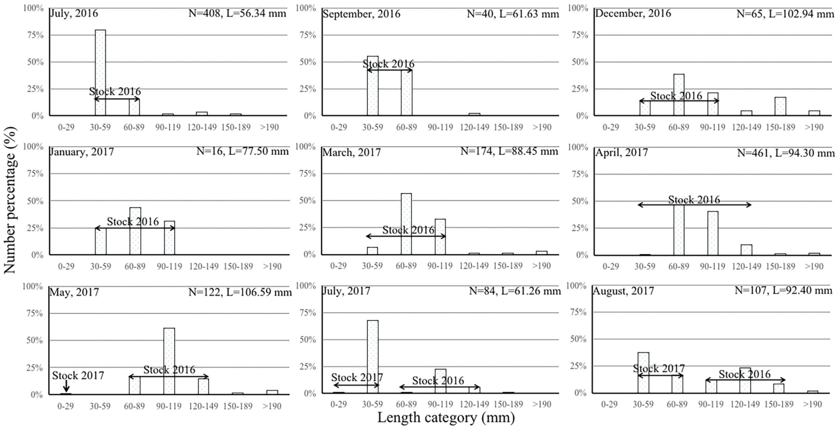

FIGURE 3
Percentage (%) of *Sebastes schlegelii* against length category (mm) from 0–29 mm to >190 mm in 30 mm length intervals from July 2016 to August 2017 in a ~2 km^2 area of the sea cucumber farm. N denotes total number of individuals; L denotes average length of individuals.

schlegelii and other fish groups such as benthopelagic fishes, reef-associated fishes, and demersal fishes were all top predators. The increasing biomass of *S. schlegelii* caused a decrease in the biomass of crustaceans, but had a positive and negative impact on mollusks and *H. otakii*, respectively (Figure 4).

Food web structure and energy flow distribution of the farm

In terms of food web structure, phytoplankton and macroalgae were the primary producers. Oysters and *A. japonicus* relied largely on detritus and, in addition to marcoalgae and phytoplankton, were the main prey of zoobenthic and zooplanktonic species. Secondary consumers such as the Gobiidae, crustacea, and top predators such as Octopodidae, were the key functional groups (Figure 5).

The transfer efficiency ranges in total flow, producer and detritus were 9.09-12.45%, 8.52-12.31% and 7.46-13.75%, respectively, varying among trophic levels II to VII. The transfer efficiency of producers in trophic levels II and III was higher than that of detritus, but an opposite result was observed in trophic levels IV and V. Most of the biomass in the ecosystem was in trophic levels I to III. The biomass in trophic levels II and III was 239.60 and 106.10 t km^{-2} $year^{-1}$, respectively. The range of biomass in trophic levels V to VII was 0.006-0.86 t km^{-2} $year^{-1}$. The first and second highest catches were 2.21 t km^{-2} $year^{-1}$ in trophic level III and 1.25 t km^{-2} $year^{-1}$ in trophic level IV. The energy flow value in the throughput item was equal to the sum of the import item, consumption item, export item, flow to detritus item and respiration item. In trophic level I, the first and second highest energy flow values were for the consumption item (3744.00 t km^{-2} $year^{-1}$) and export item (1883.00 t km^{-2} $year^{-1}$).

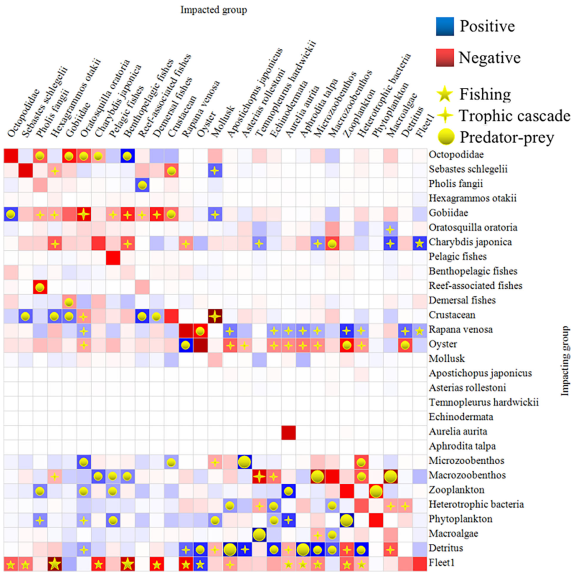

FIGURE 4
The result of a mixed trophic impact (MTI) analysis of the sea cucumber aquaculture farm ecosystem from 2016 to 2017. Impacting and impacted groups are shown along the vertical and horizontal axes, respectively. Positive and negative impacts are shown as blue and red squares, respectively. The squares should not be interpreted in an absolute sense: the impacts are relative, but comparable between groups. The darkness of the color indicates the degree of the impact. Trophic relationships of fishing, trophic cascades and predator-prey interaction are denoted by a yellow five-pointed star, a yellow cross-star and a yellow circular symbol. Impact values of 0.1–0.4 and > 0.4 are denoted by small and large symbols, respectively.

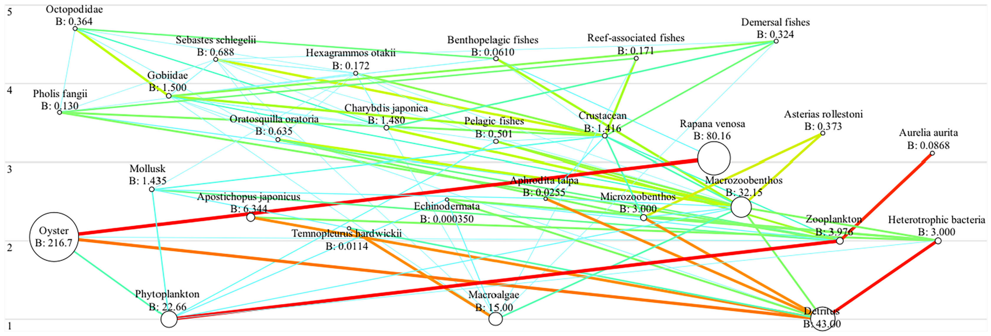

FIGURE 5
A flow diagram representing the food web structure in terms of functional groups and fractional trophic levels in the sea cucumber farm (~2 km^2) in Xiangyun cove, Bohai Bay, the Bohai Sea, China in 2016-2017. Circles are distributed on the Y-axis according to trophic level (1-5), the size of the circle being proportional to each group's biomass, the thickness of lines represented by different colors showing the strength of trophic links. Biomass is given in t km^{-2}.

In trophic levels II to VII, the energy flow value in the respiration item was highest, and the second highest item was the flow to detritus item. Energy flows in the throughtput item decreased with increasing trophic level (Table 4).

Discussion

Aquaculture farm infrastructures have been shown to enhance wild populations of sessile and mobile species, including bivalves, macroalgae, bryozoans, other mollusks, and tunicates. For example, The increased numbers of large crustaceans contribute perhaps 2.80 t ha^{-1} yr^{-1} to production on oyster reefs (zu Ermgassen et al., 2016). The productivity of invertebrates in the vicinity of *Perna canaliculus* mussel beds in New Zealand was 3.5 times greater than on nearby soft sediments (McLeod et al., 2013). The epifauna on an artificial reef in Delaware Bay, USA was found to be between 147 and 895 fold greater than in the surrounding area (Foster et al., 1994). However, there have been very few reports discussing the

TABLE 4 Transfer efficiency, biomass and energy flows at various TLs (I-VII) showing the contribution of detritus and primary production to the trophic network in Xiangyun wan, Bohai Bay, Bohai Sea, China.

	Trophic level						
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**
Transfer efficiency							
Producer%	–	10.62	10.19	7.46	10.56	13.75	10.23
Detritus%	–	10.24	8.52	11.65	10.68	12.18	12.31
Total flow%	–	10.34	9.09	10.27	10.65	12.45	11.65
Biomass							
Living	37.66	239.60	106.10	8.05	0.86	0.09	0.006
Detritus	43.00	–	–	–	–	–	–
Catches	0.00	0.08	2.21	1.25	0.38	0.06	0.004
Energy Flows							
Import	1193.00	–	–	–	–	–	–
Consumption	3744.00	639.00	59.18	6.61	0.68	0.05	0.004
Export	1883.00	0.08	2.21	1.25	0.38	0.06	0.004
Flow$_{td}$	1612.00	1354.00	208.60	23.04	2.82	0.24	0.013
Respiration	0.00	1758.00	405.40	45.63	6.12	0.51	0.026
Throughput	8432.00	3751.00	675.40	76.53	10.00	0.86	0.048

Flows: t km^{-2} year^{-1}. "-" means no data available.
Flow$_{td}$: flow to detritus. Transfer efficiency: dimensionless unit. Biomass: t km^{-2} year^{-1}.

production increase and successful recruitment of wild *S. schlegelii* populations in sea cucumber farms. Fish production is defined as the net increase in biomass (Bohnsack and Sutherland, 1985), and recruitment is defined as the process of larval settlement and post-settlement survivorship to breeding age (Richards and Lindeman, 1987). In this study, we are the first to show that sea cucumber farms in the Bohai Sea are used as spawning grounds by economically important fishes such as *S. schlegelii*. Newborn larval *S. schlegelii* are reported to be 3.80-4.70 mm in length (Lin et al., 2014), and we obtained individuals 3.90-5.50 mm in the farm in early May 2020. The results provided an example of the successful coexistence of wildlife within sea cucumber farms.

Second, it was reported that communities on new artificial reefs might take 10-15 years to fully develop (Fager, 1971). However, Hueckele and Buckley (1987) found that new foraging species continued to appear even during the fourth and fifth years following reef construction. In this study, we obtained *S. schlegelii* larvae in the farm 3-5 years after its creation, but not on artificial reefs nearby created only 1-2 years previously. We argue that the presence of a wild *S. schlegelii* larvae indicates a healthy community on an artificial reef once the habitat changes from an unproductive barren substrate to a highly productive environment (Stone et al., 1979; Matthews, 1985). On this evidence, we speculate that a sea cucumber farm can reach community maturity within 3-5 years and that successful recruitment of *S. schlegelii* larvae can result from the creation of sea cucumber aquaculture farms in shallow waters.

Third, water temperature is one of the major factors influencing fish spawning (Wootton, 1990) such as *S. schlegelii* wild population, from the primordial germ-cell stage to the larval stages (Beldade et al., 2017). In the winter of 2020, the Bohai Sea suffered from more sea ice, over a longer period than usual (Xinhua News Agency, 2020). The average water temperature in the farm between December 2020 and March 2021 was 1.42-2.77°C (unpublished data), lower than during the same period from 2016 to 2017. Local fishermen reported very low production of *S. schlegelii* in the farm in 2021, and also we obtained very few number of larvae in this year.

Regarding the reason why that natural *S. schlegelii* population spawns and inhabits sea cucumber farm, aquaculture farms introduce a considerable number of hard physical structures (both at the sea bottom and in the water column), creating an environment which marine species such as *Acetes chinensis* find attractive. Pan et al. (2020) found that *A. chinensis* formed the major part of their diet in the study area. The *A. chinensis* population uses the shallow waters of Bohai Bay as a nursery ground in May, and remain there from May to October, after which the percentage of *A. chinensis* by weight in the diet of *S. schlegelii* decreases from 100.00% in June to 1.39% in November (Pan et al., 2020). Complex 3-D fixed structures of sea cucumber aquaculture farm may result in aggregation or high productivity of prey species (Coen et al., 2007; Mann and Powell, 2007; Powers et al., 2009) and therefore increase the food available for juvenile *S. schlegelii*. In addition, wild population in Haizhou Bay in the Yellow Sea preferred natural reefs, mixed sand/artificial reef bottoms, mixed bottoms of boulder, cobble, and gravel, and artificial reefs (Zhang et al., 2015). Thus, it is therefore reasonable that wild *S. schlegelii* choose sea cucumber aquaculture farms constructed using oyster reefs as spawning and nursery grounds.

Final, understanding food web structure is an important component of ecosystem-based aquaculture management. Oysters are major grazers of planktonic organisms and consumers of organic detritus suspended in the water. Bivalve species, including oysters, have the potential to affect the plankton community structure through their grazing activities (Loret et al., 2000). However, they can have a positive influence on phytoplankton population growth through their nutrient producing activities, releasing large amounts of ammonia and other inorganic compounds into estuarine ecosystems (Dame and Libes, 1993). Oysters may be important sources of nutrients for estuarine primary producers in the farm. The detritus consumer *A. japonicus* and bivalve predators such as *R. venosa* and *C. japonica* are economically important species and secondary consumers which support fishery production in our study. Asterias spp. in the farm can cause ecological catastrophes in coastal areas. For example, the excessive spread of *Asterias amurensis* caused massive death of cultured bivalves, including oysters and clams, in Jiaozhou Bay, the Bohai Sea in March 2021 (Zhao, 2021). *A. amurensis* ate 100,000 t of *Ruditapes philippinarum* in aquaculture farms in Qingdao in 2007 (Zhao, 2021). It is therefore important to understand the dynamic interactions of wild animals in aquacultural settings to promote the sustainable development of aquaculture farms. It is necessary for farm managers to weigh the close relationship between aquacultural practices and the benefits to the wild stocks through their provision of ecosystem structures. Aquacultural management decisions should not harm wild populations or the long-term sustainability of the ecosystem.

Conclusions

We summarize the main conclusions as follows.

1. Sea cucumber aquaculture farms in the Bohai Sea, China provided the spawning and first-year nursery grounds for populations of the economically important fish *S. schlegelii*.
2. Aquacultural infrastructures constructed by artificial reefs can be regarded as an effective fisheries management tool to enhance *S. schlegelii* populations.

In this study, we constructed an Ecopath model using high quality inputs to perform a mixed trophic impact analysis to understand the food web structure and energy flow distribution of a sea cucumber aquaculture farm constructed by oyster reefs. We argue that increasing the number of sea cucumber farms can increase the amount of spawning and nursery habitat for wild fishes. In future studies, we plan to compare the ecosystem attributes and quantitative indices of community maturity of such farms created in different years and estimate their environmental capacity for wild organisms. We will use stable isotope methods to verify the actual trophic levels of wild organisms in the farms to formulate medium- and long-term predictions of their ecosystem dynamics.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

MX, Z-LQ, Z-LL, W-MQ, Y-LZ, QZ: contributed to the development, planning, data collection; Y-LZ, HL: contributed to data analysis and interpretation. All authors contributed to the writing of the manuscript.

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Conflict of interest

Authors QZ and Y-LZ are employed by Tangshan Marine Ranching Co. Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.911399/full#s upplementary-material

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