Hindawi
Aquaculture Research
Volume 2023, Article ID 4729267, 10 pages
https://doi.org/10.1155/2023/4729267

Research Article

Sediment Properties and Benthic Fauna Associated with Stock Enhancement and Farming of Marine Bivalve Populations in Xiangshan Bay, China

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Received 1 November 2022; Revised 8 January 2023; Accepted 18 January 2023; Published 4 February 2023

Academic Editor: Hamed Ghafarifarsani

This study was designed to examine the impacts of ark shell *Scapharca subcrenata* enhancement and suspended oyster *Crassostrea sikamea* farming on sediment properties and fauna communities in the benthic zone of Xiangshan Bay, China. Ark shell enhancement took place over 319 days, after which sediment samples were collected from each of the three treatment sites: the enhancement area, an oyster farm, and a control area. Sediments from the oyster farming area had significantly higher moisture and acid volatile sulfide content, and the highest Cu, Zn, Pb, and Cd concentrations in all three treatment areas. The ark shell enhancement area has the lowest total organic carbon. No significant difference was found between the mean grain size in each of the three areas. Benthic fauna communities in the ark shell enhancement area were similar to those in the control area. Among the 16 faunal taxa identified, *Ruditapes philippinarum* was the most abundant species, followed by *Glycera onomichiensis* and *Musculus senhousia*. Suspension feeders were not found in the sediment beneath the oyster rafts. Cluster analysis showed a distinct separation between the benthic fauna communities in the oyster farming area and the other two treatment areas. Ark shell enhancement was thus shown to have minimal impact on the benthic environment, while the raft-farmed oysters had significant effects on the sediment structure and the benthic fauna after more than ten years of farming. The differences between the impacts of ark shell enhancement and oyster farming could be attributed to the slowing of water flow caused by oyster rafts.

1. Introduction

Shellfish play an important ecological role in coastal ecosystems. Many studies have demonstrated that coastal filter-feeding bivalve populations, such as oyster reefs, mussel beds, and clams, can regulate coastal nutrient fluxes, sedimentation, and primary productivity [1, 2]. By removing particulate suspended solids from the water, bivalves can decrease phytoplankton abundance and/or chlorophyll concentration, and enhance the water clarity [3]. Because bivalves can assimilate land-derived nitrogen and phosphorus from the ambient environment, they are also used to remediate water eutrophication [4–7]. Moreover, through the calcification process, filter-feeding bivalves can convert bicarbonate into carbonate and thus play an important role in the global carbon cycle [8].

Filter-feeding bivalve farming is growing rapidly worldwide [9]. China generally leads the world in aquaculture production of shellfish but it has continued to increase, reaching 1.46 million metric tons of annual production in 2019 [10]. Bivalve farming usually occurs in coastal bays and lagoons due to ease of access and high primary production, which results in faster growth and increased production [11, 12]. Bivalve shellfish aquaculture can be viewed as a disturbance that modifies the coastal ecosystem [13], but the impact of this disturbance can be positive or negative and differs depending on the system. Concentrated bivalve biomass with high filtration rates can generate large amounts of fine particulate, feces, and pseudofeces that are enriched with organic matter, resulting in intense biodeposition on sediments [12, 14, 15]. Large accumulations of such organic matter beneath bivalve farms can change the benthic environment, including oxygen depletion, sulfide accumulation, and nitrification inhibition [12, 14, 16]. Due to their sedentary habits and weak mobility, benthic faunas are sensitive indicators of environmental change [17, 18]. The accumulation of organic-rich biodeposits may influence the abundance, biomass, and diversity of benthic fauna communities [19–21]. Sediment properties affect the distribution of heavy metals. It has been found that the heavy metal content in sediments is negatively correlated with the sediment particle size and positively correlated with the organic matter content [22, 23]. Due to the higher specific surface area and organic matter content, the large amounts of fine particulate generated by filter-feeding shellfish might absorb more heavy metals. Conversely, some studies have found that shellfish farming has little impact on sediment properties or benthic communities [18, 24, 25].

Due to anthropogenic pressures such as overfishing, coast reclamation works, and habitat loss, the natural resources of shellfish in China have been severely depleted, resulting in decreased annual harvests of wild shellfish [10, 26]. Releasing cultured organisms into natural coastal systems has been shown to be an effective way to enhance, conserve, or restore fisheries [27]. In China, to solve the problem of natural resource depletion, corresponding measures, including summer fishing moratorium and seed enhancement, have also been adopted [28]. From 2006 to 2015, China invested approximately 0.87 billion dollars in the release of 300 billion various aquatic fries and shellfish juveniles [29]. Numerous studies have acknowledged the beneficial role of shellfish stock enhancement [30–32]. Currently, the assessment of the effectiveness of shellfish stock enhancement is focused on productivity, and social and economic benefits [33], while few studies have investigated its environmental effects.

Xiangshan Bay is a long and narrow bay in northern Zhejiang Province, China, around 70 km long and 4 km wide. At its center, the average depth is 7 m, and there are large areas of mudflat [34]. Xiangshan Bay is a traditional fishing area and provides a habitat for many marine fish and shellfish species [35]. The ark shell, *Scapharca subcrenata*, was one of the most widely distributed native shellfish species in Xiangshan Bay, China. According to the survey results in 1995, the distribution area of *S. subcrenata* in Xiangshan Bay is about 4,433.3 hectares, mainly in Tiegang inner bay and Huangdungang inner bay, with an average density of 20.95 g/m^2 (2.095 individuals/m^2) and an estimated biomass of 927 tons [36]. However, the wild fishery resources, which this species relies upon, have declined due to overfishing and land reclamation. In 2011, the highest biomass (ash-free dry mass) of *S. subcrenata* in Tiegang inner bay was only 1.51 g/m^2 [37]. In response, the local government has initiated stock enhancement of *S. subcrenata* through the release of hatchery juveniles. Xiangshan Bay also serves as an important shellfish mariculture zone, where the suspended culture of oyster (primarily *Crassostrea sikamea* and *Ostrea plicatula*) has been conducted since the 1990s. The area farmed has recently increased from 3.7 in 2006 to 7.5 km^2 in 2016 [18]. A previous study showed that raft-farmed oysters in Xiangshan Bay significantly reduced phytoplankton abundance and chlorophyll a [38], implying a strong biodeposition.

Xiangshan Bay is an ideal place to study and compare the ecological effects of shellfish stock enhancement and artificial culturing. In this study, two species of bivalve molluscs, *S. subcrenata* and *C. sikamea*, which inhabits in the benthic zone and are farmed on rafts at the surface of the water in Xiangshan Bay, respectively, were selected. The potential effects of large-scale marine bivalve enhancement and suspended oyster farming on benthic fauna and substrate properties were studied.

2. Materials and Methods

2.1. Ethical Statement. This study was performed in accordance with the standard operating procedures for the use of experimental animals of the East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. These field studies did not involve endangered or protected species.

2.2. Experimental Area. The survey was conducted in Tiegang inner bay, located at the head of Xiangshan Bay, China (Figure 1). Samples were taken from an area where ark shell stock enhancement (the ark shell enhancement area, AEA) occurred which was about 1.0 km^2, with an average water depth of 3–4 m. The hatchery juveniles (average shell length = 3.71 ± 0.70 mm) were cultivated in a local nursery farm and then released in the study area on December 5, 2018, at densities ranging from 50 to 100 individuals/m^2. An oyster (*C. sikamea*) farm located in the northern part of Tiegang inner bay was selected for sampling (the oyster farming area, OFA), which was approximately 1.5 km^2 with an average water depth of 6–7 m. Suspended (long-line) oyster culture has been conducted in Tiegang inner bay since the 1990s [18]. The oyster farm has been operated for more than 10 years. Oyster seeds were collected using bicycle tires as a metamorphosis attachment substrate in an estuary close to the farm, from July to August each year. When the oysters attached to the bicycle tires grew to around 0.5 cm in shell length, the tires were transported to the farm and hung on the underside of the rafts situated at the surface of the water for a 2-year farming period. Each raft contained 300–350 tires. The distance between the rafts was 5 m. Oysters were farmed throughout the year. An area with no ark shell and oyster farming was selected as the control area (CA). Actually, both the control area and the enhancement area were formerly *S. subcrenata* habitat, but the resources have declined rapidly in recent years due to overfishing.

2.3. Sample Collection. In each treatment area, 4 sampling sites were selected. The sampling sites were evenly distributed, with an average distance between each station of about 200 m. At each sampling site, four benthic samples were collected with a 0.1 m^2 Van Veen grab sampler on October 20, 2019. For benthic fauna communities, the sediment

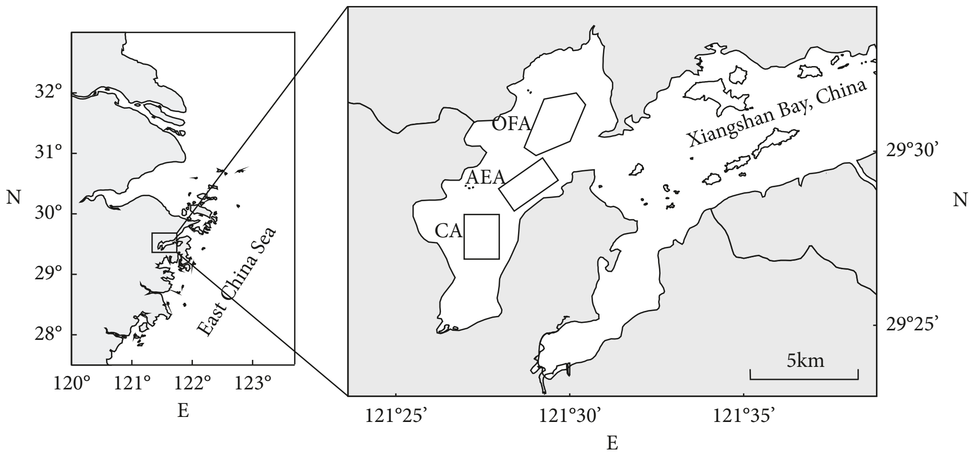

Figure 1: Map showing the study site in Xiangshan Bay, China. CA is the control area; AEA is the ark shell enhancement area; OFA is the oyster farming area.

samples were sieved through a 500 μm mesh, and all organisms retained were preserved in a 5% formaldehyde solution. Ark shells in each sample were counted, shell length was measured with electronic Vernier calipers, and wet weight was assessed using an electronic scale. After 319 days of the enhancement, the mean shell length of the ark shell increased from 3.71 ± 0.70 mm to 24.01 ± 2.98 mm, and the wet weight increased from 0.007 g to 3.02 ± 0.57 g. The densities of ark shells from the four sampling sites were 16.60, 89.03, 84.73, and 9.27 individuals/m^2. No ark shell was found in the oyster farming area. In the control area, the average concentration of the ark shell was 0.29 individuals/m^2. Benthic fauna was sorted and identified to the lowest possible taxonomic order, generally species, and enumerated. The nomenclature was referenced using the World Register of Marine Species (WoRMS: https://www.marinespecies.org). For physical and chemical property analysis, the central part of each sediment sample was taken and immediately capped and frozen using carbon dioxide ice. All samples were then transferred to the laboratory in iceboxes and stored at −20°C.

2.4. Sample Analysis. Acid volatile sulfide (AVS) contents were assayed according to the method of Allen et al. [39]. The sediment sample (about 3 g) was placed into a reaction flask and sparged with continuous pure N_2 flow and was then acidified for 20 minutes by adding 20 mL of 6 M HCl at room temperature. The generated H_2S was collected in a NaOH solution, and the concentration of AVS was determined spectrophotometrically at a wavelength of 670 nm. All reagents used during the assay were of Merck analytical grade, and all glass vessels were acid-cleaned before use.

For heavy metal analysis, sediment samples were digested for heavy metal analysis using HNO_3 and $HClO_4$. According to Sun et al. [40]; Cu, Pb, Zn, and Cd were the main heavy metal contaminants in the sediments in Xiangshan Bay, and concentrations of Cu, Pb, Zn, and Cd were determined using atomic absorption spectroscopy (AAS SOLAAR M6, Thermo Electron Corp., Watham, MA, USA) according to the method of China National Standard GB17378.5-2007, which used a flame atomic absorption method for the analysis of Cu, Zn, and Pb. The graphite furnace atomic absorption method was used for the analysis of Cd concentrations. A duplicate sample analysis was run for 10% of the total samples, and national standard reference materials (GBW07314) were used to ensure the precision of the analysis. When conducting the analysis, the relative standard deviation of duplicate samples was less than 5%, and the recovery rates of the standard reference were around 90–110%.

Sediment samples were treated with a 0.5 M sodium hexametaphosphate solution for 24 h, and the particle size distribution was measured using a particle size analyzer (Malvern, Britain). The samples were dispersed and homogenized using an ultrasonic vibrator before analysis. Grain size parameters, including median particle diameter (MD), were calculated according to the matrix method [41]. The moisture content of the sediments was determined by calculating the weight loss after drying at 105°C for 24 h. For total organic carbon (TOC) analysis, the inorganic carbon (mainly in the form of carbonate) was removed with 10% diluted hydrochloric acid. The samples were then washed repeatedly with deionized water, freeze-dried, and carefully crimp-sealed in tin capsules. Carbon analysis was conducted using an elemental analyzer (TOC-5000A, Shimadzu, Kyoto, Japan) with a measuring accuracy of 0.1%.

2.5. Statistical Analysis. The seawater environmental parameters, sediment moisture content, TOC, particle size, sulfide, and heavy metal data were analyzed using ANOVA (SPSS®, v20.0). Differences in mean values were assessed using a post hoc least-significant difference (LSD) test at the 5% level of significance ($p < 0.05$). Percentage data were square arcsine-transformed prior to analysis to meet the assumptions of equal variance.

Benthic fauna community data were analyzed using multivariate statistical analysis, and the Shannon–Wiener

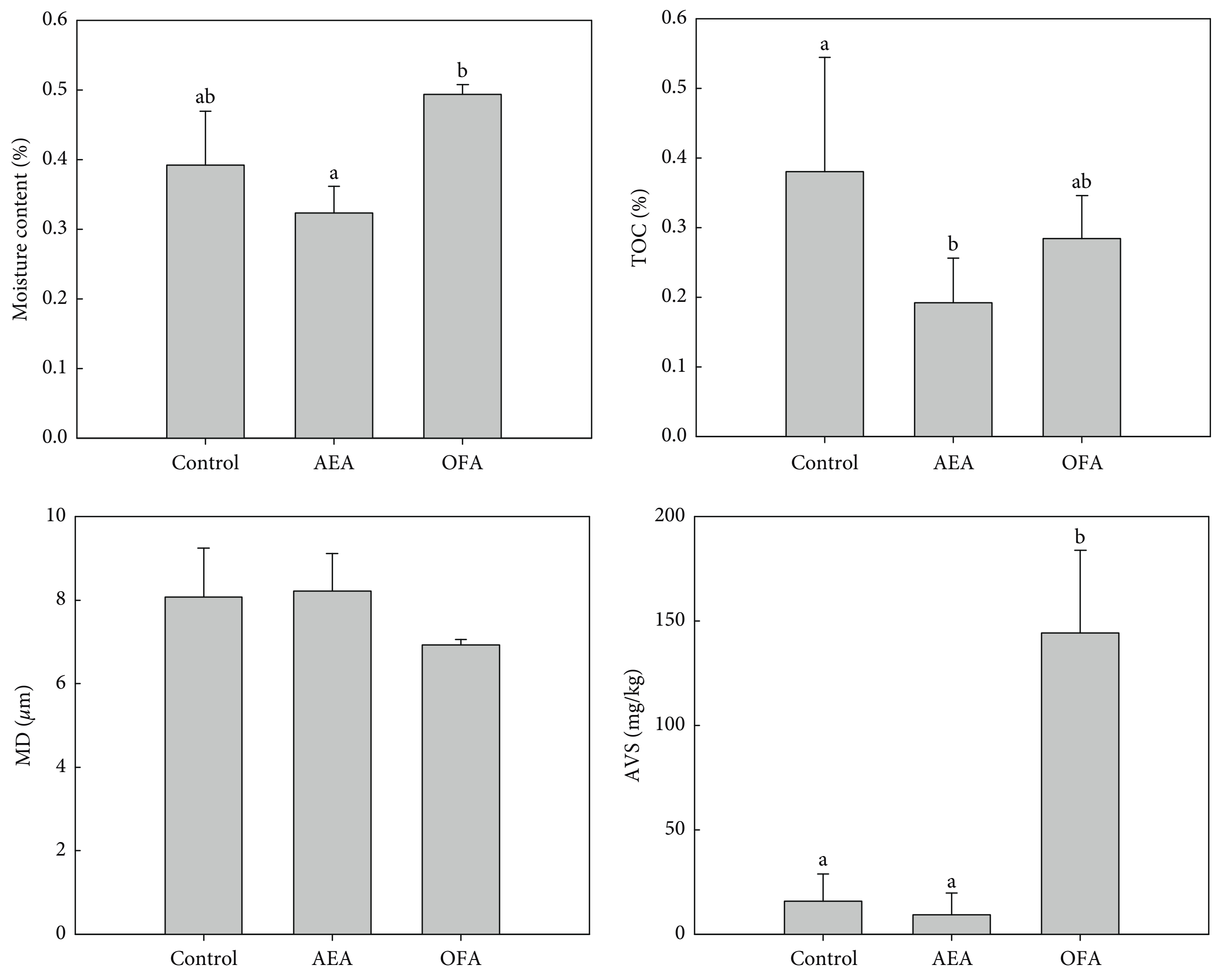

Figure 2: Properties of sediments in different treatment areas. *Note*. Bars denoted with different letters are statistically different ($p < 0.05$). CA is the control area; AEA is the ark shell enhancement area; and OFA is the oyster farming area.

diversity index was calculated using PRIMER 6.0 [42]. A hierarchical cluster analysis was conducted, and a similarity matrix was constructed based on the Bray–Curtis index on log-transformed (ln $(x+1)$) abundance data. Differences in species abundance between the ark shell enhancement, oyster farming area, and control area were examined using the analysis of similarity (ANOSIM). If global R-statistics were statistically significant, ANOSIM was then used to examine paired differences between the treatment areas.

3. Results

3.1. Sediment Properties. Sediment moisture content was significantly higher in the oyster farming area (OFA) than in the ark shell enhancement area (AEA) ($p < 0.05$, $F = 10.887$, $n = 11$) but neither differed from the control area (CA) (Figure 2). Total organic carbon (TOC) was significantly lower in the AEA than that in the CA ($p < 0.05$, $F = 3.307$, $n = 11$) but there was no difference between that in OFA and CA. No significant difference was found between the mean grain size (MD) present in each of the three areas, though the mean MD value in the OFA was the lowest. Acid volatile sulfide (AVS) contents in the sediments were >10 times higher in the OFA than in the AEA and CA ($p < 0.01$, $F = 37.435$, $n = 11$). The oyster farming area also had significantly higher Cu, Zn, Pb, and Cd concentrations, but no significant difference in these metal concentrations was observed between the AEA and CA ($p < 0.01$) (Figure 3).

3.2. Benthic Fauna Communities. In this study, 16 faunal taxa were identified. The main taxonomic groups were as follows: Annelida (8 taxa), Echinodermata (3 taxa), Mollusc (2 taxa), and others (3 taxa) (Table 1). The dominant taxa were different across the three treatment areas. Echinodermata, crustaceans, and molluscs were collected in the ark shell enhancement and control areas, but not in the oyster farming area. Nemerteans and the annelids *Aglaophamus dibranchis* and *Sternaspisscutata* were sampled only in the OFA. *Ruditapes philippinarum* was the most abundant species, followed by *Glycera onomichiensis* and *Musculus senhousia*. No significant difference was found between species richness in the different treatment areas.

The densities of benthic fauna in the AEA and CA were significantly higher than those in the OFA ($p < 0.01$, $F = 12.298$, $n = 11$), resulting in no significant difference in the Shannon–Wiener diversity index amongst treatment areas.

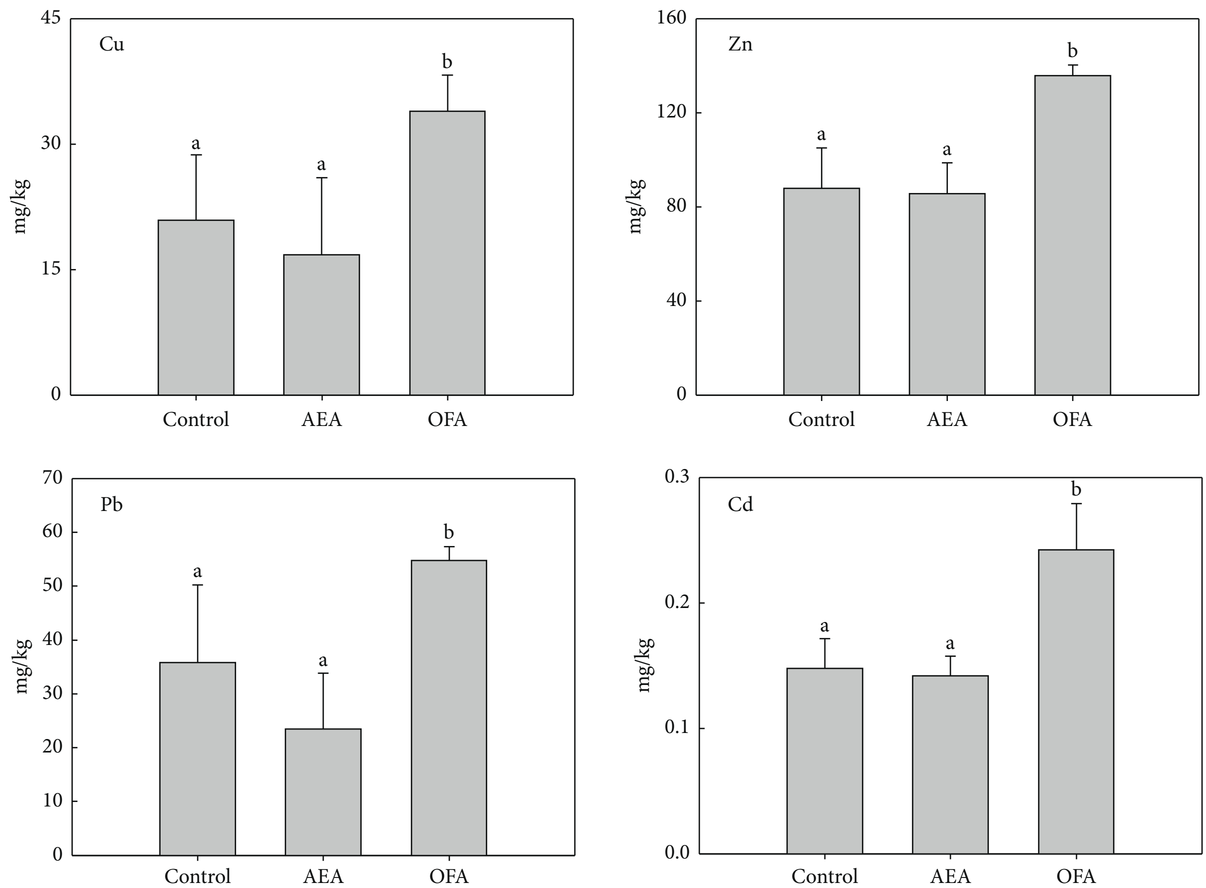

Figure 3: Heavy metal concentrations in different treatment areas. *Note*. Bars denoted with different letters are statistically different ($p < 0.05$). CA is the control area; AEA is the ark shell enhancement area; and OFA is the oyster farming area.

Cluster analysis of the community data showed that the average similarity between the treatment areas was less than 5% (Figure 4). Communities in the CA and AEA were generally similar but distinct from those in the OFA.

ANOSIM pairwise comparisons demonstrated similar groupings with significant differences between the OFA and both that in the CA and AEA treatments (ANOSIM: $R = 0.64$, $p < 0.05$) (Table 2).

4. Discussion

The present study indicates that the ark shell enhancement had less impact on the benthic environment in Xiangshan Bay, while the raft-farmed oysters had significant effects on sediment properties, heavy metal accumulation, and benthic fauna communities after more than ten years of farming.

4.1. Effects of Ark Shell Enhancement and Oyster Farming on Sediment Properties. In this study, sediments in the oyster farming area studied here had a significantly higher moisture content, acid volatile sulfide, and heavy metal concentrations, while the physical and chemical properties of sediments in the ark shell enhancement area and control area were similar.

Moisture content was lower in the oyster farming area, a sediment characteristic that has been reported to be correlated with other components in sediments, including organic matter, sediment-water permeability, and grain size and is usually inversely proportional to the grain size [43]. Biologically mediated sedimentation processes can enhance the deposition of fine sediments in estuaries and coastal areas [44]. Suspension-feeding bivalves can promote the sedimentation of particles via biodeposition [45]. The hanging ropes and rafts of shellfish long-line culture systems can reduce the water current velocity [46], which can also promote the process of deposition. The deposit rate in mussel farming areas were around 2-3 times higher than that in areas without mussels, and the deposits in shellfish farming areas usually displayed a finer structure, lower density, and higher moisture content [47–49]. This may explain the lower MD value and significantly higher moisture content in the oyster farming area in the present study.

Organic carbon has been used as an indicator of organic matter enrichment in sediments. Increased suspension-feeding bivalve production can result in a proportional increase in organic matter biodeposition [16, 50]. In the River

Table 1: Mean abundance of species of benthic fauna in different treatment areas (individuals/m^2).

Group	Species	CA	AEA	OFA
Echinodermata	*Amphiura vadicola*	0	31.25	0
	Phyllophorus ordinatus	25	0	0
	Protankyra bidentata	6.25	12.5	0
Crustacean	*Typhlocarcinus nudus*	31.25	12.5	0
Mollusc	*Musculus senhousia*	12.5	50	0
	Ruditapes philippinarum	37.5	50	0
Annelida	*Aglaophamus dibranchis*	0	0	25
	Eunice sp	0	6.25	12.5
	Glycera onomichiensis	31.25	37.5	0
	Lumbrineris pterignath	12.5	6.25	18.75
	Marphysa sanguinea	25	0	0
	Pherusa bengalensis	18.75	12.5	0
	Sternaspis scutata	0	0	6.25
	Sthenolepis japonica	25	0	0
Nemertea	*Nemertinea*	0	0	25
Sipuncula	*Sipunculus nudus*	0	37.5	0

CA, the control area; AEA, the ark shell enhancement area; OFA, the oyster farming area.

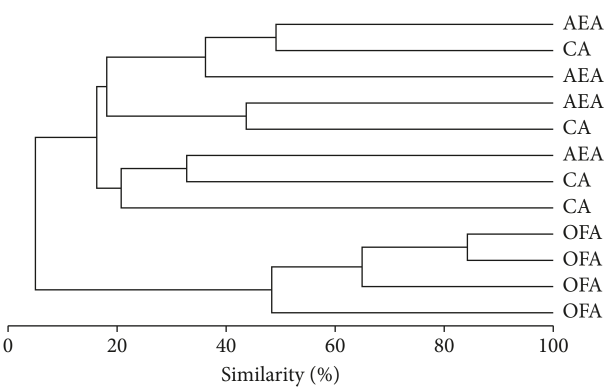

Figure 4: Cluster analysis of benthic fauna abundance in different treatment areas using the Bray–Curtis similarity index. CA is the control area; AEA is the ark shell enhancement area; and OFA is the oyster farming area.

Exe estuary in England, oyster (*C. gigas*) farming rafts significantly reduced the water currents, which doubled the sedimentation rate and increased the organic content of the sediments [51]. No significant difference was found in TOC between the treatment areas in this study, suggesting that the organic matter in biodeposits was not retained in the sediments. Researchers examining sediments at another oyster farm in Xiangshan Bay also found that TOC in sediments was not significantly different between treatment areas [18]. In South St. Simon Bay, Canada, the organic content in sediments collected from an oyster farming area was not significantly higher than the reference area, although the oysters may contribute to the high deposition rate [52]. Crawford et al. [53] found that there was no significant difference in the organic carbon content between sites inside and outside of oyster farms. Highly hydrodynamic environments can reduce the impact on benthic sediments derived from shellfish culture [54–56]. In this study, the biodeposits in the ark shell enhancement area might be washed away by tidal currents, while in the oyster farming area, biodeposits may be lost due to organic matter degradation or other mineralization processes [57]. Generally, organic matter in marine sediments is transformed into dissolved inorganic carbon (DIC) with the absence of microbial communities, which can then be released to the upper water layer, or can form carbonate by combining with calcium and magnesium ions [58, 59]. The mineralization processes include oxidation, denitrification, Mn (IV)-oxide reduction, Fe (III)-oxide reduction, and sulfate reduction [57]. In this study, the average water depth in the oyster farming area was 6–7 m, increasing to 10 m at high tide. In an anaerobic environment, the accumulation of organic matter can result in sulfate reduction and increase the level of sulfide [47]. This could explain the significantly higher AVS values found in this study in the oyster farming area, suggesting that biodeposition from farming oysters formed a large component of organic enrichment in sediments. Yan et al. [60] also reported increased sediment AVS in oyster (*C. plicatula*) farms in a different area of Xiangshan Bay, China. Sulfate reduction rates at a long-line Pacific oysters (*C. gigas*) farm in South Korea were 2.4–5.2 times higher than those at the control site [12].

In this study, the Cu, Zn, Pb, and Cd concentrations in the oyster farming area were significantly higher than those in the other two treatment areas. Heavy metals are highly persistent and toxic to humans due to the potential for bioaccumulation throughout the food chain. Many molluscs have been employed as contamination indicators due to their ability to accumulate heavy metals [61, 62]. The common mussel *Mytilus edulis* has been found to have heavy metal concentrations from 10^3 to 10^6 times higher than the concentrations in the surrounding water [63]. Oysters have also been deployed as biomonitors and shown to uptake a variety of heavy metals [61, 64, 65]. Adiyiah et al. [66] reported that the sediment with the finest grain size (<45 μm) had the highest concentrations of heavy metals due to having a larger surface area and higher adsorption capacity. This might be one of the reasons for the significantly higher Cu, Zn, Pb, and Cd concentrations in the oyster farming area in this study. Alternatively, oyster farming may accelerate heavy metal accumulation in sediments through biodeposition. In Hailing Bay, China, concentrations of Cr, Ni, Cu, Zn, As, Pb, and Cd, in the surface sediments from aquafarming areas, were significantly higher than those from nonaquafarming areas [67]. In this study, we found no significant difference in heavy metals between the ark shell enhancement area and the control area, suggesting that the enhancement has a minimal impact on the substrate environment.

4.2. Effects of Ark Shell Enhancement and Oyster Farming on Benthic Fauna Communities. Benthic fauna has been widely used to assess the impact of anthropogenic activities on marine environments such as environmental pollution and mariculture in the marine environment due to their environmental sensitivity, sedentary habits, and ease of access [18, 21, 68]. In this study, the filter-feeding bivalve *R. philippinarum* was the most abundant species found in the ark shell enhancement area and control area, and the cluster analysis showed that the benthic communities in both areas were similar. This suggests that the enhancement of ark shell has less impact than oyster farming on the benthic community. Similarly, Mantovani et al. [24] reported that in the Po River Delta, Italy, the presence and density of cultivated Manila clams (*Tapes philippinarum*) had little impact on faunal community abundance and functional group composition. Open-sea mussel culture in the Western Adriatic Sea appears to have few detrimental effects on zoobenthic communities [25]. However, the clear community separation between the oyster farming area and the other two treatment areas in this study indicates that oyster farming may have a larger impact on the benthic community. This might be demonstrated from another study where macrobenthos species richness and abundance beneath an oyster farm in Xiangshan Bay increased significantly 3 years after oyster farming ceased, and more than 75% of dominant taxa were re-established [15]. Costa and Nalesso [69] revealed that the sediment microbenthic community in long-line mussel farms in Anchieta, Southeast Brazil, showed significantly higher diversity and richness. Dubois et al. [70] found that in the Bay of Veys in France, oyster culture structures had minor effects on macrofauna density, but had profound effects on the composition of microbenthic assemblages. They also found that suspension feeders were not found beneath oyster tables, which is consistent with the findings of this study. Besides the temporal cumulative effects of farming over 10 years, the significant change in benthic communities could be due to the reduction of current velocity and accumulation of organic matter via biodeposition. Surprisingly, Liao et al. [18] reported that in another oyster (*O. plicatula*) farm in Xiangshan Bay, the oyster culture had little impact on the microbenthic community, contrary to our results, suggesting that the impact of oyster culture on benthic fauna may differ between farms, and could be regulated by a variety of factors, such as sediment type, hydrodynamics, water depth, or the history and density of cultured oysters.

5. Conclusions

In conclusion, this study indicates that enhancement of ark shells had less impact on the benthic environment than oyster farming, which had profound effects on the sediment structure, geochemical processes, heavy metal accumulation, and benthic fauna communities in Xiangshan Bay, China. The differences observed between the impacts of ark shell enhancement and oyster farming could be attributed to the slowing of water flow caused by oyster rafts, which in turn promotes the sedimentation of particles and an increase in the organic matter mineralization rate in sediments. This study presents useful information for monitoring the environmental impact of clam enhancement and oyster farming to ensure the sustainable development of shellfish culture and resource restoration in the future.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Xianyin Ping and Hui Zhang were involved in the sample collection, sediment's physical and chemical properties analysis, and drafting of the manuscript. Yazhou Jiang was involved in ark shell enhancement and benthic fauna analysis. Jianzhong Ling and Peng Sun were involved in the sample collection. Baojun Tang was involved in the experimental design, ark shell enhancement, and manuscript revision. All authors have read and agreed to the published version of the manuscript. Xianyin Ping and Hui Zhang contributed equally to this work.

Acknowledgments

The present work was sponsored by the National Key Research and Development Project (grant no. 2020YFD0900804) and https://doi.org/10.13039/501100009997Modern Agro-Industry Technology Research System of China (CARS-49). The authors appreciate very much the assistance from Mr. Tianqi Jiang and Ms. Yang Zhang in the sample collection.

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