Environmental Impacts of Aquaculture Ponds on Coastal Wetlands in the Yellow River Estuary

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Abstract

Yellow River estuary, located in the northeast of Shandong Province, Northern China, has vast coastal wetlands which provide important ecological services. Due to the regional geographical characteristics, large amounts of aquaculture ponds were established in the coastal area in the last two decades. This study investigated the potential environmental impacts of two typical aquaculture activities (sea cucumber and shrimp pond farming) through the analysis of spatial differences of typical pollution indicators and biochemical tracers in water and sediment, and dietary characteristics of dominant macrobenthos among stations with varied distances to aquaculture facilities. As a result, some key environmental impacts and monitoring indicators were identified which have the potential usage in assessing the impacts of sea cucumber and shrimp pond aquaculture in subsequent studies.

Impacts of sea cucumber farming on coastal wetlands

For sea cucumber pond farming, water column in the study area was considered not remarkably affected by aquaculture activities as no significant spatial differences of selected variables between farming and non-farming area or among stations with a gradient distance to the aquaculture pond were detected. Conversely, sediments that historically recorded the accumulation of organic and dissolved inorganic wastes showed decrease tendencies of discharged waste from sea cucumber pond to the adjacent tidal flat. Moreover, the detectable dispersion distance of aquaculture waste in the adjacent tidal flat was restricted to an area within 50 m distance from sea cucumber pond as determined by most variables. The overall results showed that pond farming for *Apostichopus japonicus* in the Yellow River estuary altered the local environment to a certain extent.

Impacts of shrimp farming on coastal wetlands

As for shrimp farming, both water and sediment in the adjacent tidal flat were considered not affected by farming activity as no significant regular changes of selected variables were found among stations with a gradient distance to the aquaculture pond. However, a certain environmental variable such as organic matter concentration in water corresponded well with the degree of stress from shrimp aquaculture, which highlighted that it could potentially serve as an effective index for the estimation of environmental disturbs. These findings affirmed the hypothesis in the other studies that predominant effects of shrimp aquaculture waste were present in water processes, rather than sediment processes.

Impacts of aquaculture on food sources for dominant macrobenthos

Food contribution shifts in both farming areas indicated that aquaculture activities might modify the dietary characteristics of benthic animals to a certain extent. Bayesian mixing models indicated that BMA (benthic microalgae) in the sea cucumber farming area had a larger contribution, while $\mathrm{POM}_{\mathrm{(marine)}}$ showed a smaller contribution to the diets of *Helice tridens* and *Macrophthalmus abbreviates* compared to those in the non-farming area. This founding contradicts to the general view that POM serves as important food sources for some small-sized crustaceans. However, in shrimp pond area, $\mathrm{POM}_{\mathrm{(pond)}}$ came to be the dominant food sources for both crabs, and salt marsh plant contributed the largest proportion of the diet of *H. tridens*. These shifts might indicate that although no significant impacts of farming activities on the adjacent tidal flat were detected by analysis of distribution patterns of environmental variables, the aquaculture wastes have potentials to be widely spread, and consequently absorbed by the primary producers or directly utilized by the dominant consumers. This study also confirmed the utilization of the salt marsh plant by *H.tridents* and found that the herbivory increased with the nutrient enrichment in salt marshes. These foundings suggests that biological accumulation would be an effective environmental indicator as it has the advantage of recording historical accumulation of organic matter that assimilated by the creatures. Biological accumulation would

detect the effect of aquaculture waste, particularly under the circumstances that clear conclusions cannot be drawn on the basis of traditional environmental parameters.

The overall results showed that sea cucumber and shrimp pond farming in the Yellow River estuary altered the local environment to a certain extent. For methodological consideration, sediment biogeochemical characteristics as a historical recorder much more efficiently reflected aquaculture waste accumulation from sea cucumber farming. Water biogeochemical tracers were more effectively in clarification of disturbs of shrimp aquaculture activities. Besides, stable isotope approaches are efficient in tracing the origin and extent of various allogenous sources, and the carbon stable isotope signatures of organisms (especially salt marsh plant) can be used as bioindicators for monitoring the aquaculture pollution.

Keywords: aquaculture pond; environmental impact; aquaculture waste; stable isotope; Yellow River estuary

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Contents

Chapter 5. General Discussion 90

Chapter 1

General Introduction

1.1 Basic information of mariculture in China

Along with the world population booming, mariculture has rapidly grown in recent years with an increasing demand for seafood production (Naylor et al., 2000; Tanner and Fernandes, 2010) to compensate the decline of wild fish stocks induced by overfishing, environmental pollution, and habitat losses.

1.1.1 High production of mariculture in China

(1) High production

China has a long history of mariculture and is the only fishery country with aquaculture production surpassed capturing yield (FAO, 2006). In 2015 the mariculture output and cultivation area reached 18.6 million tonnes and 2317,760 ha, respectively. Total mariculture value reached 293.77 billion yuan (China Fisheries Yearbook, 2016).

(2) High proportion of cosmopolitan mariculture yield

China has the world's largest mariculture industry and produces 67.9% of the global mariculture production (Fig. 1.1) in 2014 (FAO, 2016). It was the world second largest producer of finfish mariculture, and the top producer of crustaceans, mollusks and other farmed species (FAO, 2016). Schnoor (2013) has estimated that aquaculture would be the key strategies to feed 10 billion people in 2050, while China is predominating in the way.

1.1.2 Main culturing manners and species

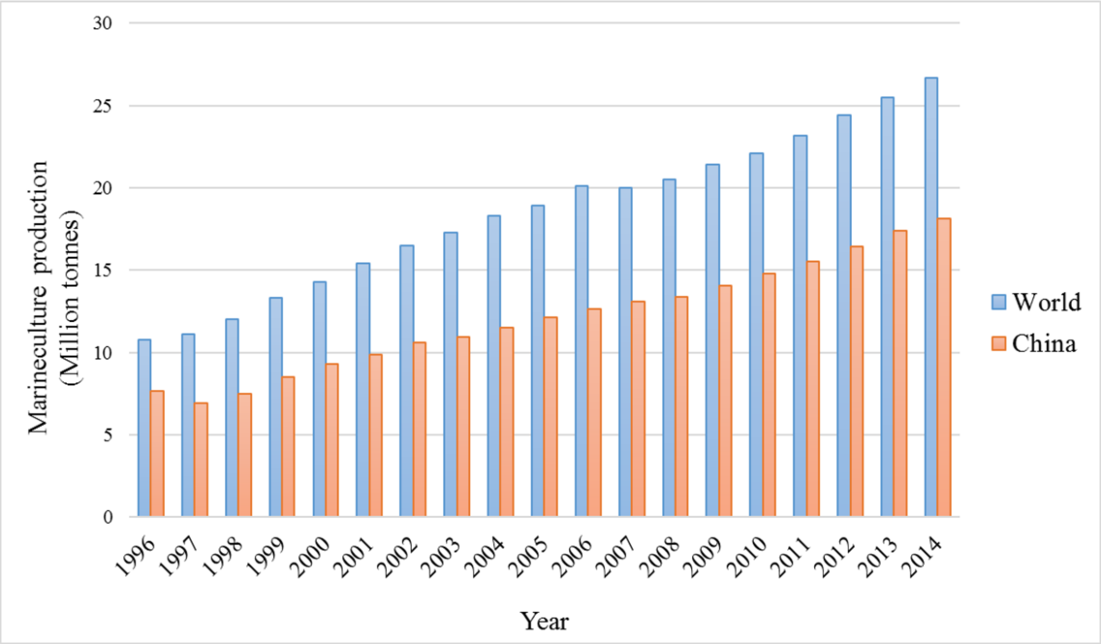

Fig. 1.1. Mariculture production (million tonnes) of China and The World (FAO, 2016).

(1) Main culturing manners

China is very diversified in terms of farming systems and aquaculture species. Marine cultivable areas in China are estimated to occupy more than 1.33 million ha (China Fisheries Yearbook, 2016), including shallow seas, mudflats, and bays. Most marine plants and animals can be cultured within the 10 m isobath using current culture technologies. The main culturing manners were shown in Table 1.1.

1) Bottom sowing

Bottom sowing was the largest productive mariculture manners (5275,773 tonnes) in China as it does not require large quantities of food and little effect on the environment. The main farming organisms are benthic species such as Manila clam (*Ruditapes philippinarum*), hard-shelled clam (*Meretrix meretrix*), blood cockle (*Tegillarca granosa*), razor clam (*Sinonovacula constricta*) and seaweeds such as *Porphyra spp*.

2) Floating raft culture

This system was the second productive culturing manner (5191,099 tonnes) that are used for a variety of species such as seaweeds (kelp and laver), filtering organisms (scallop, oyster, and mussel) and abalone, combined with culture in lantern cages. Better growth rates and quality are the main advantages of this culture manner.

3) Pond culture

Aquaculture ponds are the most common production systems on a worldwide basis (Culberson and Piedrahita, 1996). In China, mariculture ponds (2350,782 tonnes) were the third largest breeding manners, which accounted for 12.5% of the total mariculture production. The main culture animals in China are shrimp, crab or marine finfish. Some benthic species such as Manila clam and razor clam are also cultured in the ponds. In recent years, sea cucumber (*Apostichopus japonicus*) has rapidly developed in Liaoning and Shandong provinces. Animals cultured in ponds are fed by commercial feed, or fertilized to promote the production of food for cultured species

Table 1.1 Production and area of main culturing manners of China in 2015.

Mariculture manners	Production (tonnes)	Area (hectare)
Bottom sowing	5275,773	1098,625
Floating raft culture	5191,099	316,250
Pond culture	2350,782	455,029
Net-cage culture	1855,452	62935,556

Table 1.2 Production of main culturing species in China in 2015.

Main groups	Species	Production (tonnes)
Molluscs (top three)	Total	13,583,816
	Crassostrea gigas	4,573,370
	Chlamys Farreri	1,785,342
	Ruditapes philippinarum	4,009,484
Marine fish (top three)	Total	1,307,682
	Larimichthys crocea	148,616
	Scophthalmus maximus	131,837
	Lateolabrax japonicus	122,542
Crustaceans (main)	Total	1,434,917
	Penaeus vannamei	893,182
	Penaeus monodon	75,682
	Fenneropenaeus chinensis	44,799
	Penaeus japonicus	46,329
	Scylla serrata	141,040
	Portunus trituberculate	117,772
Seaweeds (top three)	Total	2,089,153
	Thallus Laminariae	1,411,289
	Gracilaria sjoestedtii Kylin	270,149
	Undaria pinnatifida	192,502
Echinoderms (main)	Total	340,763
	Apostichopus japonicus	205,791
	Hemicentrotus pulcherrimus	7,265
	Aurelia aurita	78,613

in ponds. The construction, operation, and management of aquaculture ponds play an important part in the coastal ecosystems as the building of ponds not only causes the direct loss of coastal wetlands but also indirectly affected the coastal environment by waste discharge.

4) Net-cage culture

The net-cage culture (inshore and offshore) was the fourth productive culturing manners (1855,452 tonnes) in China. The advantages of this culturing manner were low investment and easy routine management. However, this system is one of the main sources of inshore pollution and a contributor to red tides. In northern China, the main species cultured are *Lateolabrax japonicus, Paralichthys olivaceus, Sciaenops ocellatus, Sebastodes fuscescens, Hexagrammos otakii* and *Fugu sp*.

(2) Main culturing species

China is very diversified regarding aquaculture species. The principle marine species cultured in China are listed in Table 1.2.

1.2 Key problems in environmental impacts of mariculture

1.2.1 Key problems

(1) How to evaluate the environmental impacts of mariculture objectively

The key and general environment impacts of mariculture industry is the environment degradation induced by aquaculture discharges with high levels of nutrients and solid waste. The classical and objective approach to evaluating the effects is the measurement of the key environmental indicators and ecological processes (Burford et al., 2003). The selected parameters should provide robust and meaningful information about environmental impacts, and can differentiate aquaculture discharges from other loads. The challenge is to develop practical, effective monitoring techniques and indicators to reflect the process and impacts.

On the other side, mariculture is carried out in a wide variety of systems.

Features of cultured species, typology of cultivation and management practice have been considered as main factors that affecting the surrounding environment (Sarà, 2007). Therefore, the evaluation of environmental impacts should be conducted in according to different culturing manners, cultured species and management practice.

(2) Proposal of effective monitoring techniques

Marine environmental pollution and overdevelopment of natural sources have come to be the main factor that restricts further development of mariculture in China. Conventional analysis methods which widely applied in aquaculture environmental monitoring shows disadvantages of high cost, complicated operation and low effectiveness. Mariculture as an emerging and leading industry in China, practical, cost-effective monitoring techniques are urgently required for its sustainable development. Effective monitoring techniques could not only monitor the environmental changes but also could contain the pollution source identification, tracking and quantitative analysis. Given this, the development of new technologies and indicators are the main challenge to effective environmental monitoring.

1.2.2 Potential significance in solving these problems

(1) Aquaculture is becoming a significant threat to coastal ecosystems

The coastal area sustains most of the ecological consequences induced by mariculture development. The wide-scale expansion of mariculture like pond culture not only lead to the direct loss of coastal wetlands but also reduces valuable ecosystem services which it provided like nutrient removal, carbon sequestration, shoreline protection, and habitats for diverse species of fish, birds, and invertebrates (Deegan et al., 2012; Primavera, 1997, 2006). Along with the loss of coastal wetland and degradation of the ecological functions and services it provided, apparent and potential adverse impacts induced by the expansion of coastal aquaculture have prompt widespread criticism and efforts for the healthy ecosystem and sustainable natural resources.

(2) Requirement for a sustainable aquaculture industry

Water and sediment degradation caused by excess feed and feces and other anthropogenic activities may induce self-pollution or stress in culturing species and predispose them to disease. Degraded environmental quality causes for contagious diseases (such as in shrimp and fish farming) and marine disaster (outbreaks of harmful algae) are significant threats for the aquaculture. Environment monitoring and early warning can effectively support the sustainable development of aquaculture industry.

1.3 Gaps in current knowledge and requirement for a sustainable aquaculture industry

1.3.1 Previous studies

Among the key areas of environmental concern, nutrient and organic enrichment in the water and sediment caused the greatest attention as they were the most widespread issues in the worldwide scope and urgent problem needed to be solved for sustainable development of aquaculture.

The majority of studies on aquaculture impacts focus on water quality assessments or the quantification of effluent fluxes from intensively and extensively managed shrimp and fish farming (Alongi et al., 1999; Costanzo et al., 2004; Funge-Smith and Briggs, 1998; Herbeck et al., 2013; Samocha et al., 2004; Trott and Alongi, 2000; Trott et al., 2004). Environmental impact monitoring objects were mainly conducted on water quality (nutrient dynamics, nitrate sources, discharge flux) or partially on sediment characteristics (release of nutrients, organic matter) of mangrove and receiving tidal creeks. The main parameters studied in this field was shown in Table 1.3.

Some of these studies have shown different results of variable nutritional characteristics. Costanzo et al. (2004) addressed that shrimp farming discharges elevated nutrient concentrations, especially of dissolved nitrogen in the outlet channels. McKinnon et al. (2002) found that small-scale releases of farming effluent

Table 1.3 Main chemical parameters measured in aquaculture environmental monitoring.

Indicators			Main references
Water	Nutrients	Dissolved inorganic nitrogen (DIN: NO_3^-, NO_2^-, NH_4^+)	Trott et al., 2000; McKinnon et al., 2002; Burford et al., 2003; Costanzo et al., 2004
		Dissolved inorganic phosphate - P (PO_4^{3-})	Trott and Alongi, 2001; Costanzo et al., 2004
		Dissolved organic carbon (DOC)	Herbeck, L. S.(2013)
		Dissolved organic nitrogen (DON)	Trott and Alongi et al., 2001
		Dissolved organic phosphate (DOP)	Trott and Alongi et al., 2001
		N: P ratio	Jackson et al. 2003
		C: N ratio	Burford et al., 2003
		NH_4^+ uptake/regeneration	Burford et al., 2003
	Total suspended solid (TSS)	Particulate organic matter (POC)	Herbeck et al., 2013
		Particulate N (PN)	Jackson et al., 2003; Costanzo et al., 2004
		Particulate P	Jackson et al., 2003; Costanzo et al., 2004
	Chl*a*		Costanzo et al., 2004; Adélaïde et al., 2015
	BOD	Biochemical oxygen demand	Trott et al., 2000
	DO	Dissolved oxygen	Trott et al., 2000; Biao et al., 2004;
Sediment	SOM	$\delta^{13}C$, $\delta^{15}N$	Adélaïde et al., 2015
		C: N ratio	Adélaïde et al., 2015
		Chl*a*	Adélaïde et al., 2015
		Fatty acid	Adélaïde et al., 2015
	Nutrients	DIN	Burford et al., 1998
		PO_4^{3-}	Burford et al., 1998
	Denitrification		Burford et al., 2003
	O_2, CO_2 fluxes		Burford et al., 2003

did not elevate dissolved nutrient concentrations, but elevated particulate nutrients and suspended solids levels in the effluent channel. Other few studies found that the symptoms of aquaculture effluent are only measurable in close proximity (Hensey, 1992; Samocha and Lawrence, 1997). However, changes in water or sediment quality parameters overcomplicated the environmental effects of aquaculture discharges (Burford et al., 2003). More robust and efficient measures should be combined to reflect the source and process of the impact.

On the other side, some studies focusing on ecological aspects have reported that aquaculture farming waste can drastically change the density and biodiversity of benthic faunal communities in near-shore habitat (Capone et al., 1996; Gao et al., 2005; Tomassetti et al., 2009). The macrobenthos who feed on the fish farm waste (δ^{15}N-enriched fish meal and δ ^{13}C-reduced cereals) were observed with enriched δ^{15}N and depleted δ^{13}C values in contrast to the same species samples collected outside of the farm area (Yokoyama and Ishihi, 2007). Kon et al. (2009) found that the shrimp farming organic waste had higher contributions to the deposit feeder crabs inhabit in the mangrove near the shrimp farm than those no shrimp farm around.

However, no matter environmental monitoring or ecological assessment, salt marshes with high productivity are less reported. The characteristics of the receiving receptor like mangrove and salt marshes are entirely different (Paez-Osuna, 2001). In general, there is a general lack of studies that comprehensively assess impacts of aquaculture farming on the adjacent environment.

1.3.2 Main gaps

(1) Details of environmental variables are insufficient, particularly in China with extremely high productions, which hindered the further analysis.

(2) It seems there are no empirical conclusions can be made based on previous studies. Environmental impacts of mariculture are closely related to local hydrographic conditions and thus need to be analyzed specifically.

(3) For methodological consideration, new and more effective methods need to be developed to enable a cost effective and more comprehensive understanding of potential influences from aquaculture.

1.4 Main purpose of this study

1.4.1 Study area

Yellow River estuary (36°55′–38°16′N, 117°31′–119°18′E) was located in the northeast of Dongying City, Shandong Province, Northern China, which faces the Bohai Sea in the North (Fig.1.2). It has a warm-temperate continental monsoon climate. It has a mean annual temperature of 11.9°C, a frost-free period of nearly 210 days; a mean annual precipitation of 592 mm, mean annual evaporation of 1,962 mm and average annual relative humidity of 68%.

The Yellow River is the mother river of Chinese people and has an estimated length of 5,464 km. It was the second longest river in China and sixth longest river in the world. The Catchment area is about 752,443 square kilometers. The Yellow River plays a lead role in forming and maintaining the regional hydrology. It has the highest silt concentration in the world. It has an average annual sediment load of 1.049 billion tons and a mean silt concentration of 25.5 kg m^{-3}. On account of the large quantity of siltation carried out from the Yellow River, the delta spreads into the sea at a rate of 2.2 km $year^{-1}$ with an accretion of 3,240 ha land per year which is all located within the National Natural Reserve.

Since a course change from 150 years ago, the Yellow River has deposited large quantities of sediment on its new Delta in Dongying city before flowing into the Bohai Sea. The formation of the new wetlands was at an average rate of 18 km^2 per year. Older sediments have matured to constitute the land upon which Dongying was built.

Wetlands in the Dong ying (Yellow River estuary) is mainly marine and coastal wetlands, including marine waters, intertidal mudflat, and intertidal reed marshes. However, estuarine delta as a typical transitional zone between the land and ocean is

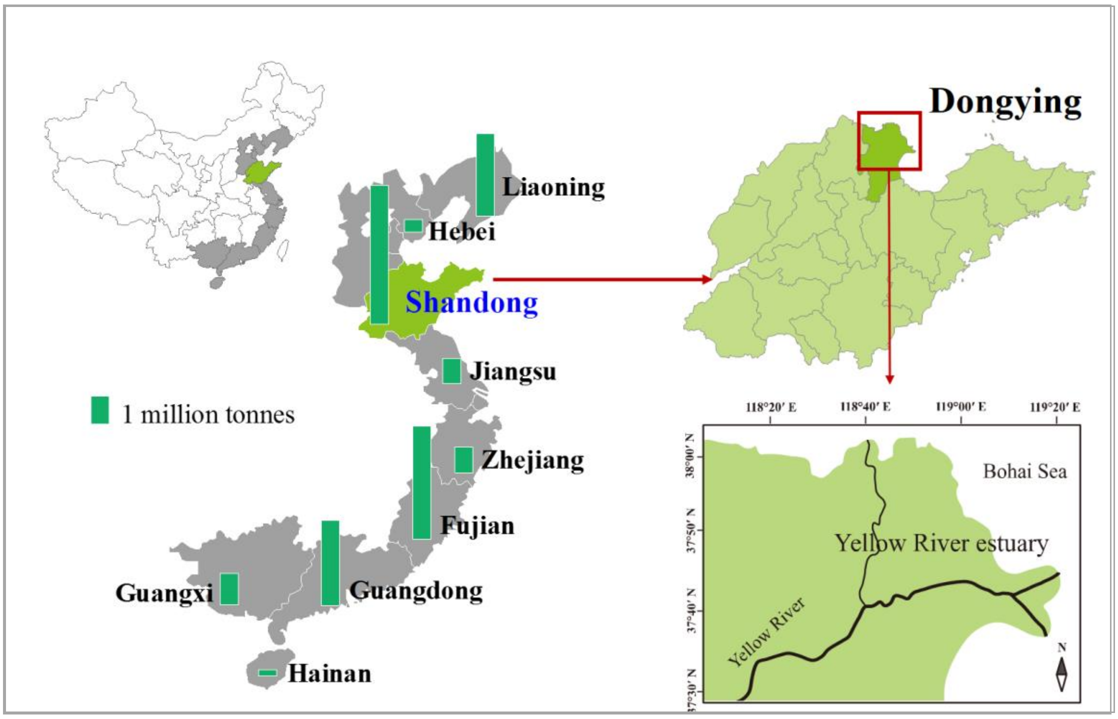

Fig. 1.2. Study area. Green columns present mariculture production in each coastal province (China Fisheries Yearbook, 2016)

a multifunctional and complex ecosystem which provides special ecological values and precious resources. It often became the reclamation activities populated areas. In the last two decades, with the rapid expanding of mariculture encouraged by the government, a vast area of coastal wetlands was transformed into aquaculture ponds.

The wetlands of the Yellow River estuary has been facing a situation common in many developing countries (LAND, 2010), where the pressure for rapid economic growth poses threats to the health and survival of the natural ecosystems. Can the wetlands in the Yellow River estuary coexist with the mariculture development? Whether and how mariculture has an impact on the local wetland environment? How can mariculture activities its aspiration to combine sustainable environment and resources with sound management?

1.4.2 Reasons for the selection of the present study area

(1) Growing urgency of sustainable resource and environmental management

The biggest development of mariculture in Shandong Province was from 1979 with the government incentive policy. Since 2002 onwards, mariculture has stably replaced freshwater aquaculture with the largest aquaculture production in Shandong province which was the leading producers of mariculture throughout all the provinces in China (Fig. 1.3).

Among the cities in Shandong Province, Dongying city (where the Yellow River estuary located) had the largest mariculture area and reached 30.1% in 2014 (Fig. 1.4). The large proportions of the mariculture area in Dongying city were contributed from the Yellow River estuary where holds abundant wetland and marine resources for the development of this industry. From 1970 to 2015, the vast area of 1708.5 ha natural wetlands (tidal flat, salt marshes) in the Yellow River estuary was transformed to the aquaculture ponds (Fig. 1.5). However, there are no detailed studies on the effect of those activities on coastal wetlands in this area. It is important and urgent to develop an understanding of the range and extent of environmental impacts of this industry before it reaches a size which adverse impacts become pervasive and unsustainable.

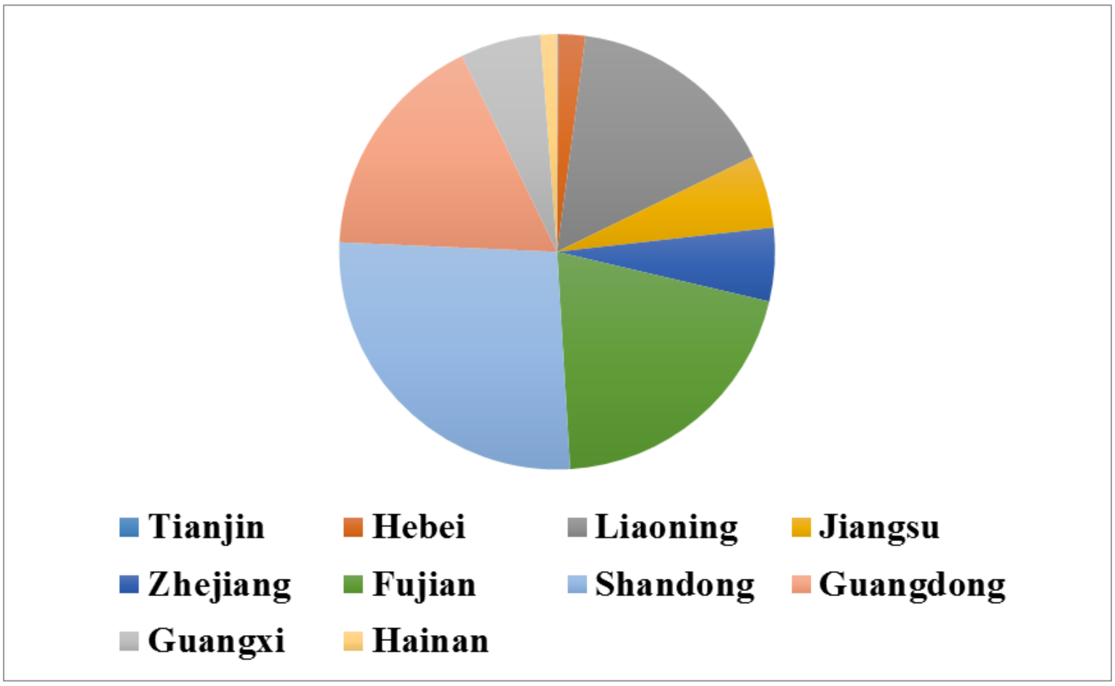

Fig. 1.3. Mariculture production composition in each coastal province of China (China Fisheries Yearbook, 2016).

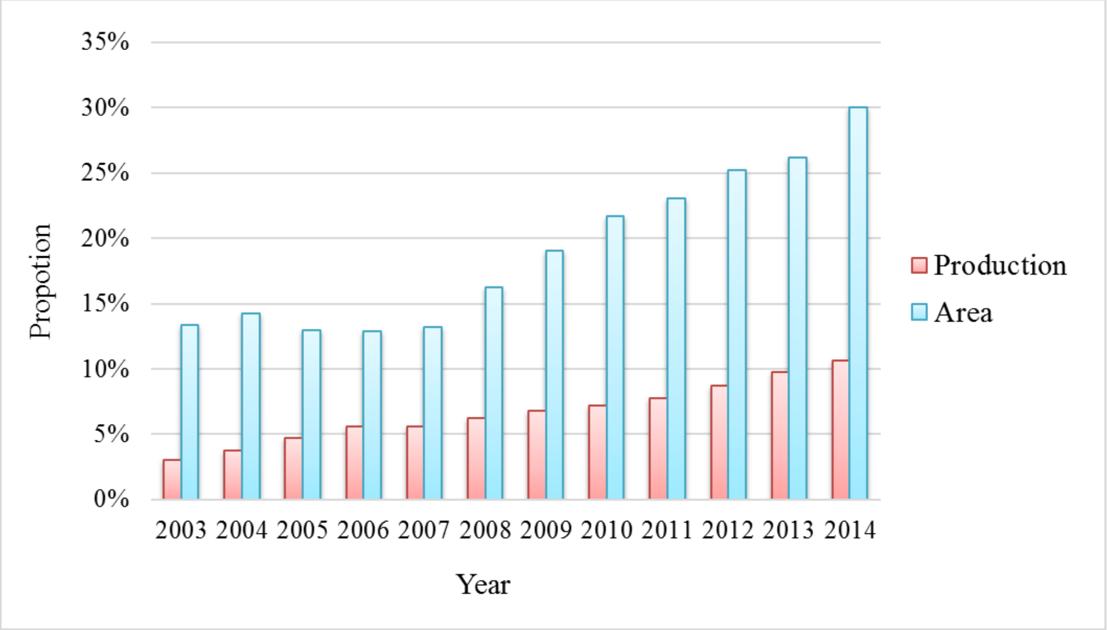

Fig. 1.4. The percentage of mariculture production and area of Dongying city accounted for the entire province (Dongying Fisheries Yearbook, 2015).

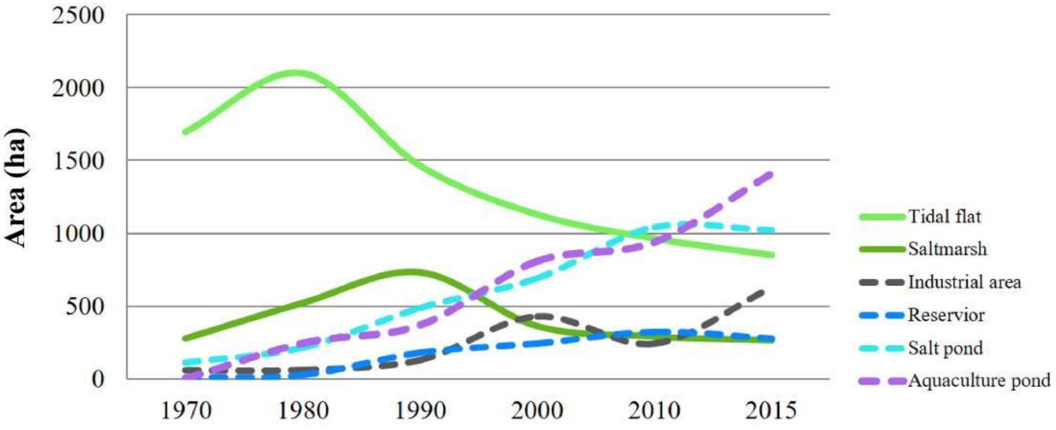

Fig. 1.5. Land use changes in recent 50 years of the Yellow River estuary.

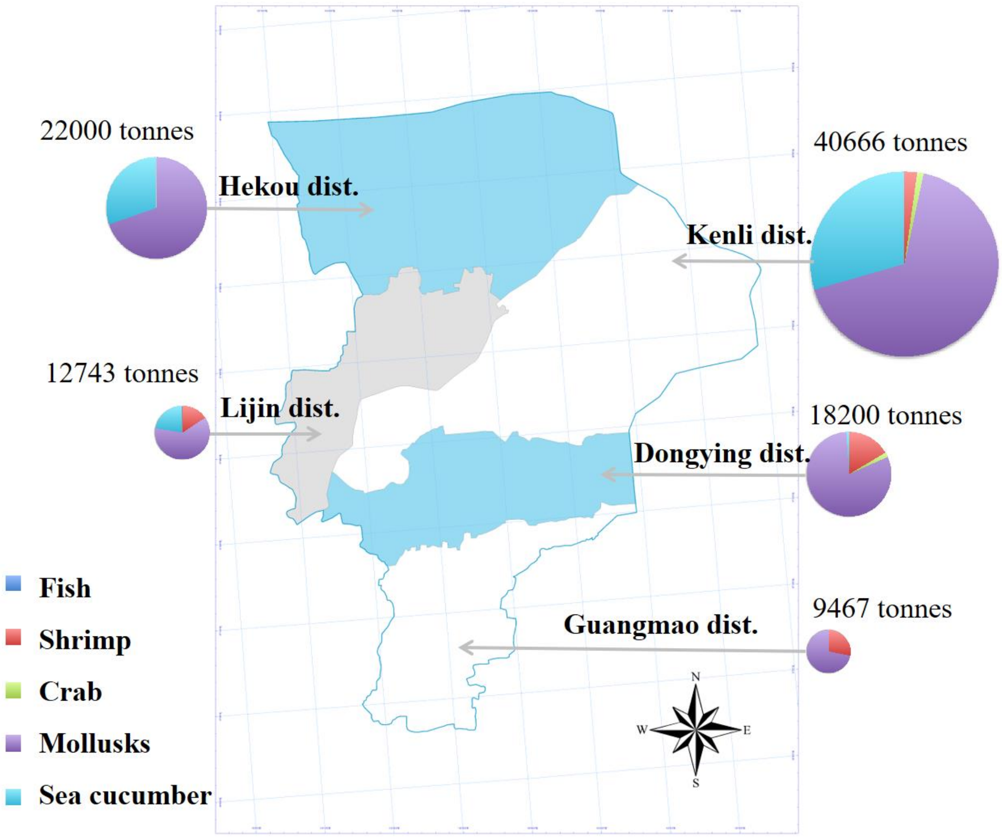

Fig. 1.6. Main mariculture types in each district of Dongying city. The pie size presents the production in each district (Dongying Fisheries Yearbook, 2015).

Besides, cultivation practices and related effluent management may vary between regions, and therefore environmental impacts of aquaculture need to be evaluated specifically.

(2) High production of the target species

The Yellow River estuary is one of the three top sea cucumber (*Apostichopus japonicus*) production bases and important shrimp (*Penaeus japonicas*) production area in China. The sea cucumber farming area increased from 21.3 ha in 2003 to 21734 ha in 2014, and now provided 11241 tonnes of products (dry weight) annually (Dongying Statistical Year Book, 2015). While the shrimp farming area occupied 8540 ha and provided 7889 tonnes of products in 2014 (Dongying Statistical Year Book, 2015). Studies in the high-yield area for those two representative species will be of great importance in economic growth and sustainable development of aquaculture industry.

(3) Possibility of comparisons between cultivated species in same culturing manners

The main organisms cultured in Dongying city were shrimp, fish, bivalve, crab and sea cucumber. The main types of culture techniques were pond and bottom sowing (Fig. 1.6). Except for the bivalve culture using bottom sowing technique, other organisms like shrimp, sea cucumber, fish, and crab were all raised in ponds. These two target species are the representative farming species which are mainly cultured in ponds and dominate this industry with large productions. This limited area enables comparisons of environmental impacts of them

1.4.3 Main objective of this study

Despite much progress in the study of environmental impacts induced by aquaculture activities, no attempts have been made to comprehensively examine their impacts by combining different culturing manners with environmental and ecological variables. The overall objectives of this study are to (1) Evaluated the impacts of two culturing manners; (2) Proposal for scientific and useful evaluation and monitoring methods.

To address these research objectives, the specific research contents are presented as follows:

1) Examine the impacts of sea cucumber pond discharge on adjacent tidal flat by evaluating spatial differences (between farming and non-farming area or among stations with a gradient distance to cultured pond) of environmental variables in water and sediment;

2) Examine the impacts of shrimp pond discharge on adjacent tidal flat by evaluating spatial differences of environmental variables in water and sediment;

3) Detect differences in feeding strategies of dominant macrobenthos between farming and non-farming areas.

4) Explore the different environmental effects induced by two aquaculture activities.

This study attempts to answer the first two questions by field investigations in the sea cucumber farming area, shrimp farming area, and natural reserve area. Water and sediment samples were collected as environment variables. Ordination techniques and multiple comparison analysis will illustrate how these variables are related. To answer the third question, biological surveys are conducted in the above three areas, SIA analysis will be performed to explain the contribution proportions of producers to the diet of consumers. The results of this study enhance our current understanding of how environment parameters and ecological indicators are perceived under the farming and non-farming influences. Besides, it will provide comparable environmental and ecological information, effective monitoring techniques for the future management of the aquaculture activities.

1.5 Methodologies

1.5.1 Principle components analysis (PCA)

Principle components analysis (PCA) (Manly, 1986) was performed to explore the relationships among stations that driven by sediment and water variables. PCA is a mathematical procedure that transforms the possibly correlated original variables into new the linearly uncorrelated variables as we called the principal components (PCs). This technique also reveals the internal structure of the data and finds the indices which could best explain the variance in the data set. PCA also provides the most meaningful parameters which interpret the whole data set, thus reducing the dimensionality of the transformed data and summarize the statistical correlation among constituents with minimum loss of original information (Kazi et al., 2009). For the purpose of this study, PCA was used as an exploratory technique to group samples with similar environmental conditions and isotopic compositions in PC space. The outcomes of the analysis can be visualized using 3D plots to illustrate the differences between sample groups. The contribution of certain characteristics which will determine the position of each sample in the scores space.

1.5.2 Hierarchical cluster analysis

Hierarchical cluster analysis was used in concert with PCA for providing more comprehensive information than using either method alone (Xue et al., 2011). Hierarchical cluster analysis was conducted to classify the samples into meaningful environment associations, aided by water and sediment parameters and biological indicators. For the cluster analysis, Ward’s method was selected. This approach minimizes an increase in the sum of the squares of distances from each sample to the centroid of the group it belongs to (McCune et al., 2002). Ward’s method is known to be an effective and useful tool, as one of the few space-conserving linkage methods.

1.5.3 Similarity analysis

As dendrogram might not give a powerful illustration of the degree of relationships among stations，analysis of Similarities and Similarity of Percentages (ANOSIM & SIMPER) (Henry et al., 2014) was used to measure the degree of similarities or differences among stations and find out which environmental factor contributes most to the differences. As such, ordination was computed via hierarchical cluster analysis based on the Euclidean distance of studied environmental variables and isotopic compositions.

1.5.4 C: N ratio and Stable isotope techniques

Stable isotopes enable to confirm relative contributions of multiple organic matter sources to the bulk organic matter (OM) pool of surface sediments (Aschenbroich et al., 2015). C: N ratio and stable isotope techniques were commonly utilized in recent years as efficient tools to trace and verify the origin, extent, and fate of nutrient sources in the environment and ecosystem (Andrews et al., 1998; Sakamaki et al., 2010). In this study, C: N ratio and carbon stable isotope signatures will be used to trace the origin of organic matter in the aquaculture pond and the adjacent tidal flat. $\delta^{13}C$ signatures of the organic matter in the particulate organic matter have proven to be an effective tool to trace the carbon cycle in the aquatic ecosystem and to evaluate the allochthonous carbon sources from aquaculture waste. Besides, it was widely used in the trophic ecology as they can trace the energy flow in the food webs. For example, $\delta^{13}C$ values have been used to trace food chains and to differentiate the ratio of autochthonous and allochthonous OM sources from anthropogenic nutrient (Moschen et al., 2009) inputs induced by aquaculture waste (Fry and Sherr, 1989; Kon et al., 2009; Sakamaki et al., 2010). Organic matter in the water column and sediment normally is a mixture of various sources like terrestrial plants, phytoplankton, and zooplankton. The diverse origins of organic matter transformed into the water or preserved in the sediment would have largely different $\delta^{13}C$ values (Gao et al., 2012; Meyers and Ishiwatari, 1993). Thus the variations of stable carbon isotope signatures will indicate the origin of organic matter in the water and sediment and reflect the whether the influence of aquaculture discharge reached the adjacent environment.

1.5.5 Bayesian stable isotope mixing model

The Bayesian stable isotope mixing model (Parnell et al., 2010) was used to determine proportional contributions of potential food sources to dominant consumers. Relative contributions of potential food sources were determined using the Bayesian stable isotope mixing model (Parnell et al., 2008). Previous mixing models have been some limitations, Bayesian approach in SIAR avoid the limitations in the previous mixing models by allowing for multiple dietary sources as well as associated uncertainties in both isotopic values (Parnell et al., 2010). Likewise, Bayesian mixing models are more robust in their results than other modeling approaches; it generates the potential dietary compositions with true probability distributions (Inger and Bearhop, 2008; Parnell et al., 2010).

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Chapter 2

Impacts of Sea Cucumber Farming on Water and Sediment Biogeochemical Characteristics

2.1 Introduction

Mariculture of sea cucumber has increased tremendously encouraged by the strong and growing market demand (Chen, 2003; FAO, 2008; Han et al., 2016) for its enormous profit and high nutritional and pharmaceutical values (Bordbar et al., 2011). Consequently, much attention had been recently focused on its culturing manner for better production and development (Dong et al., 2010; Liu et al., 2010; Paltzat et al., 2008; Yokoyama, 2013). Although a variety of studied related to sea cucumber industry, such as hatchery production, culturing manner, sociological issues of stocking, were conducted in previous studies (Han et al., 2016; Ren et al., 2012), there existed a relative paucity of details concerning the environmental effects of sea cucumber farming activities (Li et al., 2014; Ren et al., 2010; Zheng et al., 2009). One of the most important reason probably lies in that most sea cucumbers are deposit feeders. They always are considered as the “environmental cleaners or scavengers” (Yang et al., 1999), and therefore no exogenous nutrients were introduced. The exogenous nutrients input mainly depend on culturing manners. In most areas of China, commercial feeds which mainly composed of fish meal and soybean meal (accounting for 15% and 30% of total weight, respectively) were commonly used in sea cucumber ponds. The excessive feeds always make a significant amount of dissolved and particulate waste which result in nutrients enrichment of water and sediment in the pond and adjacent environment.

Sea cucumber farming in China increased tremendously along coasts, and the yield reached 205,791 tons (dry weight) in 2015 (China Fisheries Yearbook, 2016).

For the present study area Yellow River estuary, sea cucumber production exponentially increased in the past ten years. The farming ponds area increased from 21.3 ha in 2003 to 21734 ha in 2014 with an annual production of 11241 tons (dry weight) annually. Among the sea cucumber species cultured (nearly ten species) around the world at present, *Apostichopus japonicus* was the largest species in production, which was mainly artificially cultured in China (Han et al., 2016; Purcell et al., 2012). The annual hatchery production of juvenile of this species was approximately 6 billion in China, and the sum exceeded the total production of all other reared sea cucumber species (Purcell et al., 2012). The rapid growth of *Apostichopus japonicus* farming promoted a number of studies focusing on genetics (Li et al., 2009), energetic (Liu et al., 2009), neurology (SEO and LEE, 2011) and feeding ecology and physiology (Sun et al., 2013). However, few studies reported the impacts of *Apostichopus japonicus* farming on pond sediment (Ren et al., 2012; Zheng et al., 2009) or coastal waters (Kang and Xu, 2016), no comprehensive investigation of water and sediment characteristics was conducted to date.

Studies of sea cucumber farming with material from Yellow River estuary also had other remarked significance. The aquaculture area of *A. japonicus* in the Yellow River estuary represents one of the typical temperate waters, and thus significantly differed from breeding environments for most raised sea cucumber species (tropical waters). Because environmental effects of mariculture activities were closely related to local hydrographic conditions, research results in temperate areas should contribute largely to our understanding of environmental impacts sea cucumber farming.

Environmental variables selected in this research were all universally recognized by previous studies. Some of them were specially emphasized as highly informative descriptors of heterotrophic loadings from aquaculture, such as dissolved inorganic nutrients (DIN) like nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+) and phosphate (PO_4^{3-}) in water (Sarà, 2007), available phosphorus (AP) and acid volatile sulphide (AVS) in sediment (Yokoyama, 2003). Other studied variables like total organic carbon (TOC) and total nitrogen (TN) has been widely used as important indices to represent organic matter contents in water and sediment (Gao et al., 2012). Similarly, C: N ratio, δ^{13}C, and δ^{15}N were commonly utilized in recent years as efficient tools to trace and verify the origin, extent, and fate of nutrient sources in the environment and ecosystem (Andrews et al., 1998; Sakamaki et al., 2010). The combination of these biogeochemical indicators will sufficiently describe and interpret the local environment variations (Kollongei and Lorentz, 2014) induced by aquaculture activities.

The main purposes of this study were to (1) examine the impacts of sea cucumber pond discharge on local tidal flat by evaluating spatial differences (between farming and non-farming area; (2) examine the dispersal distance of aquaculture waste in the adjacent tidal flat environment. We hypothesized that significant spatial differences exist for contents of selected environmental variables by aquaculture activities with varying degrees. Besides, stable isotopes serve as an effective tool for revealing the source and fate of aquaculture induced organic matters in the environment.

2.2 Materials and methods

2.2.1 Study area

This study was conducted in the north part (37°50′–38°10′ N, 118°20′–119°00′ E) of the Yellow River estuary (Fig. 2.1) which is located in the northeast of Shandong Province, China (Fig. 2.1a). The study site is characterized by a warm-temperate continental monsoonal climate, with mean annual temperature and annual mean precipitation of 11.9 ^{o}C and 640 mm, respectively (Gao et al., 2014).

A total area of 66.67 km^2 was constructed for sea cucumber ponds along the north coast of Yellow River estuary. Each pond was built with 3 to 6 ha in size and 1.5 to 3 m in depth. *Apostichopus japonicus* (juvenile) are cultured in ponds with a density of 10 individuals m^{-2}. Commercial feed (fish meal 15%, soybean meal 20%, peanut flour 10% and seaweed meal 40%) is added to ponds 1–2 times daily. Water exchange is managed uniformly twice a month when spring tide occurs. Pond influent

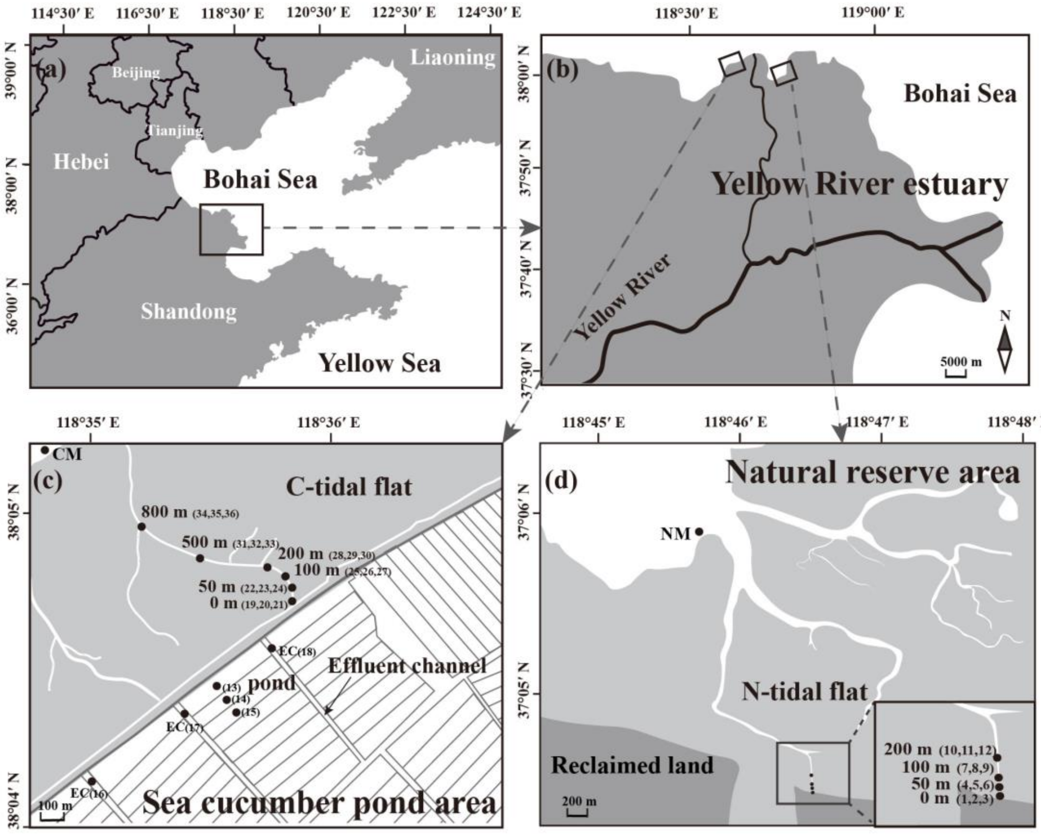

Fig. 2.1 Map of the study area (a, b) showing sampling stations in sea cucumber farming area (c) and non-farming area. Sea cucumber farming area: sea cucumber pond (pond), effluent channel (EC), C-tidal flat (0 m to 800 m) and the coastal area of C-tidal flat (CM); Non-farming area (d): N-tidal flat (0 m to 200 m) and the coastal area of N-tidal flat (NM).

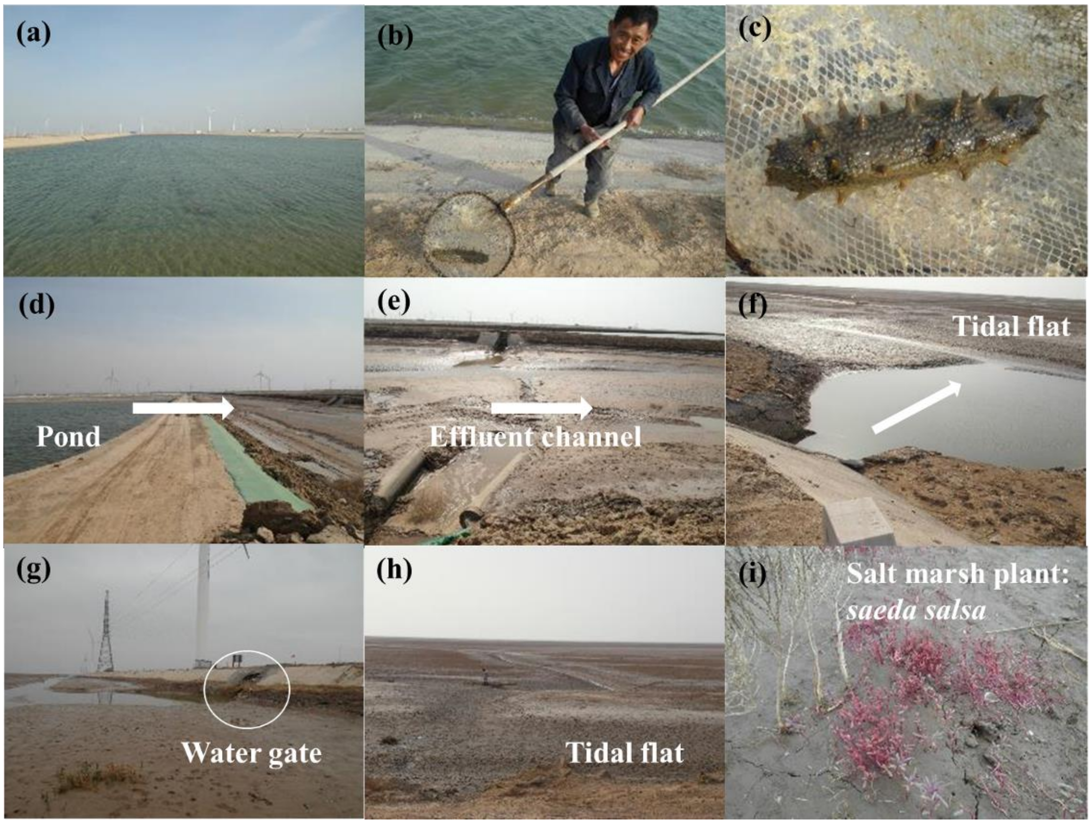

Fig. 2.2. Photos of sea cucumber farming area showing sea cucumber pond (a), cultured species: *Apostichopus japonicus* (b and c), sea cucumber pond and its effluent channel (d), main effluent channel (e), water gate (f and g), adjacent C-tidal flat (h) and dominant salt marsh plant (i).

water (Fig. 2.2) is taken from one tidal creek using pumping systems. Pond effluents are released into unified drainage channels then discharged into adjacent wetland directly through a sluice gate.

The tidal flat (C-tidal flat; Fig.2.2h) adjacent to the sea cucumber pond area is separated from sea cucumber ponds by a 7 m width road. It is dominated by salt marsh plant *Suaeda salsa* and two macrobenthic species, *Helice tridens* and *Macrophthalmus abbreviates* which are widely distributed in this locality. In order to eliminate potential effects of farming activities, we investigated the environment of a referential tidal flat (N-tidal flat) in Natural reserve area (non-farming area) which is 15 km departing from the C-tidal flat and was strictly considered with similar natural characteristics (such as sediment type, tidal characteristics, and elevation).

2.2.2 Field sampling and laboratory procedures

Field sampling was conducted in the sea cucumber pond area, C-tidal flat and N-tidal flat from May 1 to May 21, 2016. In the C-tidal flat, there were no other allochthonous nutrients loading except aquaculture effluent. In the sea cucumber pond area, two stations (Fig. 2.1c) were positioned in the sea cucumber pond and effluent channel, respectively. Two gradient-based sampling were set up in C-tidal flat and N-tidal flat. Six stations (Fig. 2.1c) in the C-tidal flat creek were set at 0 m, 50 m, 100 m, 200 m, 500 m and 800 m distances from the sluice gate using an electronic distance meter (Nikon COOLSHOT 40i Laser Rangefinders) and a GPS receiver (Garmin GPS map64s). Four stations (Fig. 2.1d) in the N-tidal flat creek were set at 0 m, 50 m, 100 m and 200 m distances from the reclaimed land. Other two stations (CM and NM) were set at the marine area of C-tidal flat and N-tidal flat (Fig. 2.1c, d) for water analysis (organic analysis only).

At each station (except for CM and NM stations), both water and sediment samples were collected for analyses of TOC, TN, C: N ratio, $\delta^{13}C$ and $\delta^{15}N$, and dissolved inorganic nutrients (NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-}/ AP). The chemical items analyzed in this study were shown with their abbreviations in Table 2.1. Dominant consumers and primary producers were sampled at 0 m, 50 m and 100 m stations in

Table 2.1 Chemical items analyzed in the present study and their abbreviations.

Analyzing items	Water	Sediment
Particulate organic matter		
in the pond area	$POM_{(pond)}$	
in the tidal flat area	$POM_{(tidal\ flat)}$	
in the coastal area	$POM_{(marine)}$	
Total organic carbon	$TOC_{(POM)}$	$TOC_{(S)}$
Total nitrogen	$TN_{(POM)}$	$TN_{(S)}$
Carbon to nitrogen ratio	C: N $ratio_{(POM)}$	C: N $ratio_{(S)}$
Stable carbon isotope ratio	$\delta^{13}C_{(POM)}$	$\delta^{13}C_{(S)}$
Stable nitrogen isotope ratio	$\delta^{15}N_{(POM)}$	$\delta^{15}N_{(S)}$
Nitrate	$NO_3^-{}_{(water)}$	$NO_3^-{}_{(S)}$
Nitrite	$NO_2^-{}_{(water)}$	
Ammonium	$NH_4^+{}_{(water)}$	$NH_4^+{}_{(S)}$
Phosphate	$PO_4^{3-}{}_{(water)}$	$AP_{(S)}$
Acid volatile sulfides		AVS

each tidal flat. All of these samplings were conducted in the ebb tides.

At each station, three replicate water samples were collected by a bucket from the surface layer of the water for the analyses of particulate organic matter (POM) and dissolved inorganic nutrients. Marine POM ($POM_{(marine)}$) in CM and NM stations were collected at the sea surface of each adjacent offshore. POM samples were collected by filtering 0.4 to 3 L surface seawater through a 125 μm mesh (to remove zooplankton) followed by glass-fiber filters (Whatman GF/F, φ47 mm). To remove carbonates, POM samples were treated with 1.2 N HCl overnight and then oven dried at 60 ^{o}C for stable isotope analysis. The remained filtered water samples were processed immediately for nutrient analysis using SAN^{++} continuous flow analyzer (SKALAR, Netherlands).

To detect the biogeochemical characteristics of deposited sediment, three replicate surface layer (0 to 1 cm) sediments were collected by using a 48 mm (inner diameter) acrylic tube. An aliquot of the samples was used for the analysis of acid volatile sulfide (AVS) concentration to present sediment condition affected by aquaculture (Yokoyama, 2010). The remainder was divided into two groups (group A and group B). Group A was used for elemental (TOC & TN) and stable isotope analysis ($\delta^{13}C$ & $\delta^{15}N$), group B was used for the analyses of dissolved inorganic nutrients in sediment. Sediment samples of group A were weighed, dried at 60 ^{o}C for 72 h, then weighed again to determine the water content, and ground to a fine powder. Prior to analyses, samples for carbon isotope analysis were soaked in 1.2 N HCl overnight to remove carbonates, filtered on a Nuclepore polycarbonate track-etch membrane filter (pore size = 0.2 μm) and dried again. Aliquots of samples not soaked in 1.2 N HCl were used for nitrogen isotopic analysis. Group B samples used for dissolved inorganic nutrients analysis (Jia et al., 2017) were air-dried at room temperature and passed through a 2 mm mesh sieve. Samples for NO_3^- and NH_4^+ analysis (5.0 g) were extracted with 2 mol l^{-1} KCL (25 ml), and samples for AP (1.2 g) were extracted with 0.5 mol l^{-1} $NaHCO_3$ (25 ml) for determining the exchangeable nitrogen and phosphorous. Aftershock extraction for 30 min, the samples were filtered and analyzed by SAN^{++} continuous flow analyzer to determine the concentrations of

NO_3^-, NH_4^+, and AP.

Benthic microalgae (BMA) samples (n=3) were extracted from the surface sediment in each tidal flat (Couch, 1989; Riera and Richard, 1996). The sediment was brought back to the laboratory within 30 min and then spread on a flat tray (1 cm depth) and covered by a nylon screen (63 μm mesh). A 4 to 5 mm layer of combusted sand (acid-washed and burned, 125–500 μm) was spread over the nylon screen. The sand was kept wet using filtered seawater and then cultured under fluorescent light for 12 to 15 h. The top 2 mm of sand was then removed and filtered through a 63 μm fiber sieve by washing with distilled water. Water containing the microalgae was filtered onto GF/F (φ25 mm) filters, and oven dried at 60 ^{o}C for stable isotope analysis.

The dominant salt marsh plant *Suaeda salsa* was collected by hand. Leaves from 10 plants were taken as a single sample. Terrestrial debris was collected by hand nets. The dominant macrobenthic animals (two crabs) were caught by a long-handled scoop around the sampling station within 5 m distance. Each sample was then preserved on ice and returned to the laboratory for sorting and sampling. Muscle tissue of crab was taken for animal isotope sample. All animal and plant samples were first treated with 1.2 N HCl to remove carbonates and then oven dried at 60 ^{o}C and ground to fine powder for isotope analysis.

The elemental and isotope analysis of samples were analyzed using a Delta XP plus isotope ratio mass spectrometer linked to a Conflo IIII interface (Thermo Fisher Scientific, USA) with a Flash EA1112 elemental analyzer

Stable isotope ratios were expressed in the conventional notation (δ^{13}C or δ^{15}N), defined by the following equation:

$$\delta^{13}C \text{ or } \delta^{15}N = (R_{sample}/R_{standard} - 1) \times 10^3$$

$$\text{and } R = {}^{13}C/{}^{12}C \text{ or } {}^{15}N/{}^{14}N$$

The standard reference materials were Pee Dee Belemnite (PDB) and atmospheric N^2 for carbon and nitrogen isotopes, respectively.

2.2.3 Data analysis

Principle components analysis (PCA) (Manly, 1986) was conducted in R V3.3.1 to explore the relationships among stations that driven by sediment and water variables using the function prcomp and princomp of R package stats. To evaluate spatial differences in N-tidal flat, C-tidal flat, effluent channel and sea cucumber pond, a multivariate technique was applied using the PRIMER 6.0 ecological software package developed by the Plymouth Marine Laboratory. Ordination was computed via hierarchical cluster analysis (CLUSTER) based on the Euclidean distance of studied environmental variables and isotopic compositions. Seriation test and SIMPER (Similarity Percentages) analysis were performed to analyze the community structure. One-way ANOSIM was carried out to test the differences in the studied areas.

Multiple comparisons of each environmental variable between stations were conducted to detect spatial differences between farming and non-farming areas or among stations with a gradient distance to the aquaculture pond. One-way ANOVA was used when data is normally distributed (Shapiro and Wilk, 1965) and homoscedastic (Bartlett, 1937), followed by Bonferroni post hoc test (Rice, 1989). Kruskal-Wallis test (Kruskal and Wallis, 1952) was performed when the data is not normally distributed or homoscedastic, followed by Gao post hoc test (Gao et al., 2008; Konietschke et al., 2015). Statistical significance level was considered at 5%. Statistical analyses were performed using the function Wilk-Shapiro test, Bartlett’s test, Bonferroni test and Kruskal test of the R package stats and the function Gao of the R package nparcomp (Ihaka and Gentleman, 1996; Shirley, 1977) and oneway_test function of the R package coin (Ihaka and Gentleman, 1996) in R V3.3.1. As no significant differences (ANOVA with Bonferroni's post hoc tests, $p > 0.05$) were observed in the N-tidal flat along the gradient (0 m, 50 m, 100 m, 200 m), the data from N-tidal flat sampling stations were calculated together to represent a reference location expressed as N-tidal flat.

2.3 Results

2.3.1 Environmental variables

Each sampling location (sea cucumber pond, effluent channel, C-tidal flat and N-tidal flat) were clearly separated from each other by PCA analysis (Fig. 2.3a) with seventeen water and sediment variables (Table 2.1). The first three principal components (PC1, PC2, and PC3) explained 73% of the total variance.

The main contributions to PC1 included sediment variables $TOC_{(S)}$, $TN_{(S)}$, C: N $ratio_{(S)}$, $\delta^{13}C_{(S)}$, $NO_3^-{}_{(S)}$, $NH_4^+{}_{(S)}$, $AP_{(S)}$ (with significant positive coefficients), $\delta^{15}N_{(S)}$ and $\delta^{13}C_{(POM)}$ (with negative coefficient). Vector plots of sediment variables and $\delta^{13}C_{(POM)}$ showed that PC1 represented axes of increasing organic accumulation and nutrients concentrations in sediment and decreasing $\delta^{15}N_{(S)}$ and $\delta^{13}C_{(POM)}$ from tidal flat to effluent channel and then followed by sea cucumber pond. As for PC2 (Fig. 2.3b), POM variables (except for $\delta^{13}C_{(POM)}$) and $PO_4^{3-}{}_{(water)}$ in water were closely related to PC2 as indicated by significant positive coefficients ($TOC_{(POM)}$, $TN_{(POM)}$, C: N $ratio_{(POM)}$, $\delta^{15}N_{(POM)}$) and negative coefficient ($PO_4^{3-}{}_{(water)}$), respectively. PC2 represented axes of increasing organic accumulation in POM and decreasing $PO_4^{3-}{}_{(water)}$ concentration from sea cucumber pond area to N-tidal flat. The main contribution to PC3 (Fig. 2.3c) was from $DIN_{(water)}$ that showed highly positive coefficients ($NO_3^-{}_{(water)}$, $NO_2^-{}_{(water)}$, $NH_4^+{}_{(water)}$). As the vector plots of $DIN_{(water)}$ that PC3 represented, however, no obvious gradient variations were found across stations.

The cluster analysis (Fig. 2.4) showed that studied localities could be distinguished into three groups (sea cucumber pond, the combination of C-tidal flat and effluent channel, N-tidal flat) with the threshold Euclidean distance more than 7.0 (Fig. 2.4). The results of the pairwise test in ANOSIM showed that significant differences were present between N tidal flat and sea cucumber pond ($R = 1$, $p < 0.01$), N tidal flat and the combination of C tidal flat and effluent channel ($R = 0.529$, $p < 0.01$), sea cucumber pond and the combination of C tidal flat and effluent channel ($R = 0.991$, $p < 0.01$). With the subsequent SIMPER analysis, the primary contributors to three groups were $TOC_{(S)}$ (89.60% contribution), $TOC_{(POM)}$ (63.16% contribution)

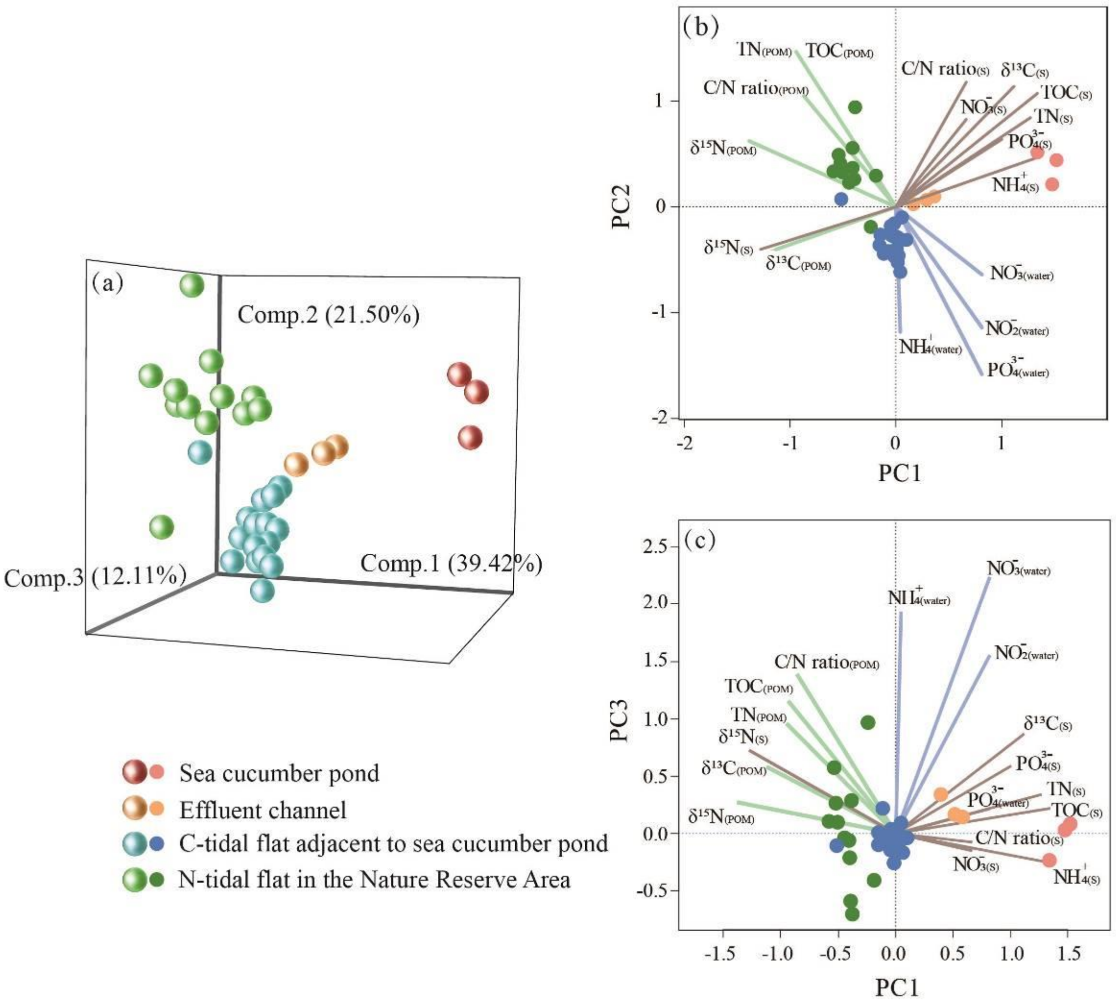

Fig. 2.3. Principal component analysis (PCA) based on the seventeen sediment and water parameters. (a) 3D scores plot (PC1, PC2 and PC 3) of the environmental variables to the samples. (b) PC1 and PC2. (c) PC1 and PC3.

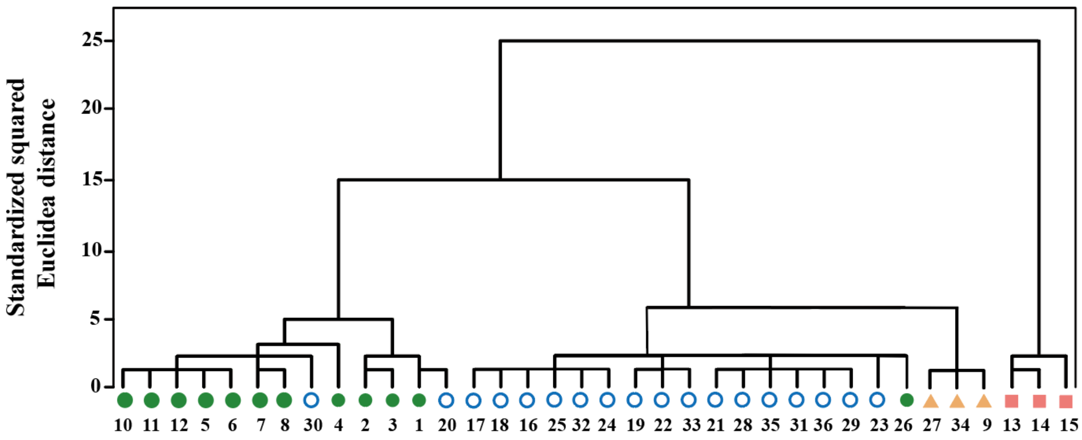

Fig. 2.4. Dendrogram showing cluster relationship between samples. Different sampling locations were shown in different symbols (filled circle: N-tidal flat, open circle: C-tidal flat, triangle: effluent channel, square: sea cucumber pond). See Fig. 2.1 for the sample numbers.

and $TOC_{(S)}$ (97.82% contribution).

2.3.2 Water and sediment biogeochemical characteristics

(1) Water biogeochemical characteristics

$TOC_{(POM)}$ and $TN_{(POM)}$ concentrations (Figs. 2.5a, b) detected in farming area (mean ± SD = 0.07 ± 0.02 and 0.05 ± 0.2 mg l^{-1}, respectively) were significantly lower than those in N-tidal flat (1.4 ± 0.6 and 0.2 ± 0.1 mg l^{-1}, respectively). C: N ratios of POM (Fig. 2.5c) ranged from 4.7 to 9.1 and showed significant differences between sea cucumber pond and N-tidal flat (ANOVA with Bonferroni's post hoc tests, $p < 0.001$). $\delta^{13}C_{(POM)}$ in sea cucumber pond (–20.6 ± 1.0‰), effluent channel (–19.9 ± 1.2‰) and C-marine stations (–21.1 ± 0.1‰) showed significantly lower values than those in C-tidal flat (–16.7 ± 1.6‰), N-tidal flat (–16.9 ± 1.2‰) and N-marine (–18.4 ± 0.4‰) stations (Fig. 2.5d). $\delta^{15}N_{(POM)}$ values in the sea cucumber pond (–2.0 ± 0.2‰) was significantly lower than those in other stations (Bonferroni's post hoc tests, $p < 0.001$). No remarkable differences were found within C-tidal flat stations (0.9 ± 0.6‰).

NO_3^- and NH_4^+ showed no significant differences (ANOVA, $p > 0.05$) among stations (Figs. 2.5f, h), whereas significant differences were present in NO_2^- and PO_4^{3-} (Fig. 2.5g, i; Kruskal-Wallis test, $p < 0.01$ and $p < 0.001$, respectively). The NO_2^- concentration in sea cucumber pond (53 ± 2 μg l^{-1}) was clearly higher than that in N-tidal flat (40 ± 9 μg l^{-1}), but no distinct differences were found among stations in the farming area or between C-tidal flat and N-tidal flat. The PO_4^{3-} concentrations in sea cucumber pond (32 ± 1 μg l^{-1}), effluent channel (33 ± 1 μg l^{-1}) and C-tidal flat (29 ± 3 μg l^{-1}) were clearly higher than that in N-tidal flat (23 ± 2 μg l^{-1}), but no distinct differences were found within stations in the farming area.

(2) Sediment biogeochemical characteristics

AVS concentration detected in sea cucumber pond was 0.1 ± 0.5 mg g^{-1} (dry sediment) higher than those in the effluent channel, C-tidal flat and N-tidal flat (all

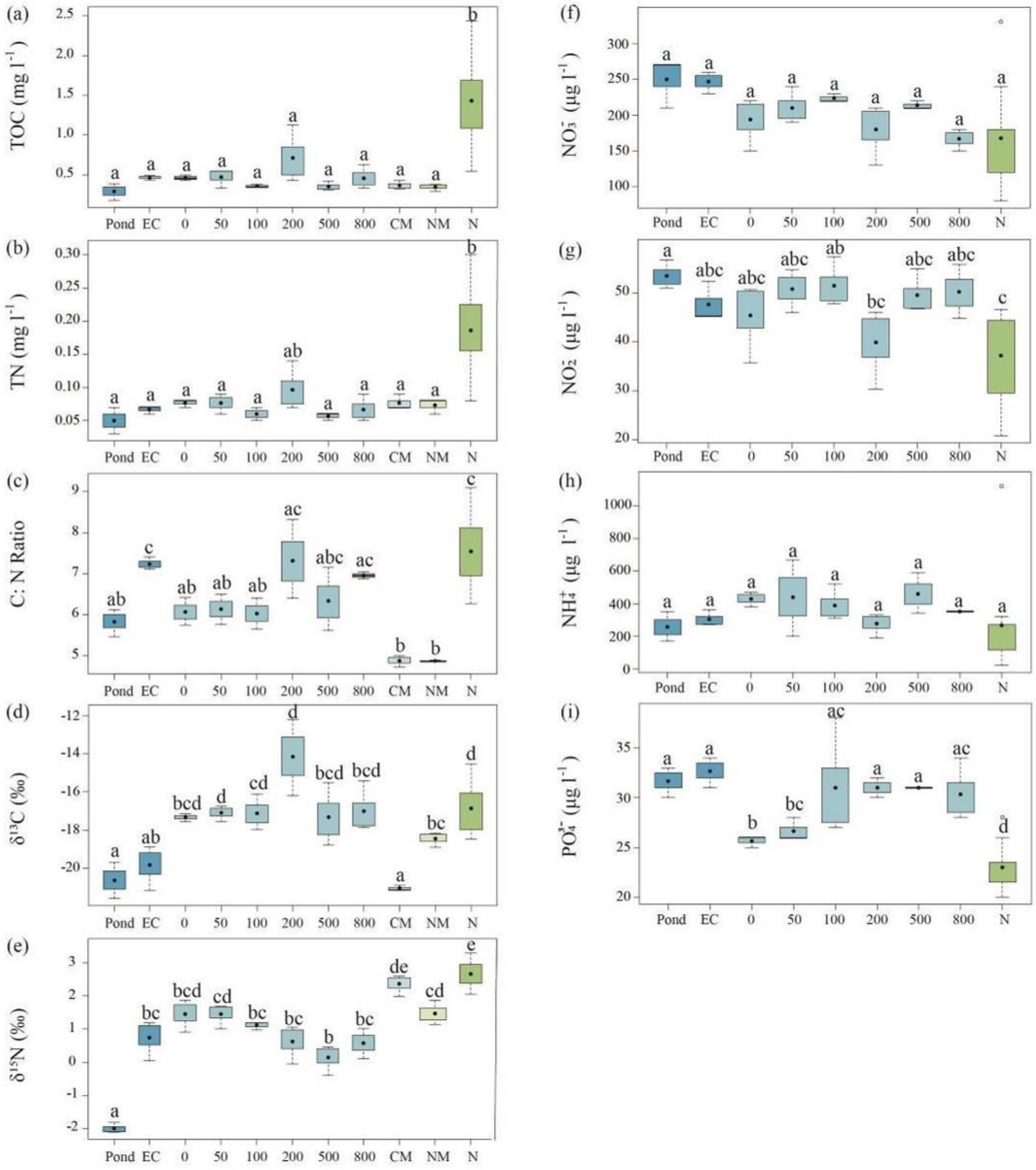

Fig. 2.5. Comparisons of water parameters including TOC (a), TN (b), C: N ratio (c), $\delta^{13}C$ (d), $\delta^{15}N$ (e), NO_3^- (f), NO_2^- (g), NH_4^+ (h) and PO_4^{3-} (i). Different letters above bars indicate significant differences ($p < 0.05$) by Bonferroni post hoc test (TOC, TN, C: N ratio, $\delta^{15}N$, NO_3^-, NH_4^+) and Gao post hoc test ($\delta^{13}C$, NO_2^-, PO_4^{3-}). Boxes represent 25% – 75% percentiles; range bars represent the 5% and 95% percentiles, solid dots in the boxes represent mean values, hollow dots represent values outside of 95% confidences interval.

less than 0.01 mg g^{-1}). For $TOC_{(S)}$ and $TN_{(S)}$, significant differences (Kruskal-Wallis test, $p < 0.01$ and $p < 0.001$, respectively) were observed among stations (Figs. 2.6a, b). $TOC_{(S)}$ and $TN_{(S)}$ in the sea cucumber pond (5.9 ± 1 mg g^{-1} and 0.8 ± 0.3 mg g^{-1}, respectively) and effluent channel (2.95 ± 0.43 mg g^{-1} and 0.51 ± 0.08 mg g^{-1}, respectively) were significantly higher than those in the C-tidal flat (1.2 ± 0.3 mg g^{-1} and 0.3 ± 0.1 mg g^{-1}, respectively) and N-tidal flat (1.8 ± 0.2 mg g^{-1} and 0.3 ± 0 mg g^{-1}, respectively). Both $TOC_{(S)}$ and $TN_{(S)}$ contents showed decreasing trends from sea cucumber pond to 0 m station and no gradient variations along the dispersal distance in the C-tidal flat.

C: N ratios (Fig. 2.6c) in the pond, effluent channel, 0 m station and N-tidal flat had significant differences from other stations in the C-tidal flat (50 m, 100 m, 200 m and 800 m). A decreasing trend was found from pond to 0 m. $\delta^{13}C_{(S)}$ and $\delta^{15}N_{(S)}$ values of the sea cucumber pond were remarkably different from the effluent channel, C-tidal flat and N-tidal flat (Fig. 2.6d, e). A decreasing trend of $\delta^{13}C_{(S)}$ was found from pond to 0 m station. $\delta^{13}C_{(S)}$ of 0 m station showed an extensive range of standard deviation and no significant differences with the effluent channel and other stations in C-tidal flat and N-tidal flat.

No significant differences (Fig. 2.6f) among stations were found for $NO_3^-{}_{(S)}$ (Kruskal-Wallis test, $p > 0.05$). Both $NO_3^-{}_{(S)}$ and $NH_4^+{}_{(S)}$ were found decreasing trends from pond to 0 m station. The concentration of $NH_4^+{}_{(S)}$ ranged from 1.8 to 18.1 mg kg^{-1} (Fig. 2.6g) and varied across stations (Kruskal-Wallis test, $p < 0.001$). The highest $NH_4^+{}_{(S)}$ concentration found in sea cucumber pond (15.8 ± 2.0 mg kg^{-1}) was significantly greater than those from effluent channel (8.6 ± 2.9 mg kg^{-1}), C-tidal flat (5.7 ± 2.6 mg kg^{-1}) and N-tidal flat stations (4.3 ± 0.5 mg kg^{-1}). $NH_4^+{}_{(S)}$ content in effluent channel showed no significant differences with C-tidal flat stations (except for 500 m) but was obviously higher than that in the N-tidal flat. AP contents (Fig. 2.6h) in pond (8.2 ± 0.9 mg kg^{-1}) and effluent channel (10.4 ± 3.9 mg kg^{-1}) were significantly higher (Bonferroni's post hoc tests, $p < 0.001$) than those in both C-tidal flat (3.6 ± 0.6 mg kg^{-1}) and N-tidal flat (3.4 ± 2.1 mg kg^{-1}).

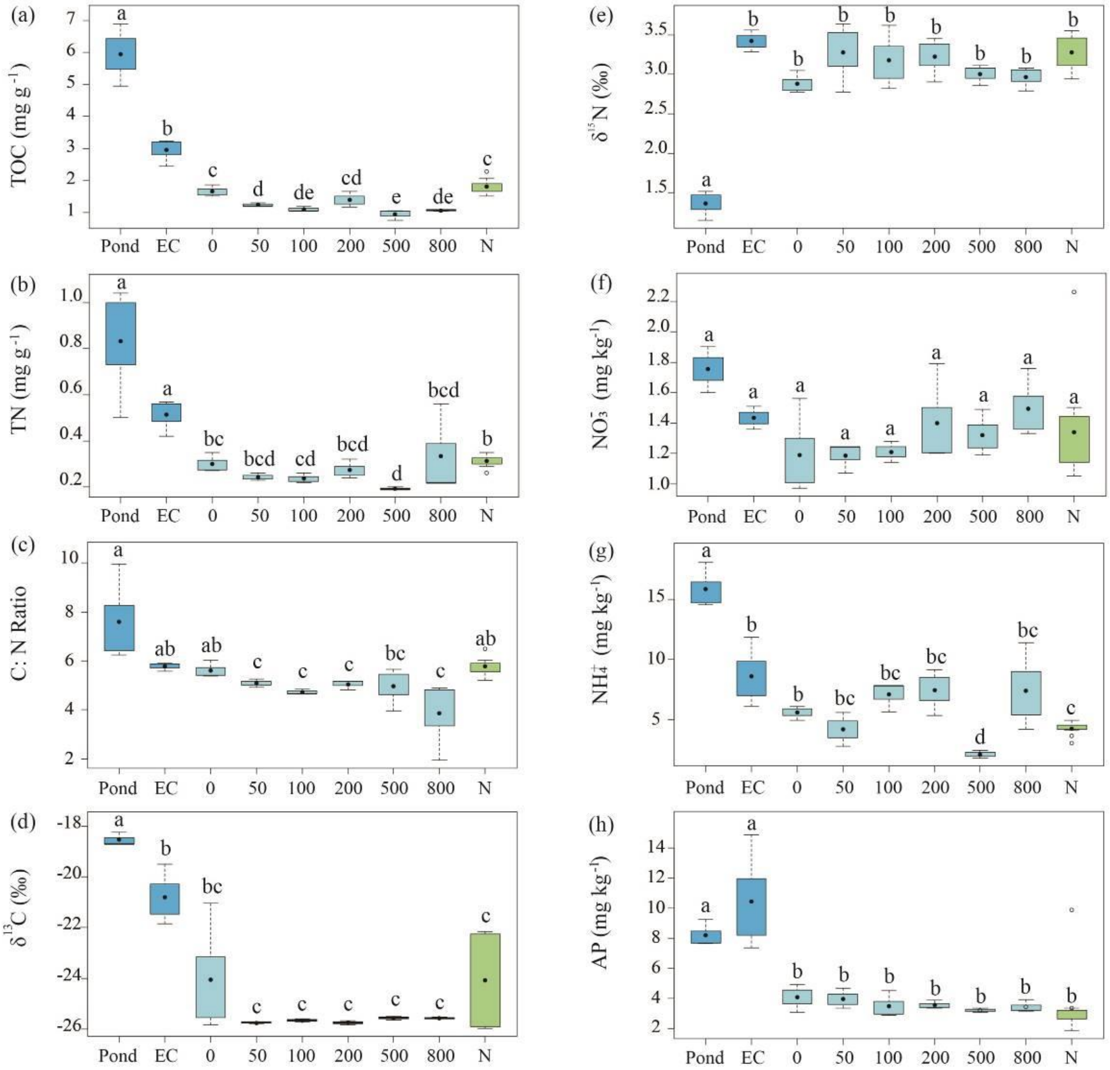

Fig. 2.6. Comparisons of sediment parameters including TOC (a), TN (b), C: N ratio (c), $\delta^{13}C$ (d), $\delta^{15}N$ (e), NO_3^- (f), NH_4^+ (g) and AP (h). Different letters above bars indicate significant differences ($p < 0.05$) by Bonferroni post hoc test ($\delta^{15}N$, AP) and Gao post hoc test (TOC, TN, C: N ratio, $\delta^{13}C$, NO_3^-, NH_4^+). Boxes represent 25%–75% percentiles; range bars represent the 5% and 95% percentiles, solid dots in the boxes represent mean values, hollow dots represent values outside of 95% confidences interval.

2.4 Discussion

2.4.1 Differences in distance-based environmental variables

Overall, this study intended to evaluate the environmental impacts of sea cucumber farming based on two kinds of analyses. The first one was the detection of discrepancies among distance-based pollution indicators in the water column and sediment. The second one was the utilization of biochemical tracers ($\delta^{13}C$ and $\delta^{15}N$) to trace the origin of organic matters. Both methods had been widely confirmed as reliable tools to evaluate environmental effects of aquaculture (Gowen and Bradbury, 1987; Sarà, 2007; Sarà et al., 2004; Wai et al., 2011). A total of eight typical environmental variables (TOC, TN, C: N ratio, AVS, NO_3^-, NH_4^+, NO_2^-, PO_4^{3-} and AP) were studied in this paper. For the studied parameters in the water column, they showed no normal variation tendencies. For example, $NH_4^+{}_{(water)}$ and $NO_3^-{}_{(water)}$ contents were similar between farming and non-farming areas, or among stations within the farming area (such as pond and its adjacent tidal flat). $TOC_{(POM)}$, $TN_{(POM),}$ $NO_2^-{}_{(water)}$ and $PO_4^{3-}{}_{(water)}$ contents in the farming area were different from those in N-tidal flat, while they did not differ among farming stations. In our study, distribution patterns of studied parameters may have many potential reasons. One possible reason lies in that eutrophication, related to the rapid development of mariculture (Bouwman et al., 2013) or other anthropogenic activities, are widely present in the Yellow River estuary and its adjacent waters (Chen et al., 2013; Kang and Xu, 2016; Zhang et al., 2008). High background values hinder the statistical differences.

In previous studies, $NO_3^-{}_{(water)}$ was recognized as the typical dominant part of DIN in the studied coastal waters (Gong, 2012; Zhang et al., 2008). However, our study found the same result as Kang (2016), that $NH_4^+{}_{(water)}$ represented the relative major proportion of DIN than $NO_3^-{}_{(water)}$ in the mariculture zone. This result corresponded well to the meta-analysis of Sarà (2007) that $NH_4^+{}_{(water)}$ appears to be the most affected compound by aquaculture loadings than $NO_2^-{}_{(water)}$, $NO_3^-{}_{(water)}$ and followed by $PO_4^{3-}{}_{(water)}$. However, discrepancies among distribution patterns of studied parameters prevented us from drawing much more clear conclusions.

Despite the distinct distribution patterns of nutrients in the water column, $TOC_{(S)}$, $TN_{(S)}$ $NO_3^-{}_{(S)}$, $NH_4^+{}_{(S)}$, $AP_{(S)}$ and AVS contents in sediment showed uniform distance-based differences. They had significantly higher values in farming area than those in non-farming area. Within the farming area, a decrease transect from pond to 0 m station was detected. These findings clearly demonstrated the presence of aquaculture waste from the sea cucumber farming, and additionally implied a restricted dispersion distance (approximately 50 m in the C-tidal flat) of aquaculture waste that accumulated in sediment. It was also worth noting that AVS content in sea cucumber pond in this study was below the threshold (0.2 mg g^{-1}), which in some rules ensuring a sustainable aquaculture (Uede, 2008; Yokoyama, 2003; Yokoyama et al., 2007). However, the content was much higher than those reported from other natural sediments. Similar cases were also found in $TOC_{(S)}$ and $TN_{(S)}$ concentrations in sea cucumber pond. This phenomenon was also considered as a result of sea cucumber aquaculture.

2.4.2 Detection of origins of organic matters and their implications

In the water column, the result of C: N $ratios_{(water)}$ and isotopic composition analysis revealed that sea cucumber pond had different organic origins from adjacent tidal flat and non-farming area. For example, C: N $ratios_{(POM)}$ detected in pond, 0 m, 50 m, 100 m and 500 m stations were close to the Redfield ratio (C: N = 106:16) for marine plankton (Andrews et al., 1998; Burkhardt et al., 1999; Redfield, 1963), which might indicate that the main contributor of POM in these stations was marine phytoplankton . The relatively higher values of C: N $ratios_{(POM)}$ in the effluent channel, 200 m, 800 m and N-tidal flat suggested that POM resulted from a mixture of marine phytoplankton and other organic matter sources. Low C: N $ratios_{(POM)}$ detected in N-marine and C-marine areas might be attributed to the contribution of marine zooplankton with low C: N ratio (Båmstedt, 1986; Walve and Larsson, 1999). The lowest $\delta^{13}C_{(POM)}$ and $\delta^{15}N_{(POM)}$ values found in sea cucumber pond maybe due to the allogenous sources. Terrestrial plant (soybean and peanut) and seaweed flour (seaweed cultured in offshore) introduced by aquaculture (commercial feeds) had low $\delta^{13}C$ and $\delta^{15}N$ values, and the presence of allogenous sources resulted in low isotopic values. In this case, the distribution pattern of $\delta^{13}C_{(POM)}$ and $\delta^{15}N_{(POM)}$ values indicated the environmental impact of aquaculture.

In the sediments, high $\delta^{13}C_{(S)}$ and low $\delta^{15}N_{(S)}$ values were detected in sea cucumber pond. Similar to the reason of low $\delta^{15}N_{(POM)}$ compositions in the water column, low $\delta^{15}N_{(S)}$ values should be due to the allogenous organic matter input. $\delta^{13}C_{(S)}$ in pond sediment was approximately equal to $\delta^{13}C_{(POM)}$ of POM, which suggested that they had homogeneous sources. In the adjacent and natural tidal flat, deposition of organic matter from salt marsh plant ($\delta^{13}C = -29.74 \pm 1.31‰$, $\delta^{15}N = 6.1 \pm 0.91‰$) contributed to the sediment with much lower $\delta^{13}C$ and high $\delta^{15}N$ values. Therefore, high $\delta^{13}C$ and low $\delta^{15}N$ values in the pond were a reflection of the disturbance of aquaculture waste.

2.4.3 Implications for operation and management of sea cucumber farming

The overall conclusion of the present study was that sea cucumber farming slightly altered the local environment. However, this modification was restricted to a limited area (within 50 m). Up to present, there existed a relative paucity of details concerning the environmental effects of sea cucumber farming (Li et al., 2014; Ren et al., 2010; Zheng et al., 2009). Therefore, it was difficult to make a comparison of our results with the previous studies. In this study, the short dispersal distance might be attributed to the typical operation mode and management of sea cucumber pond farming in the studied region. Firstly, the main feed for *Apostichopus japonicus* is composed of much seaweed flour (40%) and fewer protein feeds (fishmeal = 15%) in comparison with the shrimp feed (around 45%) (Dierberg and Kiattisimkul, 1996; Paez-Osuna, 2001; Trott et al., 2004). As a result, it reduced the accumulation of organic matter from the waste feed. Besides, the intense of sea cucumber farming activities was moderate, with low densities (10 individuals m^{-2}) that far below the average densities (20 individuals m^{-2}) practiced by Chinese farmers (Dong et al., 2010; Kang and Xu, 2016). Also, wastewaters from sea cucumber ponds, connected by internal pipes, were gathered to bigger channels and drained intermittently by valves, which may result in a higher deposition before the discharge. The modification in sediment was restricted to 50 m distance, which indicated that farming pond and effluent channel were heavily disturbed. Consequently, high natural feeds content, low stocking densities, and line effluent channels reduced the deleterious effects of sea cucumber farming on the adjacent environment. Besides, deposited sludge should be more efficiently removed physical efforts or by filters like salt marsh plant, bivalves, and microalgae.

For the management of aquaculture activities, one of the challenges is to develop practical, cost-effective monitoring techniques that reflect the impact and processes (Burford et al., 2003). The present study provides meaningful information about environmental impacts of *Apostichopus japonicus* farming. The unique characteristics of *Apostichopus japonicus* farming discharge indicated that the predominant effects of sea cucumber farming discharge were in the sediment processes, rather than water column processes. In this case, environmental variables should be unequally treated.

In conclusion, the present study considered that pond farming for *Apostichopus japonicus* in the Yellow River estuary altered local environment to a certain extent, as determined by the discrepancies among distance-based environmental parameters in sediment ($TOC_{(S)}$, $TN_{(S)}$, $NO_3^-{}_{(S)}$, $NH_4^+{}_{(S)}$, $AP_{(S)}$ and AVS) as well as isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$). Such kind of modification covered farming pond, effluent channel, and adjacent tidal flat within 50 m range. For methodological consideration, sediment biogeochemical characteristics as a historical recorder much more efficiently reflected both organic and dissolved inorganic nutrients induced by aquaculture.

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Chapter 3

Impacts of Shrimp Farming on Water and Sediment Biogeochemical Characteristics

3.1 Introduction

Attracted by the global demand for seafood, shrimp farming with high profitability and generation of foreign exchange has extraordinarily expanded since 1970 in the world (Burford and Williams, 2001; Primavera, 1997). The expansion of this activity in or adjacent to the coastal ecosystem (A.I.Roberston, 1995) has raised global concern and widespread interests on the environmental impacts of shrimp farm effluent (Páez-Osuna et al., 1997; Tanner and Fernandes, 2010; Troell et al., 2003; Trott et al., 2004).

The effluent from shrimp ponds is typically enriched in organic matter and dissolved inorganic nutrients as uneaten feed and shrimp excrements (Primavera, 1994). The enriched effluent discharged into the adjacent environmental waters without treatment will alter the water and sediment qualities in ponds and adjacent environment, which will carry the risk of organic pollution in the sediment (Burford et al., 2003; Carroll et al., 2003; Holmer et al., 2007) and eutrophication in the coastal waters (Bouwman et al., 2013). Besides, adjacent waters always serve as influent water for the same or neighboring shrimp farms (Costanzo et al., 2004), reduce the possibility of sustainable usage of natural water resources and affect the shrimp farms themselves through self-pollution (Lin and Fong, 2008).

Herbeck et al. (2013) evaluated that POC and PN accounted more than half of the TOC and TN which exported from shrimp effluent in solid carbon and nitrogen form. Dissolved inorganic nutrients were the principal index of the pollution of the mariculture. In addition to the conventional analysis of water (Biao and Yu, 2007) and sediment qualities, some novel bioindicators such as $\delta^{15}N$ enrichment of coastal biota (Costanzo et al., 2004; Jones et al., 2001; Lin and Fong, 2008) and trophic interactions and processes (McKinnon et al., 2002a) used in recent studies, have proven useful in revealing the fate of shrimp farming waste in the adjacent mangrove ecosystem.

However, studies using certain physical or chemical parameters have ascertained that symptoms of aquaculture effluent are only focused on water, sediment, or the water-sediment interface. Besides, few studies have been conducted to assess environmental impacts on salt marshes (King and Lester, 1995; Paez-Osuna, 2001), especially the range of farm waste in the adjacent tidal flat. Subsequently, there is a need for a more comprehensive approach combined with stable isotope signatures to assessing the impact extent of shrimp farming effluent on the surrounding water and sediment biogeochemical characteristics, with the ability to establish effective monitoring tools for predicting changes in the environment following further changes in farm management practices.

China was ranked as one of the leading shrimp producers. In 2015 the area under cultivation and the output reached 258,381 ha and 1.2 million tonnes, respectively (China Fisheries Yearbook, 2016). There are nearly 100 penaeid shrimp species in China’s coastal waters. *Penaeus japonicas* was one of the seven main shrimp types cultured in China. The cultivation area and the yield reached 21,753 ha and 46,329 tonnes, respectively in 2015 (China Fisheries Yearbook, 2016). Every year nearly 43 billion tons of wastewater from shrimp farming and shrimp breeding system spill into the coastal waters (Biao and Yu, 2007). However, there has no well-documented report regarding the effect of shrimp aquaculture on its coastal environments.

In this study, a range of chemical and biological components was combined to examine the environment effect of shrimp farming. The main purposes of this study were to (1) examine the impacts of shrimp pond discharge on adjacent tidal flat by evaluating spatial differences of organic and nutrients concentrations between farming and non-farming area; (2) Determine the dispersal distance of discharge waste in the adjacent tidal flat by stable isotope tracers.

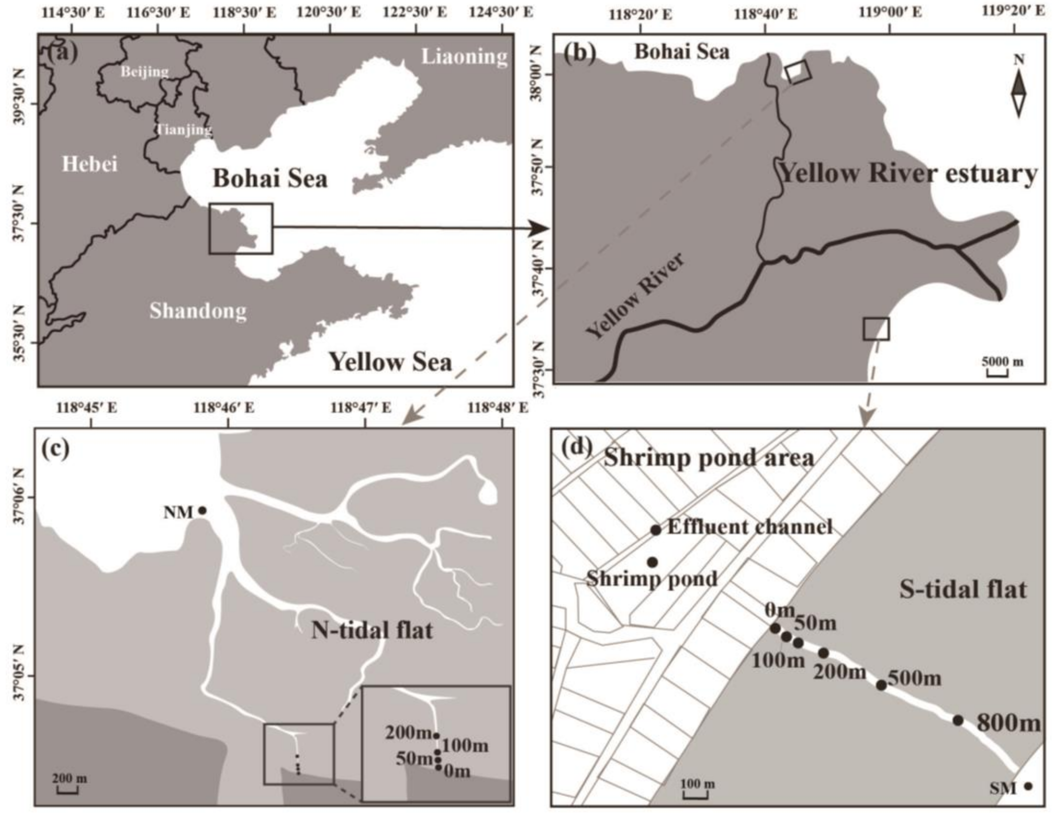

Fig. 3.1. Map of the study area (a, b) showing sampling stations in non-farming area (c) and shrimp farming area (d). Non-farming area (c) includes N-tidal flat (0 m to 200 m) and coastal area of N-tidal flat (NM). Shrimp farming area: shrimp pond (pond), effluent channel (EC), S-tidal flat (0 m to 800 m) and coastal area of S-tidal flat (SM);

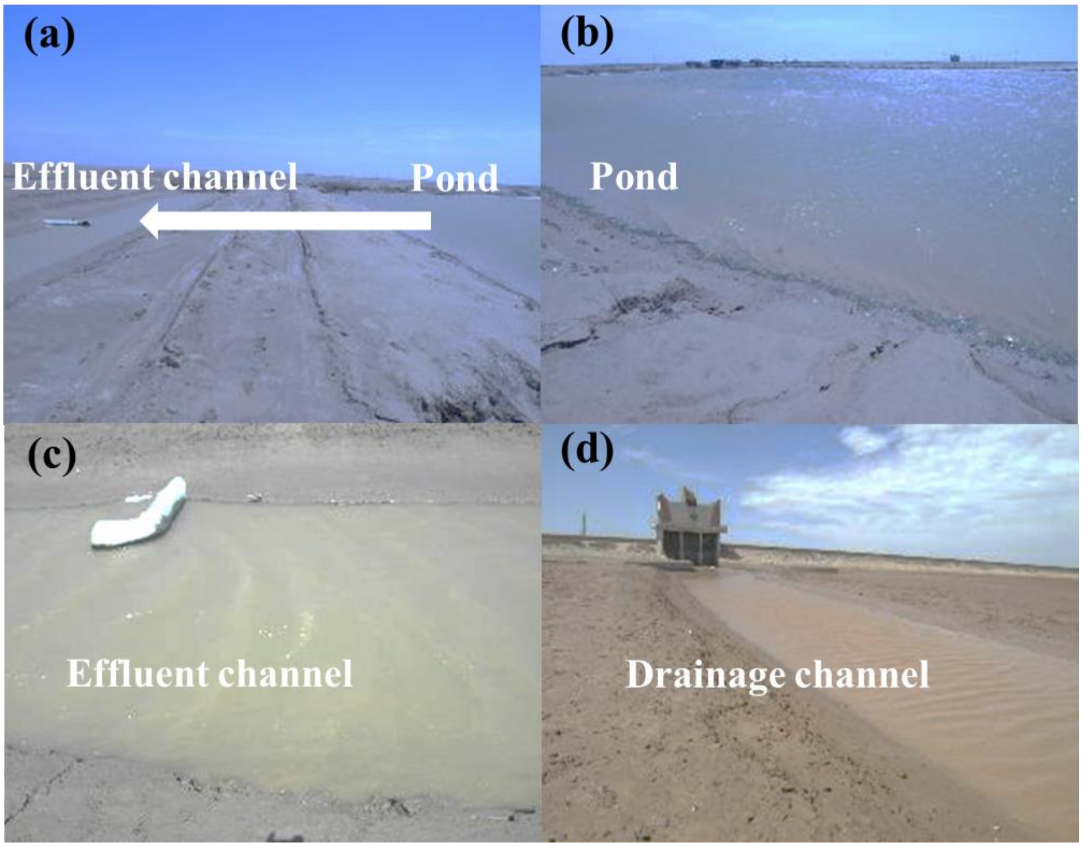

Fig. 3.2. Photos of shrimp farming area showing shrimp pond (b), shrimp pond and its effluent channel (a, c), water gate and adjacent S-tidal flat (d).

3.2 Materials and methods

3.2.1 Study area

This study was conducted in the south and north part (37°30′–38°10′ N, 118°40′–119°00′ E) of the Yellow River estuary in Shang Dong Province, China (Fig. 3.1a, b). The study site is characterized by a warm-temperate continental monsoonal climate, with mean annual temperature and annual mean precipitation of 11.9 ^{o}C and 640 mm, respectively (Gao et al., 2014).

The shrimp ponds were constructed with 3 to 6 ha in size and 1.5 to 2 m in depth (Figure 3.2). *Penaeus japonicas* (juvenile) were stocked at a density of 25–35 individuals m^{-2}. At the early growth stage, seaweed and gammarid were used to feed shrimp. Commercial feed (fish meal 30%, soy flour 20%, peanut flour 35% and wheat flour 15%) was added to ponds 1–2 times daily (with inputs increasing as shrimp grow/ satisfy the nutritional requirements of the increased biomass of shrimp) in the late growth stage. Air blowers or aerators are not used in most shrimp farms in China. Water quality was improved just by water exchange (Biao and Yu, 2007) which was managed uniformly twice a month when spring tide occurs. Pond influent and effluent water were controlled by the same sluice gate from the adjacent tidal creeks.

The shrimp ponds were positioned on the fringe of an extensive tidal flat (S-tidal flat) dominated by salt marsh plant *Suaeda salsa* and two widely distributed macrobenthic species, *Helice tridens* and *Macrophthalmus abbreviates*. The studied creek was separated from the shrimp ponds by an 8 m width road and connected by a sluice gate. It was not influenced by other anthropogenic activities except for shrimp farming discharges which allowed biogeochemical indicators to be specifically related to farming discharge.

To eliminate potential influence of aquaculture activities, the control creek in Natural reserve area (N-tidal flat, non-farming area) was strictly selected with similar natural characteristics (such as sediment type, tidal characteristics, and elevation).

Table 3.1 Chemical items analyzed in the present study and their abbreviations.

Analyzing items	Water	Sediment
Particulate organic matter		
in the pond area	$\mathrm{POM}_{(\mathrm{pond})}$	
in the tidal flat area	$\mathrm{POM}_{(\mathrm{tidal\ flat})}$	
in the coastal area	$\mathrm{POM}_{(\mathrm{marine})}$	
Total organic carbon	$\mathrm{TOC}_{(\mathrm{POM})}$	$\mathrm{TOC}_{(\mathrm{S})}$
Total nitrogen	$\mathrm{TN}_{(\mathrm{POM})}$	$\mathrm{TN}_{(\mathrm{S})}$
Carbon to nitrogen ratio	$\mathrm{C{:}\ N\ ratio}_{(\mathrm{POM})}$	$\mathrm{C{:}\ N\ ratio}_{(\mathrm{S})}$
Stable carbon isotope ratio	$\delta^{13}\mathrm{C}_{(\mathrm{POM})}$	$\delta^{13}\mathrm{C}_{(\mathrm{S})}$
Stable nitrogen isotope ratio	$\delta^{15}\mathrm{N}_{(\mathrm{POM})}$	$\delta^{15}\mathrm{N}_{(\mathrm{S})}$
Nitrate	$\mathrm{NO_3}^{-}{}_{(\mathrm{water})}$	$\mathrm{NO_3}^{-}{}_{(\mathrm{S})}$
Nitrite	$\mathrm{NO_2}^{-}{}_{(\mathrm{water})}$	
Ammonium	$\mathrm{NH_4}^{+}{}_{(\mathrm{water})}$	$\mathrm{NH_4}^{+}{}_{(\mathrm{S})}$
Phosphate	$\mathrm{PO_4}^{3-}{}_{(\mathrm{water})}$	$\mathrm{AP}_{(\mathrm{S})}$
Acid volatile sulfides		AVS

3.2.2 Field sampling and laboratory procedures

Field sampling was conducted at the early stage of shrimp farming (juvenile shrimp) from May 1 to May 16, 2016. There were three sampling areas including shrimp pond area, S-tidal flat area, and N-tidal flat area. In the shrimp pond area, two stations (Fig. 3.1c) were positioned in the shrimp pond and effluent channel, respectively. Other two gradient-based sampling were set up in S-tidal flat and N-tidal flat. Six stations (Fig. 3.1c) in the S-tidal flat were set at 0 m, 50 m, 100 m, 200 m, 500 m and 800 m distances from sluice gate of a tidal channel using an electronic distance meter (Nikon COOLSHOT 40i Laser Rangefinders) and a GPS receiver (Garmin GPS map64s). Four stations in the N-tidal flat (Fig. 3.1d) were set at 0 m, 50 m, 100 m and 200 m distances from the reclaimed land. Other two stations (SM and NM) were established in the coastal area of S-tidal flat and N-tidal flat (Fig. 3.1c, d) for water analysis (organic analysis only).

At each station, both water and sediment samples were collected for analyses of TOC, TN, C: N ratio, δ^{13}C and δ^{15}N, and dissolved inorganic nutrients (NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-}/ AP). The chemical items analyzed in this study were shown with their abbreviations in Table 3.1. All of these samplings were conducted in the ebb tides. The analysis of chemical items followed the same methods in Chapter 2.

3.2.3 Statistical analysis

All the statistical analysis was conducted using R V3.3.1 for Windows.

Multiple comparisons of each environmental variable between stations were carried out to detect spatial differences between farming and non-farming areas or among stations with a gradient distance to the aquaculture pond. All environmental data were tested for normal distribution (Shapiro–Wilk) and homoscedasticity (Bartlett test). Differences of C: N, δ^{13}C and δ^{15}N data between stations were tested using ANOVA followed by Bonferroni post hoc test (Rice, 1989). Differences of C: N, δ^{13}C and δ^{15}N data between stations were tested using Kruskal-Wallis test (Kruskal and Wallis, 1952) followed by Gao post hoc test (Gao et al., 2008; Konietschke et al., 2015). Statistical significance level was considered at 0.05. Statistical analyses were performed using the function Wilk-Shapiro test, Bartlett’s test, Bonferroni test and Kruskal test of the R package stats and the function Gao of the R package nparcomp (Ihaka and Gentleman, 1996; Shirley, 1977) and oneway_test function of the R package coin (Ihaka and Gentleman, 1996).

Stable isotopes of primary producers (food sources) and consumers were initially investigated using biplot diagrams. Because no differences in stable isotope composition of consumers and primary producers were found along the distance gradient in N-tidal flat and S-tidal flat, each of them was calculated together in each tidal flat. A *t*-test was conducted to test significant differences in stable isotope values of consumers and primary producers. The Bayesian stable isotope mixing model, SIAR v 4.0 (Stable Isotope Analysis in R) (Parnell et al., 2010) was used to determine proportional contributions of potential food sources to dominant consumers. Contributions of dietary sources were represented as mean and 95% credibility interval (Ci). The fractionations $\delta^{13}C$ and $\delta^{15}N$ for crustacean (muscle, acid treated) calculated from raw data presented in Yokoyama et al. (2005) were $\delta^{13}C = 1.93 \pm 0.40‰$, $\delta^{15}N = 3.97 \pm 0.49‰$, respectively.

3.3 Results

3.3.1 Water biogeochemical characteristics

(1) TOC and TN content of POM in water

$TOC_{(POM)}$ and $TN_{(POM)}$ contents differed significantly among stations (Figs3.3. 1a, b; Kruskal-Wallis, df = 10, $p < 0.05$). The only pond had significantly higher $TOC_{(POM)}$ and $TN_{(POM)}$ contents than those in NM station. All other stations were similar to each other (GAO post hoc test, $p > 0.05$). Decreasing trends of $TOC_{(POM)}$ and $TN_{(POM)}$ were found at the pond to 0 m station. Increasing trends were detected from 0 m to 800 m stations. Large range of standard deviations was found in the S-tidal flat stations (from 50 m to 800 m).

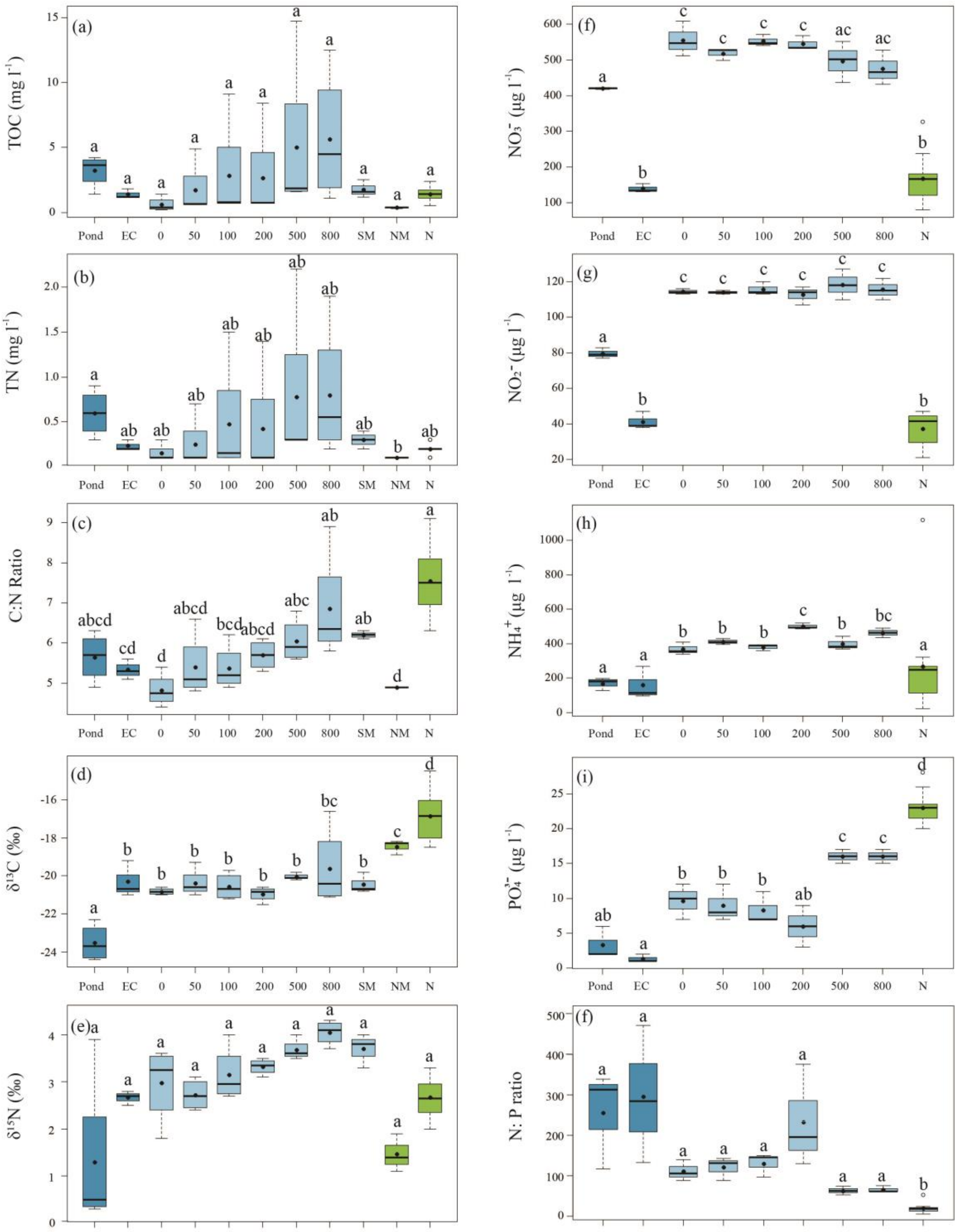

Fig. 3.3. Comparisons of water parameters including TOC (a), TN (b), C: N ratio (c), δ^{13}C (d), δ^{15}N (e) of and NO_3^- (f), NO_2^- (g), NH_4^+ (h), PO_4^{3-} (i) and N: P ratio (f). Different letters above bars indicate significant differences ($p < 0.05$) by Bonferroni test (C: N ratio, δ^{15}N, NO_3^-, NH_4^+) and Gao test (TOC, TN, δ^{13}C, NO_2^-, PO_4^{3-}, N: P ratio). Boxes represent 25%–75% percentiles; range bars represent the 5% and 95% percentiles, solid dots in the boxes represent mean values, hollow dots represent values outside of 95%.

(2) C: N ratios and stable isotope signatures of POM in water

C: N ratios$_{(POM)}$ varied significantly among stations (Fig.3.3c; Kruskal-Wallis, df = 10, $p < 0.05$). Pond (5.6 ± 0.6) had similar C: N ratio$_{(POM)}$ with S-tidal flat and N-tidal flat stations. C: N ratio$_{(POM)}$ in 0 m station (4.8 ± 0.4) and NM station (4.9) showed significant lower values than stations in 500 m (6.1 ± 0.5), 800 m (6.9 ± 1.4), SM station (6.2 ± 0.1) and N-tidal flat (7.5 ± 0.8). A decreasing trend was detected from pond to 0 m station, and an increasing trend was found from 0 m to 800 m stations.

$\delta^{13}C_{(POM)}$ and $\delta^{15}N_{(POM)}$ values of water POM varied significantly among stations (Fig. 3.3d; ANOVA, $p < 0.01$). Pond (-23.5 ± 1.0‰) had the lowest $\delta^{13}C_{(POM)}$ signatures. No significant differences were found among stations in the S-tidal flat, but they were significantly lower than that in the N-tidal flat. $\delta^{15}N_{(POM)}$ value measured in the pond was 1.3 ± 1.7‰ with a large range of standard deviations. All stations in the S-tidal flat were similar to each other. Stations in the farming area were detected with relatively lower $\delta^{13}C_{(POM)}$ values and higher $\delta^{15}N_{(POM)}$ values than those in the non-farming area.

(3) Dissolved inorganic nutrients in water

Dissolved inorganic nutrients in water (Figs. 3.3f-i) varied significantly among stations (NO_3^-, NH_4^+ and PO_4^{3-}: Kruskal-Wallis, df = 8, $p < 0.01$; NO_2^-: ANOVA, $p < 0.01$). Nutrient concentrations were considerably variable in the effluent creek over the three surveys, whereas concentrations in the influent creek were relatively uniform. Highest nutrient concentrations were recorded in the effluent creek when the ponds were full, standard errors indicating the large range of values recorded between sites within the creek (Costanzo et al., 2004).

$NO_3^-{}_{(water)}$, $NO_2^-{}_{(water)}$ and $NH_4^+{}_{(water)}$ concentrations were found highest in S-tidal flat stations with relatively consistent values, respectively, then followed by the pond and effluent channel. NO_3^- and NO_2^- concentrations in the N-tidal flat were similar to the effluent channel; they were significantly lower than the pond and S-tidal flat. While $NH_4^+{}_{(water)}$ levels in the pond, effluent channel and N-tidal flat were similar to each other (GAO post hoc test, $p > 0.05$). NO_3^- was the dominant nutrient species measured in the shrimp farming area. Moreover, NH_4^+ was the dominant nutrient species in the non-farming area. $PO_4^{3-}{}_{(water)}$ concentration was significantly highest in N-tidal flat, then followed by S-tidal flat, pond and effluent channel. The more distant stations (500 m and 800 m) in S-tidal flat showed higher $PO_4^{3-}{}_{(water)}$ concentrations than the adjacent stations (from 0 m to 200 m).

N: P $\text{ratio}_{(water)}$ in pond (256 ± 121: 1) and effluent channel (297 ± 169: 1) were significantly higher (Fig. 3.3j) than S-tidal flat (99 ± 35: 1; except for 200 m station) and N-tidal flat (20 ± 12: 1). N: P $\text{ratio}_{(water)}$ in the N-tidal flat was near to the red field ratio 16: 1. Likewise, 500 m (64 ± 10: 1) and 800 m (66 ± 8: 1) stations in S-tidal flat were detected lower values than the stations adjacent to the slice gate in the S-tidal flat (range from 89: 1 to 376: 1).

3.3.2 Sediment biogeochemical characteristics

(1) TOC and TN contents in sediment

$TOC_{(S)}$ and $TN_{(S)}$ contents in sediment varied significantly among the stations (Figs. 3.4a, b; Kruskal-Wallis, df = 8, $p < 0.001$). Pond, effluent channel and S-tidal flat (except for 800 m) had significantly higher $TOC_{(S)}$ and $TN_{(S)}$ contents than those in the N-tidal flat (GAO post hoc test, $p < 0.05$). $TOC_{(S)}$ in the pond was similar to the effluent channel, 50 m, and 800 m stations, but lower than the remaining stations in the S-tidal flat. $TN_{(S)}$ in pond showed no significant differences with the effluent channel and S-tidal flat (except for 800 m). Highest $TOC_{(S)}$ and $TN_{(S)}$ concentrations were detected around 100 m and 200 m stations with large ranges of standard deviations. Decreasing trends of $TOC_{(S)}$ and $TN_{(S)}$ contents were detected from 200 m to 800 m stations in the S-tidal flat.

(2) C: N ratios and stable isotope signatures of OM in sediment

C: N $\text{ratios}_{(S)}$, $\delta^{13}C_{(S)}$ and $\delta^{15}N_{(S)}$ values in sediment differed significantly among stations (C: N ratios and $\delta^{13}C_{(S)}$: Kruskal-Wallis, df = 8, $p < 0.001$; $\delta^{15}N$: ANOVA, $p < 0.001$). C: N $\text{ratio}_{(S)}$ of pond showed no significant difference with the effluent

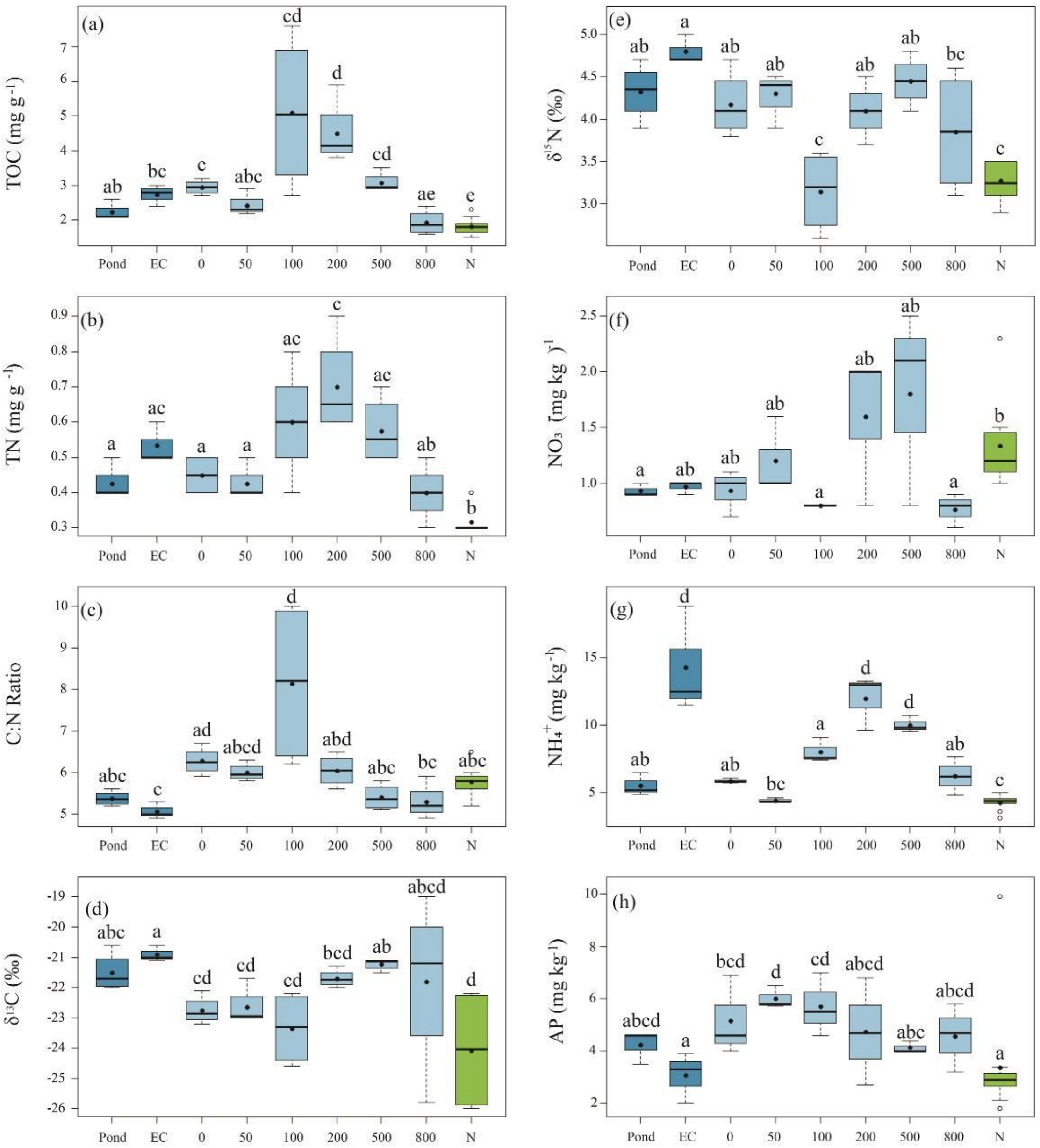

Fig. 3.4. Comparisons of sediment parameters including TOC (a), TN (b), C: N ratio (c), δ^{13}C (d), δ^{15}N (e) of and NO_3^- (f), NH_4^+ (g) and AP (h). Different letters above bars indicate significant differences ($p < 0.05$) by Bonferroni test (δ^{15}N, AP) and Gao test (TOC, TN, C: N ratio, δ^{13}C, NO_3^-, NO_2^-, NH_4^+). Boxes represent 25%–75% percentiles; range bars represent the 5% and 95% percentiles, solid dots in the boxes represent mean values, hollow dots represent values outside of 95% confidences interval.

channel, S-tidal flat (except for 100 m stations) and N-tidal flat (Figs. 3.4c). 100 m station with large standard errors showed the highest C: N ratio$_{(S)}$. $\delta^{13}C_{(S)}$ and $\delta^{15}N_{(S)}$ values (Figs. 3.4d, e) in pond showed no significant differences from the effluent channel and S-tidal flat (except for $\delta^{15}N_{(S)}$ in 100 m) stations, but significantly differed from those in the N-tidal flat.

(3) Dissolved inorganic nutrients in sediment

$NO_3^-{}_{(S)}$, $NH_4^+{}_{(S)}$ and $AP_{(S)}$ contents in the sediment differed significantly among stations (Kruskal-Wallis, df = 8, $p < 0.05$;). For $NO_3^-{}_{(S)}$ concentrations (Fig. 3.4f), only pond, 100 m and 800 m stations were found significant differences with the N-tidal flat. All other stations were similar to each other. The highest $NO_3^-{}_{(S)}$ concentrations were found around 200 m and 500 m stations in the S-tidal flat with an extensive range of standard deviations.

$NH_4^+{}_{(S)}$ contents in the pond, effluent channel and S-tidal flat (except for 50 m) displayed significantly higher concentrations than that in the N-tidal flat. $NH_4^+{}_{(S)}$ concentrations in effluent channel, 200 m and 500 m stations were considerably greater than that in the pond and other stations in the S-tidal flat. A decreasing trend of $NH_4^+{}_{(S)}$ contents was found from 200 m to 800 m stations. As for $AP_{(S)}$ contents, pond showed no significant differences from effluent channel, S-tidal flat and N-tidal flat. No significant differences were found within stations in S-tidal flat except for 50 m and 500 m stations. 0 m, 50 m, and 100 m stations had significantly higher $AP_{(S)}$ values effluent channel and N-tidal flat.

3.4 Discussion

This study represented one of the few comprehensive evaluations of shrimp farming activities on wetland environment. The selected indicators employed in this study served a purpose in establishing and understanding of various influences on the environment. It is evident that farm waste is moving outside and influencing sites within the influent creek.

3.4.1 *Organic nutrients in water and sediment*

Organic parameters showed measurable changes in water and sediment induced by shrimp farming waste and natural variations. Although $TOC_{(POM)}$ and $TN_{(POM)}$ were found no noticeable differences between farming and non-farming stations, they showed uniform tendencies with C: N $ratio_{(POM)}$. Decreasing values from pond to 0 m might reflect a reduction of shrimp pond waste, and increasing values from 0 m to 800 m station are likely due to natural environmental variation in the S-tidal flat. Large standard errors of stations from 50 m to 800 m indicated the large range of values recorded between sites within the creek.

C: N ratios detected in pond, 50 m, 100 m, 200 m, 500 m, 800 m and SM were close to the Redfield ratio (6.6) which inferred phytoplankton was the primary suspended matter source in those stations (Herbeck et al., 2013; Jackson et al., 2003). This result might indicate that the main contributor of POM in these stations was marine phytoplankton. The relatively higher values of C: N ratios detected in N-tidal flat might result from a mixture of marine phytoplankton and other organic matter sources. The comparatively low C: N ratio in 0 m station might be attributed to the contribution of marine zooplankton with low C: N ratio. $\delta^{13}C_{(POM)}$ values detected in shrimp pond was lower than those in S-tidal flat and N-tidal flat stations, which reflect the different composition of POM in water. One reason might be the attributed to the aquaculture waste which will cause the alternation of phytoplankton communities composition (Primavera, 2006) in the pond. Besides, as shrimp farmers commonly use commercial feeds that mainly composed of the terrestrial plant (soy and peanut) and seaweed flour (seaweed cultured in offshore) with low $\delta^{13}C$ values. As a result, the $\delta^{13}C_{(POM)}$ value of shrimp pond water will be reduced by the allogenous feed sources with low values. In addition, relatively lower $\delta^{15}N$ mean value found in shrimp pond might indicate the impact of aquaculture feed with low $\delta^{15}N$ value. The large standard error which made the $\delta^{15}N$ value in pond not significantly different from those in the S-tidal flat. The high variability of $\delta^{15}N_{(POM)}$ in the pond might be attributed to the high nutrient concentrations of DIN in the pond which made a high $\delta^{15}N$ value. This result was also comparable with other studies of shrimp farm effluent (Costanzo et al., 2004; Jones et al., 2001; Lin and Fong, 2008; Preston et al., 2000) that produce a $\delta^{15}N$ signature of approximately 4.2–6‰.

Sediments in the farming area (except for 800 m stations) with higher $TOC_{(S)}$ and $TN_{(S)}$ contents than N-tidal flat， might indicate the additional contribution of organic matter (OM)-rich pond effluent. Moreover, 800 m station with relatively lower values indicated a less disturbed condition similar to N-tidal flat.

However, combined with the result of C: N $ratios_{(S)}$ and isotopic composition, OM-rich sediment in the S-tidal flat were considered not affected by pond effluent as no unified grading trend were found from the shrimp pond to the downstream tidal creek. For instance, the effluent channel showed similar C: N $ratio_{(S)}$, $\delta^{13}C_{(S)}$ and $\delta^{15}N_{(S)}$ values with pond, but differed significantly from S-tidal flat (except for $\delta^{15}N_{(S)}$). Hence, the effluent channel had similar SOM origin with pond. However, S-tidal flat with OM-rich sediment might not contain origin from aquaculture waste but the result of natural variation in a tidal flat which has high organic loading originating from saltmarsh and seaweed terrestrial debris. However, it was difficult to show in this study whether the variation was caused by shrimp farming indirectly.

Hence, high $\delta^{13}C_{(POM)}$ values detected in shrimp pond water was lower than that in sediment. However, the $\delta^{15}N_{(S)}$ values in water although it showed relatively lower mean values, the broad range of standard error might reflect the influence of high nutrients concentrations in the pond, as a result of high $\delta^{15}N_{(S)}$ values. In general, no matter water or sediment in the adjacent S-tidal flat was considered not impacted by aquaculture solid waste, but the dissolved inorganic nutrients were difficult to judge whether high concentrations of nutrients were caused by aquaculture effluent or the large scale nutrient enrichment.

3.4.2 Dissolved inorganic nutrients

In general, water nutrients in both shrimp farming and non-farming area showed high concentrations and exceeded the maximum allowed concentrations of aquaculture water in China. The reason may be due to causes related to the eutrophication, which was widely present in the Yellow River estuary (Kang and Xu, 2016; Zhang et al., 2008).

The most notable observation was the high nitrogen nutrients and N: P ratio, but low $PO_{4(water)}^{3-}$ in shrimp farming area compared to those in the N-tidal flat. Most of DIN was in the form of nitrate in shrimp farming area, and ammonium in the non-farming area might result from different nutrients input. However, the dominant nutrient in shrimp farming area was same to the previous studies that the coast water of Yellow River estuary was nitrate-dominated (Gong, 2012; Zhang et al., 2008). Thus, N-tidal flat in the Natural Reserve area might also influence by anthropogenic activities with the changed nutrient species.

Some early studies showed that dissolved nutrient contents are not elevated in mangrove tidal creeks that receiving shrimp farm effluent (McKinnon et al., 2002b), and no significant difference of dissolved nutrients was found between farming and non-farming estuaries (Trott and Alongi, 2000). Conversely, In the meta-analysis of the influence of aquaculture on water nutrients, Sarà (2007) demonstrated large effect sizes of shrimp aquaculture in mixed waters on ammonium and nitrite. It was also suggested by other studies (Bouwman et al., 2013; Herbeck et al., 2013) that ammonium was the dominant nutrient species in shrimp effluent creek. Although ammonium in the study area was not the dominant species, it still showed high concentrations following nitrite closely. Hence, it was not possible to detect the impacts of aquaculture on certain nutrient species in the adjacent environment by spatial comparison. More useful indicators are needed.

The dissolved inorganic nutrients concentrations in the S-tidal flat were much higher than those in shrimp pond and effluent channel. The high concentration in S-tidal flat might because that in the early growth stage pond, many seaweeds gammarids were used as natural feeds would uptake nutrients for growth. As a result, the concentrations of dissolved inorganic nutrients in the pond were reduced. Uptake of dissolved N by plankton may also explain the absence of significant difference between estuaries in any of the dissolved N species (DIN, DON) (Trott and Alongi, 2000).

Besides, the $PO_4^{3-}{}_{(water)}$ concentrations in the pond is remarkably lower (Costanzo et al., 2004) than those in N-tidal flat and highly variable within stations (Trott and Alongi, 2000). The low value even below the nutrient concentrations of national water quality for protection area and aquaculture area. Based on the previous studies, one possible reason might lie in that shrimp has relatively low execration of phosphorus than nitrogen, and as Sarà (2007)suggested that phosphorus not be influenced by shrimp cases regarding mixed waters. Besides, the low pH and the abundance of iron and aluminum ions induced by aquaculture waste also result in phosphorus precipitation (Poer-nomo and Singh 1982). Thereby lowering the concentration in water, as a result of high contents of DIN, but low contents of phosphorus that lead to the extremely high N: P ratio in shrimp pond and effluent channel, which indicated a strong P limitation (Costanzo et al., 2004). As a result, it will limit the natural food production (algal growth) and change the phytoplankton community composition within the pond too.

Nutrients in sediment among stations did not indicate any obvious sedimentation of particulate material from shrimp effluent. $NH_4^+{}_{(S)}$ of sediment in the effluent channel with highest concentrations reflected the high accumulation of ammonium. Besides, $NO_3^-{}_{(S)}$ and $AP_{(S)}$ contents in pond showed no significant differences with stations in S-tidal flat, indicating that the sediments in the S-tidal flat were not noticeably affected by shrimp farming.

3.4.3 Implications for operation and management of shrimp farming

According to the results of water and sediment biogeochemical analysis, the tidal flat adjacent to the shrimp pond farming area was considered not affected by aquaculture effluent at the early growth period. This result might be attributed to the operation and management of shrimp pond. First, shrimp ponds were constructed with a large size and low density (25 individuals m^{-2}) that even no need to use the aerators for oxygen exchanging. It can reduce the adverse impact on the water and sediment environment. Second, the feed supply at the early growth stage is mainly natural food like gammarids and seaweeds which contributed low nitrogenous excretion and had less adverse effects on water and sediment quality compared to artificial diets. Third, artificial feed for shrimp is composed of wheat flour (15%), soybean meal (20%), peanuts powder (35%) and fewer protein feeds (fishmeal:15%) that reduced the accumulation of organic matter from high protein feed and mitigate the pressure from using capture fishes meal. Fourth, some effluent water of shrimp ponds in high salinity was used to produce salt in adjacent saltern with a special pipeline connected, which contribute to the reduction of pollutant discharge on adjacent environment. Last but the most important is the discharge design of shrimp pond effluents. Shrimp ponds were managed uniformly that they were connected by internal pipes and gathered waste water in several normal large channels to control drainage by valves, most parts of farming waste would deposit on their long way to the adjacent tidal flat. Moreover, the tidal creeks here is not like mangrove forests where water can stand for a long deposition time. Hence, the detected distance of discharged waste from shrimp ponds was limited in the adjoining effluent channels.

In conclusion, the detected environmental parameters confirmed shrimp farming activities in the Yellow River estuary did not affect the water and sediment environment in the adjacent wetland.

For sustainable aquaculture industry consideration, feeding management (Herbeck et al., 2013) like improving the method of feed supply (Paea-Osuna and others 1998) and nutrient composition (low protein) might be the most direct and efficient methods for reducing pollution in the pond and adjacent environment. Besides, wastewater utilization in the related industry like salt manufacturing, or waste water treatment by settling tank or standardized drainage system (long pipeline design) would also contribute to alleviating the pollution to the local environment.

For environmental monitoring aspect, in the vast scope of eutrophication in the Yellow River Delta, endogenous and exogenous nutrient sources in the aquaculture area should be identified by stable-isotope analysis in the future study. It would be especially critical in ascertaining whether mariculture was a significant and expanding cause of coastal eutrophication (Bouwman et al., 2013) in the Yellow River Delta.

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Chapter 4

Impacts of Aquaculture Waste on Feeding Strategies of Dominant Macrobenthos

4.1 Introduction

Salt marshes as highly productive coastal wetlands that are experiencing global coastal eutrophication (Deegan et al., 2012). Among a series of reasons, mariculture is one of the significant and expanding causes of nutrient enrichment (Bouwman et al., 2013). The salt marshes that receive aquaculture effluents, often resulting in water quality deterioration and sediment alternation. Macrobenthos as an important component of the estuarine and coastal ecosystem, are sensitive to the environment change. Some studies have reported that aquaculture farming waste can drastically modify the density and biodiversity of benthic faunal communities in relatively oligotrophic near-shore habitat. The diet changes of macrobenthos that utilizes food sources from the land and the sea can be used to judge and reflect the environment change induced by aquaculture organic waste which contains a large amount of uneaten feed. The similar modifications of food composition of dominant benthic animals by aquaculture were confirmed in previous studies of different aquaculture activities, such as fish farming (Mazzola and Sarà, 2001; Yokoyama and Ishihi, 2007) and shrimp pond farming (Kon et al., 2009). The macrobenthos who feed on the fish farm waste (δ^{15}N-enriched fish meal and δ ^{13}C-reduced cereals) were observed with enriched δ^{15}N and depleted δ^{13}C values in contrast to the same species samples collected outside of the farm area (Yokoyama and Ishihi, 2007).

Attracted by the high demand for seafood, aquaculture ponds with high operabilities and profitabilities has tremendously expanded along the coastal zones (Burford and Williams, 2001; Primavera, 1997). The effluents released from the aquaculture ponds contains a significant amount of suspended solids, dissolved nutrients which will change the resource subsidies in the recipient wetland. Kon et al. (2009) found that the shrimp farming organic waste had higher contributions to the deposit feeder crabs inhabit in the mangrove near the shrimp farm than those no shrimp farm around. However, there is no investigation comparing the effects of different aquaculture farming waste on the food availability for macrobenthos in the tidal flat.

In this study, the stable isotope analysis was used to investigate the nutritional sources of the macrobenthos. This approach has been universally recognized by previous studies to trace and verify the origin, extent, and fate of the nutrients sources in the environment and ecosystem (Andrews et al., 1998; Sakamaki et al., 2010). Besides, they were especially emphasized as highly informative descriptors of heterotrophic loadings from aquaculture, such as fish farming waste on the adjacent environment

The main purposes of this study were to (1) examine if stable isotope signatures of consumers and producers show responses to the aquaculture effluent; (2) detect differences in feeding strategies of dominant macrobenthos between farming and non-farming areas or different aquaculture activities. We hypothesized that significant isotopic differences exist for contents of consumers or primary producers by aquaculture activities with varying degrees. Besides, stable isotopes serve as an effective tool for revealing the source and fate of aquaculture induced organic matters in the environment and food webs.

4.2 Materials and methods

4.2.1 Study area

The study was conducted in the Yellow River estuary (37°30′–38°10′ N, 118°20′–119°00′ E) (Fig. 4.1a) which is located in the northeast of Shandong Province, China. The study site is characterized by a warm-temperate continental monsoonal climate, with mean annual temperature and annual mean precipitation of 11.9 ºC and

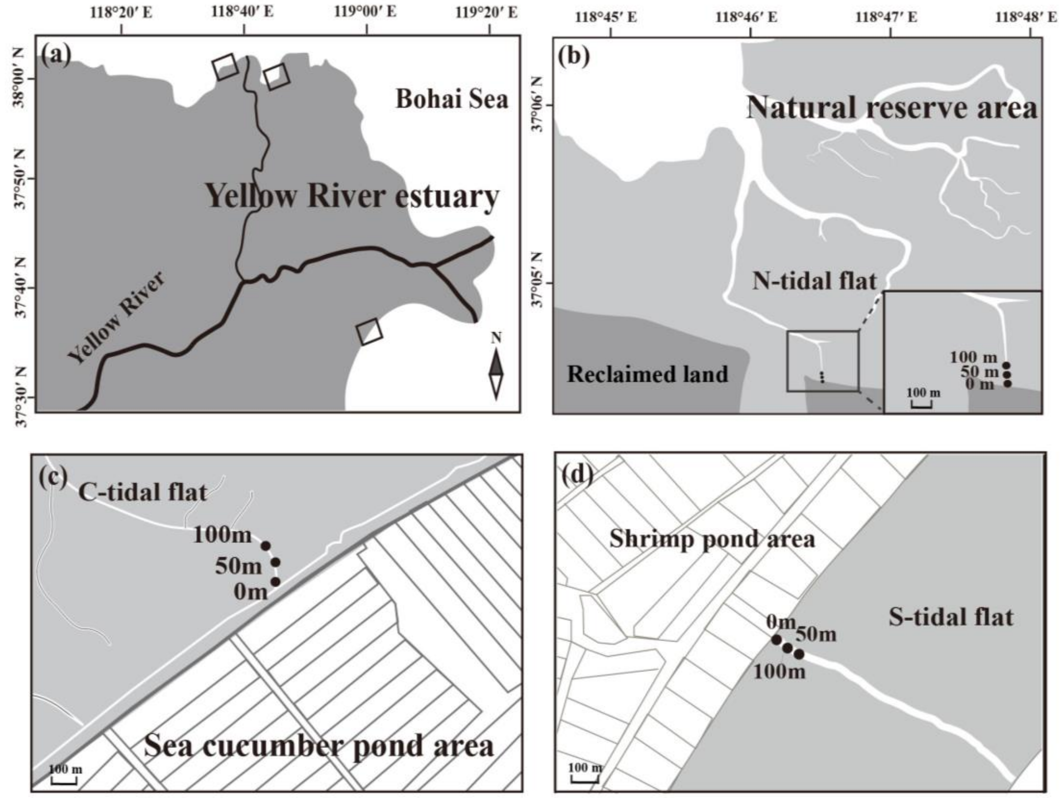

Fig. 4.1 Study area (a) and sampling locations in three areas (b, c and d). Natural reserve area (b): 0 m, 50 m and 150 m distance sampling stations in the N-tidal flat. Sea cucumber farming area (c): 0 m, 50 m and 150 m distance sampling stations in the C-tidal flat. Shrimp farming area (d): 0 m, 50 m and 150 m distance sampling stations in the S-tidal flat.

640 mm, respectively (Gao et al., 2014).

4.2.2 Field sampling and laboratory procedures

Three tidal flat areas were selected as the survey area. One tidal flat was (C-tidal flat) adjacent to the sea cucumber farming area, and another was adjacent to the shrimp farming area. Both tidal flats received farm effluent and were separated from the farming pond by a 7 to 8 m roads. Another tidal flat (N-tidal flat) in the Natural reserve area (non-farming area) was selected for the reference site. All of the sampling tidal flats were strictly considered with similar natural characteristics (such as sediment type, tidal characteristics, elevation). Three tidal flat areas were all dominated by salt marsh plant *Suaeda salsa* and two macrobenthic species, *Helice tridens* and *Macrophthalmus abbreviates* which are widely distributed in this locality.

Field sampling was conducted in the C-tidal flat, S-tidal flat and N-tidal flat from May 1 to May 21, 2016. In the C-tidal flat and S-tidal flat, there were no other allochthonous nutrients loading except aquaculture effluent. Two gradient-based sampling were set up in C-tidal flat, S-tidal flat and N-tidal flat. Three stations (Fig. 4.1c) were set respectively in each tidal flat creek with 0 m, 50 m and 100 m distances from the sluice gate (farming area) or reclaimed land (non-farming area) using an electronic distance meter (Nikon COOLSHOT 40i Laser Rangefinders) and a GPS receiver (Garmin GPS map64s). Two stations were set in the sea cucumber pond and shrimp pond for collecting particulate organic matter (POM) in the pond ($\mathrm{POM}_{\mathrm{(pond)}}$). Other three stations (CM, SM, and NM) were set in the coastal area of C-tidal flat, S-tidal flat and N-tidal flat (Fig. 4.1c, d) for the analysis of marine POM ($\mathrm{POM}_{\mathrm{(marine)}}$).

Samples for stable isotope analysis were collected from consumers (dominant crabs) and primary producers (salt marsh plant, benthic microalgae, terrestrial debris and particulate organic matter from marine, tidal flat and pond). At each sampling station, the date, location (latitude and longitude) were recorded.

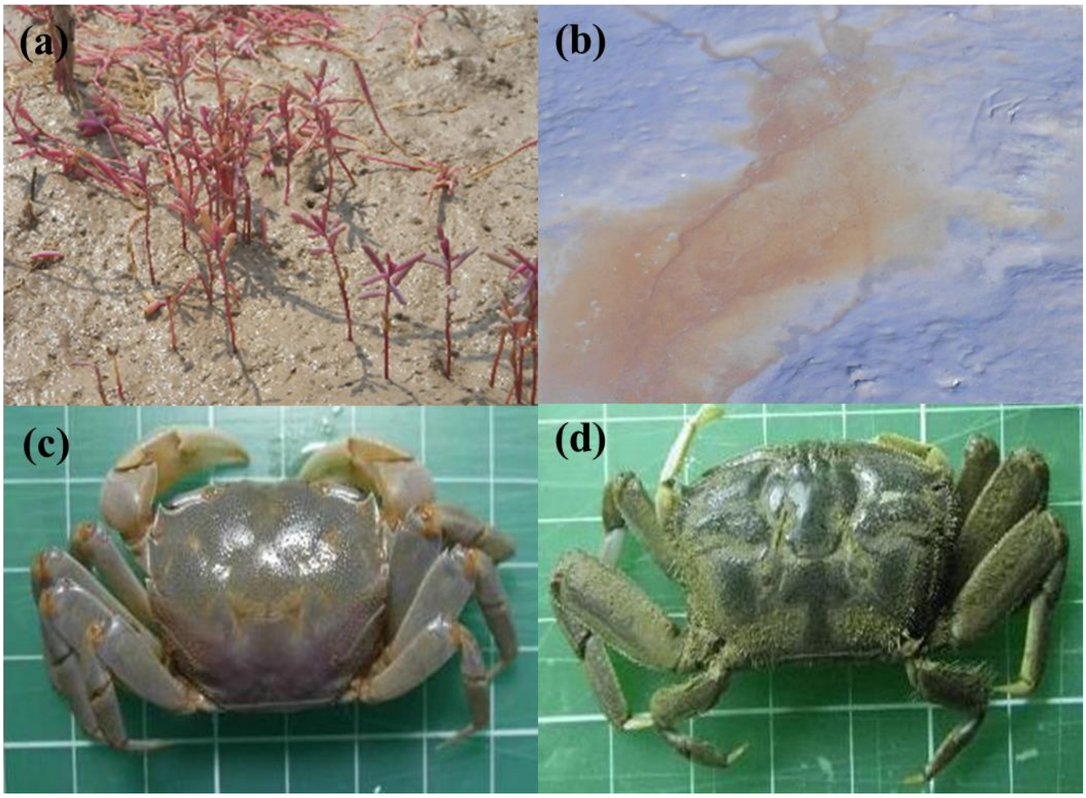

Fig. 4.2. Photos of dominant salt marsh plant *Suaeda salsa* (a), *benthic microalgae* (b), dominant macrobenthos species *Helice tridens* (c) and *Macrophthalmus abbreviates* (d) in each tidal flat.

The dominant macrobenthic animals (two crab species) were caught by a long-handled scoop around the sampling station within 5 m distance. Non-dominant macrobenthic animals and terrestrial debris were collected by excavating sediment with an area of 15×15 cm^2，25cm depth, washed through a 1mm mesh sieve in water to remove silt, clay and sand from the sample. Animal samples were then preserved on ice and returned to the laboratory for sorting and sampling. Muscle tissue of crab, whole body of the non-dominant microbenthic animal were taken for animal isotope samples.

The dominant salt marsh plant *Suaeda salsa* was collected by hand. Leaves from 10 plants were taken as a single sample. Each sample was then preserved on ice and returned to the laboratory for sorting and sampling.

All animal and plant samples were first treated with 1.2 N HCl to remove carbonates and then oven dried at 60 ^{o}C and ground to fine powder for isotope analysis.

Benthic microalgae (BMA) samples (n=3) were extracted from the surface sediment in each tidal flat (Couch, 1989; Riera and Richard, 1996). The sediment was taken back to the laboratory within 30 min and then spread on a flat tray (1 cm depth) and covered by a nylon screen (63 μm mesh). A 4 to 5 mm layer of combusted sand (acid-washed and burned, 125–500 μm) was spread over the nylon screen. The sand was kept wet using filtered seawater and then cultured under fluorescent light for 12 to 15 h. The top 2 mm of sand was then removed and filtered through a 63 μm fiber sieve by washing with distilled water. Water containing the microalgae was filtered onto GF/F (φ25 mm) filters, and oven dried at 60 ^{o}C for stable isotope analysis.

At each station, three water samples were collected by a bucket from the surface layer of the water for the analyses of particulate organic matter (POM). Besides, marine POM ($POM_{(marine)}$) in CM and NM stations were collected at the sea surface of each adjacent offshore. POM samples were collected by filtering 0.4 to 3 L surface seawater through a 125 μm mesh (to remove zooplankton) followed by glass-fiber filters (Whatman GF/F, φ47 mm). To remove carbonates, POM samples were treated with 1.2 N HCl overnight and then oven dried at 60 ^{o}C for stable isotope analysis.

The elemental and isotope analysis of samples were analyzed using a Delta XP plus isotope ratio mass spectrometer linked to a Conflo IIII interface (Thermo Fisher Scientific, USA) with a Flash EA1112 elemental analyzer

Stable isotope ratios were expressed in the typical estuary notation ($\delta^{13}C$ or $\delta^{15}N$), defined by the following equation:

$$\delta^{13}C \text{ or } \delta^{15}N = (R_{sample}/R_{standard} - 1) \times 10^{3}$$

$$\text{and } R = {}^{13}C/{}^{12}C \text{ or } {}^{15}N/{}^{14}N$$

The standard reference materials were Pee Dee Belemnite (PDB) and atmospheric N^2 for carbon and nitrogen isotopes, respectively.

4.2.3 Data analysis

All the statistical analysis was conducted using R V3.3.1 for Windows.

Carbon and nitrogen stable isotopes of primary producers (food sources) and consumers were initially investigated using biplot diagrams. Because no differences in stable isotope composition of consumers and primary producers were found along the distance gradient in N-tidal flat and C-tidal flat, each of them was calculated together in each tidal flat. A t-test was performed to test significant differences in stable isotope values of consumers and primary producers. The Bayesian stable isotope mixing model, SIAR v 4.0 (Stable Isotope Analysis in R) (Parnell et al., 2010) was used to determine proportional contributions of potential food sources to dominant consumers. Contributions of dietary sources were represented as mean and 95% credibility interval (Ci). The fractionations $\delta^{13}C$ and $\delta^{15}N$ for crustacean (muscle, acid treated) calculated from raw data presented in Yokoyama et al. (2005) were $\delta^{13}C = 1.93 \pm 0.40‰$, $\delta^{15}N = 3.97 \pm 0.49‰$, respectively.

4.3 Results

4.3.1 Sampling results

57 samples of *H. tridens* and 27 samples of *M. abbreviates* were collected as

Table 4.1 Macrobenthos species collected in each tidal flat

Location		Species	δ^{15}N		δ^{13}C		
			Mean	SD	Mean	SD	n
S-tidal flat	1	*Ashiharakani*					2
S-tidal flat	2	*Augulus compressissima*	4.1	3.9	–20.2	7.8	4
S-tidal flat	3	*Etone* sp.					2
S-tidal flat	4	*Heteromastus* sp.					1
S-tidal flat	5	*Lingula unguis*					1
S-tidal flat	6	*Mediomastus* sp.					1
S-tidal flat	7	*Neanthes* sp.	6.4	1.1	–14.5	1.4	40
S-tidal flat	8	*Potamocorbula laevis*					5
C-tidal flat	1	*Augulus compressissima*	2.8	0.8	–12.9	0.8	14
C-tidal flat	2	*Etone* sp.					1
C-tidal flat	3	*G.nibarica*	8.5		–11.9		1
C-tidal flat	4	gammarid					1
C-tidal flat	5	*Glycera* sp.					1
C-tidal flat	6	*Heteromastus* sp.	4.1		–14.2		17
C-tidal flat	7	*Leptomya minuta*	2.2	1.8	–16.5	6.2	21
C-tidal flat	8	*Mediomastus* sp.					1
C-tidal flat	9	*Neanthes* sp.	*9.0*		–12.7		2
C-tidal flat	10	*Nectroneathea oxypoda*	5.6		–12.9		2
C-tidal flat	11	*Prionospio* sp.					1
C-tidal flat	12	*Rissoina bureri*					4
C-tidal flat	13	*Sinonvacula constricta*	2.5		–19.7		1
C-tidal flat	14	*Tabanus bovinus*					3
N-tidal flat	1	*Augulus compressissima*	3.4	1.9	–15.3	3.0	11
N-tidal flat	2	*Etone sp.*					2
N-tidal flat	3	*G.nibarica,*	8.2		–14.4		1
N-tidal flat	4	*gammarid*					1
N-tidal flat	5	*Heteromastus sp.*	5.6	0.1	–15.1	0.4	39
N-tidal flat	6	*L.boschosina*	2.5		–18.4		1
N-tidal flat	7	*Leptomya minuta*	5.0		–13.7		3
N-tidal flat	8	*Neanthes sp.*	5.8	0.3	–15.4	2.2	10
N-tidal flat	9	*Orbiniidae*					2
N-tidal flat	10	*Rissoina bureri*					11
N-tidal flat	11	*Sinonvacula constricta*	2.5	0.2	–19.3	1.3	3
N-tidal flat	12	*Tabanus bovinus*					1

Table 4.2 Carbon and nitrogen isotopic compositions of consumers and their potential food sources in the tidal flat of Natural Reserve Area and sea cucumber pond area

	N-tidal flat		C-tidal flat		S-tidal flat	
Consumers / producers	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Helice tridens	–15.5 ± 3.3	5.8 ± 0.4	–13.0 ± 0.6	4.3 ± 1.1	–17.8 ± 3.2	8.2 ± 0.8
Macrophthalmus abbreviates	–12.3 ± 0.4	3.8 ± 0.9	–12.1 ± 0.6	2.1 ± 0.7	–13.5 ± 1.1	6.6 ± 0.9
POM $_{\text{(marine)}}$	–18.4 ± 0.3	1.5 ± 0.3	–21.1 ± 0.1	2.4 ± 0.3	–20.4 ± 0.4	3.7 ± 0.3
POM $_{\text{(tidal flat)}}$	–16.9 ± 1.2	2.7 ± 0.4	–16.7 ± 1.6	0.9 ± 0.6	–20.4 ± 1.0	3.3 ± 0.6
POM $_{\text{(pond)}}$			–20.6 ± 0.9	–2.0 ± 0.2	–23.5 ± 1.0	1.3 ± 1.7
BMA	–8.1 ± 0.4	2.2 ± 0.4	–10.7 ± 1.5	3.3 ± 0.4	–12.3 ± 0.9	3.3 ± 0.1
Salt marsh plant	–30.4 ± 0.5	6.3 ± 1.5	–29.7 ± 1.3	6.1 ± 0.9	–30.4 ± 0.5	10.4 ± 2.2
Terrestrial debris	–25.8 ± 0.9	0.7 ± 0.4	–17.7 ± 1.6	–0.1 ± 0.4	–25.7 ± 0.6	0.2

The mean ± SD values are given for food items with the number of samples (n) ≥ 3. BMA: benthic microalgae. Different letters ($^{a,\,b}$) on stable isotope values indicate significant difference ($p < 0.05$) by *t*-test.

dominant consumers within the study area (Table 4.1). The carbon and nitrogen stable isotope values of dominant consumers and primary producers were shown in Table 4.2.

4.3.2 Stable isotope signatures

The mean value of δ^{13}C for *H. tridens* was highest in the C-tidal flat (–13 ± 0.6‰) than that in the N-tidal flat (–15.5 ± 3.3‰) and S-tidal flat (–17.8 ± 3.2‰). The δ^{13}C value of *H. tridens* in the N-tidal flat showed a big variation within individuals (Table. 4.1). The mean value of δ^{15}N for *H. tridens* in the S-tidal flat was 8.2 ± 0.8‰, significantly higher than those in the N-tidal flat (5.8 ± 0.4‰) and then followed by the C-tidal flat (4.3 ± 1.1‰). As for the crab *M. abbreviates*, δ^{13}C value in the N-tidal flat was –12.3 ± 0.4‰, similar to that in S-tidal flat (–13.5 ± 0.6‰) and C-tidal flat (–12.1 ± 0.6‰), whereas δ^{15}N values in S-tidal flat (6.6 ± 0.9‰) was significantly higher than those in N-tidal flat (3.8 ± 0.9‰) and C-tidal flat (2.1 ± 0.7‰).

In according with the presumptive trophic shift of crustaceans (1.9 ± 0.4‰ in δ^{13}C and 4.0 ± 0.5‰ in δ^{15}N) presented by Yokoyama et al. (2005), the expected δ^{13}C and δ^{15}N values of the *H. tridens* diet would be –17.4 ± 3.7‰ and 1.8 ± 0.9‰, respectively in N-tidal flat, –14.9 ± 1.0‰ and 0.3 ± 1.6‰, respectively in C-tidal flat, and –14.9 ± 1.0‰ and 0.3 ± 1.6‰, respectively in S-tidal flat. The expected δ^{13}C and δ^{15}N values of *M. abbreviates* diet would be –17.4 ± 3.7‰ and 1.8 ± 0.9‰, respectively in N-tidal flat, –14.9 ± 1.0‰ and 0.3 ± 1.6‰, respectively in C-tidal flat, and –14.9 ± 1.0‰ and 0.3 ± 1.6‰, respectively in the S-tidal flat.

Among the primary producers, expect for terrestrial debris, organic items (salt marsh plant, $\text{POM}_{\text{(marine)}}$, $\text{POM}_{\text{(tidal flat)}}$, $\text{POM}_{\text{(pond)}}$) in the S-tidal flat displayed significant higher δ^{15}N values and lower δ^{13}C values than those in N-tidal flat and C-tidal flat. There were large differences in isotope compositions between salt marsh plant and hypothetical diet of the two crabs (Fig. 4.3). The δ^{13}C values of hypothetical diet for two crabs were between $\text{POM}_{\text{(marine)}}$, $\text{POM}_{\text{(tidal flat)}}$ and $\text{POM}_{\text{(pond)}}$ and BMA.

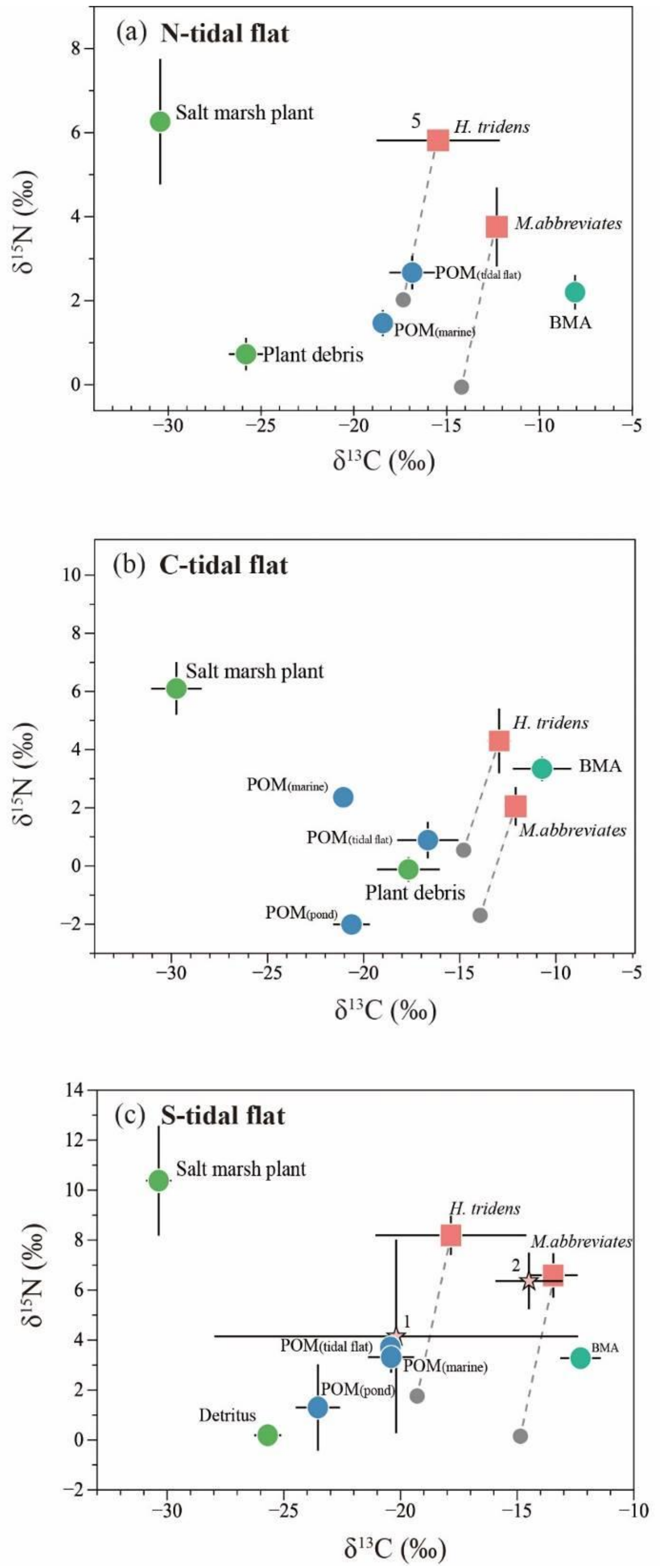

Fig. 4.3. Stable isotope biplots of δ^{13}C and δ^{15}N for dominant consumers and their potential food sources in the N-tidal flat (a), C-tidal flat (b) and S-tidal flat. Grey solid dots: expected isotope composition of the diet of each crab. Error bars represent SD.

4.3.3 Diet modeling

Proportional contributions of the primary producers to the diet of *H. tridens* and *M. abbreviates* were calculated from the SIAR model (Fig. 4.4). In N-tidal flat (Fig. 4.4a), $\mathrm{POM}_{(\mathrm{marine})}$ (25%, Ci 0 – 49%), BMA (27%, Ci 8 – 45%) and terrestrial debris (25%, Ci 8 – 42%) were important source items as they made similarly higher contributions to the diet of *H. tridens*. $\mathrm{POM}_{(\mathrm{tidal\ flat})}$ made a relatively smaller contribution (19%, Ci 0 – 37%), and salt marsh plant *Suaeda salsa* made a minimal contribution (4%, Ci 0 – 9%) to the diet of *H. tridens*.

In C-tidal flat, $\mathrm{POM}_{(\mathrm{marine})}$ made a smaller contribution to the diet of *H. tridens* (3%, Ci 0 – 7%), while BMA made a greater contribution (45%, Ci, 36 – 54%) (Fig. 4.4b). Besides, the contributions of $\mathrm{POM}_{(\mathrm{tidal\ flat})}$ (15%, Ci 0 – 32%), terrestrial debris (18%, Ci 0 – 36%) and *Suaeda salsa* (1%, Ci 0 – 2%) were relatively low. In addition, $\mathrm{POM}_{(\mathrm{pond})}$ contributed 19% (Ci 2 – 34%) of the diet of *H. tridens* in the C-tidal flat.

In S-tidal flat, significant shifts in the food contribution were detected in comparison to N-tidal flat and C-tidal flat. $\mathrm{POM}_{(\mathrm{pond})}$ (22%, Ci 4.4 – 44%) was detected as the most important food sources for *H. tridens.* Salt marsh plant *Suaeda salsa* changed to be the second important food source that made significant higher contributions (21%, Ci 13 – 28%) to the diet of *H. tridens* than that in the N-tidal flat. Besides, $\mathrm{POM}_{(\mathrm{marine})}$ (18%, Ci 0 – 35%) and BMA (16%, Ci 0 – 32%) contributed smaller proportions in S-tidal flat than those in N-tidal flat. While terrestrial debris made a minimal contribution (4.6%, Ci 0.1 – 9.1%) in S-tidal flat which was much lower than that in the N-tidal flat.

As for crab *M. abbreviates* (Fig. 4.4c, d), BMA overwhelmingly dominated the diet in N-tidal flat (51%, Ci 39 – 63%) and C-tidal falt (54%, Ci 37 – 69%). However, same with *H. tridens*, $\mathrm{POM}_{(\mathrm{marine})}$ in the C-tidal flat (6%, Ci 0 – 16%) showed the much lower contribution to the diet of *M. abbreviates* in than that in the N-tidal flat(22%, Ci 0 – 44%). Likewise, $\mathrm{POM}_{(\mathrm{tidal\ flat})}$ and terrestrial debris made small contributions, and *Suaeda salsa* made a minimal contribution to the diet of *M.*

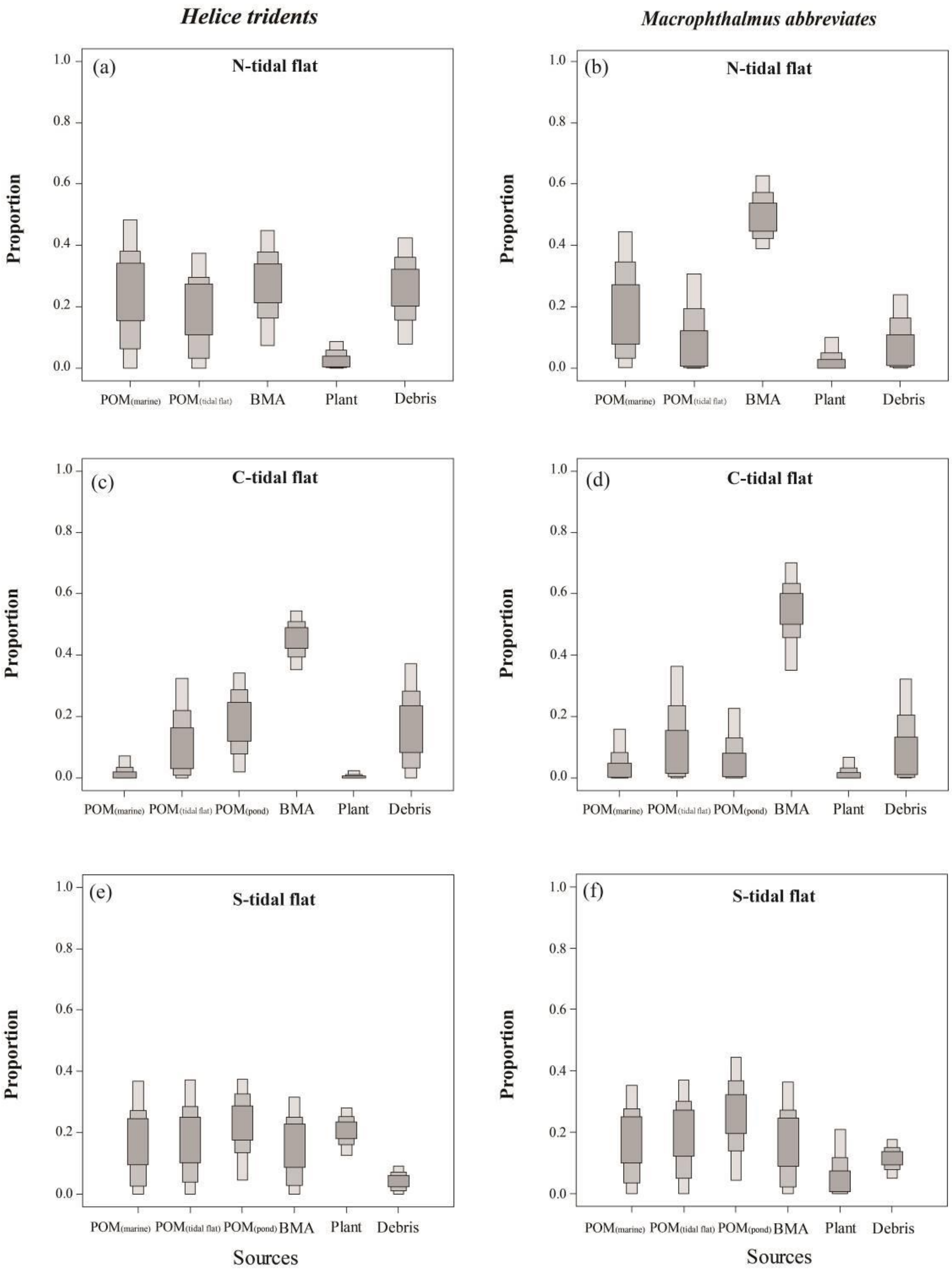

Fig. 4.4. Proportionate contribution of potential food sources to the diet of Helice tridents and Macrophthalmus abbreviates by SIAR boxplots. Plant: salt marsh plant. Debris: terrestrial debris. Dark gray, gray and light gray boxes show 50, 75, 95 credibility intervals respectively.

abbreviates in both N-tidal flat and C-tidal falt. However, $\text{POM}_{(\text{pond})}$ contributed a relatively lower amount (9%, Ci 0 – 22%) to the diet of *M. abbreviates* than it contributed to *H. tridens* in the C-tidal flat.

In contrast to N-tidal flat and C-tidal flat, $\text{POM}_{(\text{pond})}$ (25%, Ci 4.4 – 44%) was the important food source for *M. abbreviates* in the S-tidal flat. $\text{POM}_{(\text{marine})}$ (18%, Ci 0 – 35%) and BMA (18%, Ci 0 – 32%) showed lower contributions than those in N-tidal flat. While *Suaeda salsa* and terrestrial debris still made small contributions to the diet of *M. abbreviates* in S-tidal flat, but lower than that in N-tidal flat and C-tidal flat.

4.4 Discussion

SIAR analysis results revealed that BMA was the most important food resources for *H. tridens* and *M. abbreviates* in both N-tidal flat and C-tidal flat. However, for both species, contributions of BMA to the diet in the C-tidal flat were bigger than those in the N-tidal flat. This founding might imply that nutrient enrichment of surface sediment may enhance the primary production of BMA which furthermore enabled a higher contribution to the consumer's diet. However, apart from the results of N-tidal flat and C-tidal flat, $\text{POM}_{(\text{pond})}$ was the most important food sources for the diet of two crabs in shrimp farming area. These results might indicate that the POM from shrimp pond affected the diet of dominant crabs. On the one hand, POM(pond) showed the largest contributions to the diet of two dominant crabs. On the other hand, comparably higher δ^{15}N detected in most organic items (two dominant crabs, BMA, and salt marsh plant) in S-tidal flat might be attributed to the high DIN concentrations in the S-tidal flat.

In addition, salt marsh plant was found contributed minimally in N-tidal flat, while it changed to contribute more in shrimp farming area, especially the contribution to the diet of *H. tridens,* it was the second dominant contributor followed $\text{POM}_{(\text{pond})}$ closely. Hence, salt marsh plant was also an important food source to *H. tridens* in the S-tidal flat. This result also can be explained by the previous study of He and Silliman (2015) that the fertilization strongly increases herbivory in salt marshes. High nutrient concentration in S-tidal flat might increase herbivory of *H. tridens*. Hence the salt marsh plant was used much and became to be the important food source in the S-tidal flat.

Besides, salt marsh plant can be used as bioindicators for aquaculture farming as their use is cheap and less labor intensive than the traditional water/sediment sampling. What's more, they can record non-steady nutrient flows which are often missed by direct water sampling. These advantages are of peculiar importance in the long-term and large-scale environmental monitoring projects (Costanzo et al., 2004).

This differences indicated that sea cucumber farming activities might modify the dietary characteristics of benthic animals to a certain extent. The similar modifications of food composition of dominant benthic animals by aquaculture were confirmed in previous studies of another aquaculture type, such as fish farming (Mazzola and Sarà, 2001; Yokoyama and Ishihi, 2007) and shrimp pond farming (Kon et al., 2009).

In conclusion, a food availability shift was observed induced by the aquaculture waste in the dominant macrobenthos in typical salt marshes. This shift might indicate the potential effects of aquaculture waste that could spread up to the adjacent tidal flat. These foundings suggested that biological accumulation would be effective environmental indicators as they have the advantage of historical recording accumulation of organic matter that assimilated by the creatures. Biological accumulation can effectively detect the effect of aquaculture waste, even when it could not be detected by analyzing the organic matter in the environments due to relatively low concentrations of waste.

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Chapter 5

General Discussion

5.1 Summarization

Coastal wetlands receive nutrients and organic inputs from multiple anthropogenic and natural sources. Unquestionably, among which those from the rapid expansion of aquaculture activities account for a large proportion, particularly in countries with intensive aquaculture production like China. In such cases, widespread concerns have been raised about the environmental costs and sustainability of aquaculture techniques (Pusceddu et al., 2007; Tanner and Fernandes, 2010; Troell et al., 2003).

Several studies have addressed that the type of cultivated organisms (e.g. shrimps, fishes, and sea cucumbers etc.), cultivation locations (i.e. mangroves, tidal flats, or offshore facilities), cultivated biomass, stocking density, feed or farming operation and management are the major factors affecting the extent of environmental impacts (Sarà, 2007). The conclusions of previous studies have varied in different cases, which has further highlighted that there were no clear empirical conclusions. Therefore, in this present study, we provide a comprehensive estimate of the various environmental impacts induced by two typical types of aquaculture in the Yellow River estuary, Northern China.

5.1.1 Comparison of general sea cucumber farming and shrimp farming methods

There are notable differences between sea cucumber mariculture and shrimp mariculture methods.

Firstly in shrimp mariculture, juveniles and adults are commonly reared in intensive pond, pen or cage systems that mostly use 100% exogenous feed (Thongrod, 2007). The nutrients derived from uneaten feeds will cause high concentrations of dissolved inorganic nutrients and organic particulate organic matter. As a result, the increasing release of dissolved and solid nutrients potentially altered the sediment biogeochemistry and caused water quality deterioration (Trott and Alongi, 2000). By contrast, most sea cucumbers are deposit feeders. They are considered as the “environmental cleaners or scavengers (Yang et al., 1999) as they can efficiently ingest and assimilate the redundant feed and feces in the polyculture (Ahlgren, 1998; Han et al., 2016; Kang et al., 2003; Yang et al., 1999; Zamora et al., 2016). As a result, any negative environmental impacts caused by their feeding activities are generally overlooked as no exogenous nutrients are introduced during their culture.

However, similarly, commercial feeds are also commonly and largely used for sea cucumber mariculture, for example, previous studies have shown that sea cucumber mariculture altered the sediment characteristics and enhanced sediment oxygen consumption (Zheng et al., 2009), which indicated that a more detailed analysis of sea cucumber farming (particularly pond farming) is needed. However, there is a relative paucity of information concerning the environmental effects of sea cucumber mariculture (Li et al., 2014; Ren et al., 2010; Zheng et al., 2009). While on the other hand, the environmental impacts of shrimp aquaculture have been discussed for two decades (Flegel and Alday-Sanz, 1998; Herbeck et al., 2013; Martin et al., 1998; Paez-Osuna, 2001; Trott and Alongi, 2000), and a number of studies have been published on the causes, effects and alternative mitigations practices.

5.1.2 Environmental impacts of sea cucumber farming and shrimp farming

Overall, this study evaluated the environmental impacts of these two typical kinds of aquaculture (shrimp and sea cucumber) based on two types of analyses. The first one was the detection of discrepancies among distance-based pollution indicators in the water column and sediment. The second one was the utilization of biochemical tracers ($\delta^{13}C$ and $\delta^{15}N$) to trace the origin of the organic matters. Both of these methods have been widely confirmed as reliable tools to evaluate environmental effects of aquaculture (Gowen and Bradbury, 1987; Sarà, 2007; Sarà et al., 2004; Wai et al., 2011).

(1) Organic pollution

Organic nutrients in the water showed different patterns of distribution and concentrations among aquaculture types. In this study, $TOC_{(POM)}$ and $TN_{(POM)}$ concentrations in the shrimp pond were ten times higher than values in the sea cucumber pond. Besides, $TOC_{(POM)}$ and $TN_{(POM)}$ in the sea cucumber farming area showed no variation tendencies, while the shrimp farming area showed a decreasing trend from the pond to the 0 m station. These are likely to be a reflection of the disturbance caused by organic waste in the water column of the shrimp pond, and it can be detected in the water processes. Similarly, tidal flats adjacent to both farming areas were considered not affected by farming waste as no decreasing tendencies were found in the adjacent tidal flats.

On the other hand, organic nutrients in sediment were differently influenced by the aquaculture types. $TOC_{(s)}$ and TN(s) in sediment showed decreasing trends from the sea cucumber pond to 0 m, while no regular changes were observed in the shrimp farming area. As a result, historical accumulation of organic waste was found in sea cucumber farming area, and the waste dispersal distance was detected within a 50 m distance. While no effective information was obtained from the shrimp farming area, although decreasing tendencies were found from 200 m to 800 m in the shrimp farming tidal flat, it was considered a natural accumulation of organic matters from organisms.

In brief, organic matter concentrations in the water column can be effective indices for estimation of shrimp pond aquaculture, while organic matter concentrations in the sediment are effective indices for the estimation of organic pollution from sea cucumber pond aquaculture.

(2) Dissolved inorganic nutrients

For the studied dissolved inorganic nutrients (NO_3^-, NH_4^+, NO_2^- and PO_4^{3-}) in the water, only $NO_2^-{}_{(water)}$ and $PO_4^{3-}{}_{(water)}$ contents in the sea cucumber farming area were significantly higher than those in the non-farming area, while they did not differ among farming areas. On the other hand, the shrimp farming area had significantly higher $NO_3^-{}_{(water)}$ and $NO_2^-{}_{(water)}$, but lower $PO_4^{3-}{}_{(water)}$ concentrations than the non-farming area. As for NH_4^+, the shrimp pond had the same concentration level as the non-farming area. These results showed that the shrimp farming area had the highest nutrient concentration load for $NO_3^-{}_{(water)}$ and $NO_2^-{}_{(water)}$, then followed by the sea cucumber farming area and the non-farming area. $PO_4^{3-}{}_{(water)}$ was highest in the sea cucumber farming area then followed by the non-farming area and the shrimp pond area.

Similar to the previous studies on nutrients in the Yellow River estuary (Gong, 2012; Zhang et al., 2008), $NO_3^-{}_{(water)}$ was found as the typical dominant part of DIN in the shrimp farming area. However, the sea cucumber pond area was found with the same result as Kang (2016), that $NH_4^+{}_{(water)}$ represented a relatively major proportion of DIN than $NO_3^-{}_{(water)}$ in the sea cucumber farming zone. This result corresponded well with the meta-analysis of Sarà (2007) that $NH_4^+{}_{(water)}$ appears to be the most affected compound by aquaculture loadings compared to $NO_2^-{}_{(water)}$, $NO_3^-{}_{(water)}$ and followed by $PO_4^{3-}{}_{(water)}$. The different distribution patterns of studied parameters may imply the possible reasons that nutrients species were differently affected by the different aquaculture types. However, discrepancies among the distribution patterns of the studied parameters prevented clearer conclusions from being drawn.

For the studied dissolved inorganic nutrients ($NO_3^-{}_{(s)}$, $NH_4^+{}_{(s)}$ and $AP_{(s)}$) in the sediment, different distribution patterns were also found in both farming areas. Firstly, $NO_3^-{}_{(s)}$ and $NH_4^+{}_{(s)}$ were all showed the same decreasing tendencies with $TOC_{(s)}$ and $TN_{(s)}$ from sea cucumber pond to the 0 m station, and $AP_{(s)}$ in the pond and the effluent channel was significantly higher than those in the adjacent tidal flat stations. However, these nutrient concentrations did not show regular changes among stations in the shrimp farming and nonfarming areas.

Overall, both organic matter and nutrients in sediment effectively indicated the influence of waste from sea cucumber aquaculture waste on the pond and the adjacent environment. However, for the shrimp aquaculture, only organic matter in the water column can be effectively used. This result might be explained by other studies that the predominant effects of shrimp aquaculture waste were in the water processes, rather than sediment processes.

(3) Detection of the origins of organic matters and their implications

C: N ratios and biochemical tracers ($\delta^{13}C$ and $\delta^{15}N$) used in this study successfully verified the origin, extent, and fate of the nutrient sources derived from pond aquaculture. For example, on the one hand, both $\delta^{13}C_{(POM)}$ values in the water of the sea cucumber pond and shrimp pond implied the allogenous sources from commercial feeds used in aquaculture, as significantly lower values were found in ponds than those in each adjacent tidal flat station. These commercial feeds were composed of the terrestrial plant (soybean and peanut) and seaweed flour (seaweed cultured in offshore) that had low $\delta^{13}C$ and $\delta^{15}N$ values, and the usage of commercial feeds in pond farming resulted in low isotopic values. Although sediments in both farming ponds should have lower $\delta^{13}C_{(s)}$ than adjacent tidal flats, they showed higher $\delta^{13}C_{(S)}$ values (similar to $\delta^{13}C_{(POM)}$) than the adjacent tidal flats which received allogenous organic matter input from salt marsh plant with very low $\delta^{13}C$ values.

On the other hand, $\delta^{15}N$ of both water and sediment in the sea cucumber pond reflected the impact from low isotopic commercial feeds. Apart from the sea cucumber pond, $\delta^{15}N$ of both water and sediment in shrimp pond showed high values that were similar to the adjacent tidal flat. This might imply the influence of high $NO_3^-{}_{(water)}$ and $NO_2^-{}_{(water)}$ concentrations in the shrimp farming area that nutrient enrichment might affect the biotic process in the pond and adjacent tidal flat with enriched $\delta^{15}N$ signatures of POM in water and sediment. The combination of these biochemical tracers sufficiently describes and enables the interpretation of the variation of organic matter in both water and sediment environment induced by aquaculture activities.

5.1.3 Biological indicators

The literature on the impact of aquaculture waste on ecological processes in adjacent environments is generally limited，but the literature on fish farms and shrimp farming (mangroves) is more extensive. As our study in case, primary producers and dominant consumers were analyzed for comparison of isotopic signatures of organisms and dietary contributions between farming and non-farming areas. The results showed that the aquacultural waste from shrimp farming might influence the biotic processes in the adjacent tidal flat, as significantly enriched δ^{15}N signatures of consumers and producers (except for terrestrial debris) were observed in the shrimp farming tidal flat than those in the natural tidal flat and the sea cucumber farming tidal flat. In accordance with the previous studies, the stable nitrogen isotope ratio (δ^{15}N) of mangroves and macroalgae have been observed to be positively correlated with the proximity to outfalls of shrimp effluent (Costanzo et al., 2004). This suggests that salt marsh plants which are widely distributed and easily collected samples can be used as bioindicators for monitoring aquaculture-derived DIN in coastal ecosystems. Besides, salt marsh plants can also be considered as biofilters like mangroves or seaweeds to remove excessive DIN in the farming areas, so as to reduce the nutrient enrichment induced by aquaculture activities in the coastal waters of the Yellow River estuary.

As for the food composition of dominant benthic animals, SIAR (Stable Isotope Analysis in R) analysis results revealed that both the sea cucumber farming area and shrimp farming area had led to modifications in the food composition of two dominant crabs. Firstly, POM$_{(pond)}$ from the sea cucumber pond and shrimp pond were found to be used by the two crabs, and they showed large contributions to the crabs' diet in both the farmed tidal flats. Secondly, the dietary characteristics of the two crabs differed between the two farming areas. In the sea cucumber pond area, BMA made greater contributions to both crabs than that in the shrimp pond and non-farming area. While in the shrimp pond area, POM$_{(pond)}$ was the dominant food sources for both crabs, and it is noteworthy that saltmarsh plants were observed to be used by *H. tridens* to a large extent. This finding was confirmed by stable isotopic techniques for the first time that the salt marsh plant *Suaeda salsa* was utilized by *H. tridens* and the nutrient enrichment strongly increases herbivory in salt marshes (He and Silliman, 2015).

5.2 Proposals for operation and management of mariculture ponds

The overall conclusion of the present study was that sea cucumber farming altered the sediment environment of the adjacent tidal flat within a limited distance (i.e. within 50 m). Shrimp farming was considered not to impact the adjacent tidal flat. The negligible impact from aquaculture pond farming might be attributed to the typical operation mode and management of sea cucumber farming and shrimp pond farming in the studied area.

First, both the sea cucumber pond and shrimp pond were constructed with large sizes and stock reared at low densities, especially in the shrimp pond which size is large to enhance oxygen supplementation. The intensity of the sea cucumber farming activities with low densities (10 individuals m^{-2}) was far below the average densities (20 individuals m^{-2}) practiced by Chinese farmers (Dong et al., 2010; Kang and Xu, 2016). The intensity of the shrimp farming activities were also relatively low, which will contribute to the reduction of the adverse impacts from on the water and sediment environment.

Second, the feed supply and nutrient composition used are relatively natural in the sea cucumber aquaculture pond and shrimp aquaculture pond. The main feed for *Apostichopus japonicus* is composed of much seaweed flour (40%) and low protein feeds (fishmeal = 15%) in comparison with the shrimp feed (around 45%) in other studied areas (Dierberg and Kiattisimkul, 1996; Paez-Osuna, 2001; Trott et al., 2004). Low protein content reduced the accumulation of organic matter from the waste feed. For shrimp farming, live food, such as seaweed and gammarids were used at the early growth stage which contributed low nitrogenous excretion and had less adverse effects on water and sediment quality compared to artificial diets. Besides, commercial feed that is composed of wheat flour (15%), soybean meal (20%), peanuts powder (35%) and less protein feeds (fishmeal = 15%) than the common commercial feeds would reduce the accumulation of organic matter from high protein feed and mitigate the pressure from using fish meal from capture fisheries.

Third, discharge design of both the sea cucumber farming and shrimp farming is a key factor in determining environmental impacts. The studied aquaculture ponds farming were all managed uniformly that they were connected by internal pipes and gathered waste water in several uniform large channels to control drainage by valves. That is to say, waste waters from the aquaculture ponds might deposit in the effluent channel before the discharge. In this case, most parts of farming waste would deposit on their long way to the adjacent tidal flat. Moreover, the tidal creeks here are not like mangrove forests where water can stand for a long deposition time. Hence, the detected distance of discharged waste from the aquaculture ponds was limited in the adjoining effluent channels (shrimp farming) or within a distance of 50 m in the adjacent tidal flat like sea cucumber farming.

Consequently, the high natural feeds biomass, low stocking densities, and centralized drainage effluent channels would contribute to reduce the deleterious effects of aquaculture pond farming on the adjacent environment. In general, the most efficient way to reduce the impact of aquaculture ponds was to minimize the discharges from aquaculture ponds. As most nutrients discharged from the pond were originated from the aquaculture feed (Briggs and FVNGE-SMITH, 1994), development of a low protein and low pollution diet (Paez-Osuna, 2001), or natural feed that with low nitrogenous excretion would be the most direct and effective way to reduce nutrient discharge loads (Burford et al., 2003) and their adverse impacts on the local environment.

For the monitoring of aquaculture activities, a more significant challenge is to develop practical, cost-effective indicators that can reflect the impact and processes (Burford et al., 2003). The present study provides meaningful information about environmental impacts of sea cucumber and shrimp farming ponds.

5.3 Future studies

5.3.1 The requirement of larger datasets

(1) Large datasets

Ideally, conclusions drawn from a large number of replicates enables an accurate assessment of the conditions in the aquaculture areas. However, due to the cost and labors required for field collections, the number of samples often is insufficient. For example, only 60 stations were established in this study in an approximate 1600 ha aquaculture areas. Besides, because of the patch distribution pattern of some sedimentary variables and macrobenthic assemblages, large numbers of replicates (＞100) are particularly needed

(2) Regular monitoring is of great significances.

This study investigated the juvenile stage of sea cucumber farming and shrimp farming, and these activities were conducted twice a year. The environmental parameters and ecological indicators might vary with the change of seasons. Therefore, further investigation should be carried out in a different aquaculture period for long-term environment monitoring.

5.3.2 Quantitative analysis of wastes from aquaculture

This study mainly addressed what are the general distribution characteristics of environmental variables in the water and sediments of the Yellow River estuary. The contents involved are mainly focused on the detection of spatial discrepancies of environmental variables and trophic characteristics of the dominant crabs. However, the potential mechanisms for this phenomenon remain unknown. These questions are very complex, however, in the first step quantitative analysis of wastes from aquaculture is needed.

Specifically, as shown in other similar studies, the employment of sediment traps in a distance-based transect study is necessary to clarify what are the exact rates of sedimentation and its related nutrient (organic and inorganic) fluxes.

Using the SIAR method to distinguish waste feed and feces and calculate the contribution of wastes from aquaculture in the present sediment. The application of stable isotope techniques and analysis models not only reflect the environmental impact level from aquaculture discharges but also enables the implementation of an optimum ration level of feed for cost control and effective farm management (Yokoyama et al., 2010).

5.3.3 Key procedures of environmental variables and possible field experiment

(1) Stable isotope analysis in tracing nutrient sources

Biological tracers ($\delta^{13}C$ and $\delta^{15}N$) were only used in organic matter. The environmental influence induced by aquacultural waste was effectively shown by the combination of organic concentrations (TOC, TN) and stable isotope signatures. However, the isotopic technique was not used in tracing dissolved nutrients in water and sediment. Thus, the dispersal distance of nutrients produced by aquaculture waste was not successfully assessed under the high nutrient background condition. The elevated $\delta^{15}N$ values in the primary producers and consumers suggested that organism in the shrimp farming tidal flat assimilated high concentrations of the nitrogen. However, the origin of nitrogen (comes from aquaculture activities or other anthropogenic inputs) is unknown in this study. Based on this, further studies are warranted to measure the origin of nutrients by stable isotope techniques to give a thorough explanation of the causes of nutrient enrichment in the Yellow River estuary. Meanwhile, assessment of whether sea cucumber farming and shrimp farming have different impacts on certain nutrient species, like sea cucumber farming area might affect NH_4^+ and PO_4^{3-}, while shrimp farming affects more on NO_3^- and NO_2^-.

(2) Key environmental variables

In addition to the typical environmental variables studied in this study, a series of new variables should be included, such as biodeposition, oxygen consumption and nitrate release. Key environmental variables like dissolved organic nitrogen in the water column need to be detected, especially in aquacultural areas.

(3) Possible field processes

Details of benthic fluxes, including organic matters, oxygen, NH_4^+ and PO_4^{3-} need to be further clarified. Some possible transformations among variables, such as nitrification of nitrate, ammonium, and related denitrification processes need to be considered in the further studies.

5.3.4 Ecological aspects of wastes from aquaculture

(1) Modification of feeding behavior of some macroinvertebrates.

Some polychaetes had been shown to have potential as both deposit feeders and omnivores. Species of macrobenthos in the Chongming Island area have been reported to change feeding behavior from deposit feeding to terrestrial debris feeding with altered biotope. Feeding behavior of different consumers could be roundly considered in this field to explore the ecological effects of aquaculture wastes.

(2) Trophic cascade modified with altered environmental parameters.

BMA (benthic microalgae) in the sea cucumber pond was observed with higher contributions than natural and shrimp farming tidal flat. We noted that this result might be attributed to the high nutrient concentrations in this farming area, which prompted the growth of BMA. Although high nutrient concentrations were also found in the shrimp farming area, the low phosphorous contents might limit the development of BMA in this area. Hence, there might be another question that is will the N: P ratio (in different farming areas) mediate the feeding behaviors of the dominant consumers, or mediate the trophic cascade in coastal wetlands.

5.3.5 Proposals for managements

(1) Providing some basic data for the establishment of a scientific and effective mode of aquaculture mode - polyculture.

Polyculture has the advantages of reducing waste loading and environmental impacts, and increasing the productivity and efficiency compared to the intensive monoculture systems (Neori et al., 2004). As the studied species, sea cucumber (*Apostichopus japonicus*) was fed a large amount of commercial feed as a result of the poor nutritional input from local seawater. In this case, polyculture might be an appropriate method that can be conducted for higher production of sea cucumber and lower adverse environmental impacts on the pond and its adjacent environmental than by monoculture.

(2) To draw some straightforward and understandable conclusions for the related administrations aiming at scientific marine environment managements.

The major cause of marine environmental pollution and ecological environment destruction are the poor regulation during planning in the development process and the poor management of the breeding processes. Several research aspects can be considered to provide a scientific basis for sustainable marine environmental managements.

First, evaluation of coastal water carrying capacity would enable useful suggestions for proper culture area and species.

Second, farming scale and drainage system design also play an important part in reducing environmental pollution. The long effluent channel addressed in this study offers a good example that it can lessen the scope of aquaculture waste in the adjacent natural environment.

Third, analysis of feed conversion efficiency in different culturing methods could help farm managers avoid unnecessary waste from redundant feed and corresponding pollution.

Fourth, develop practical and cost-effective monitoring techniques and establish integrated and robust aquaculture environmental impact assessment systems for scientific marine environment management.

5.4 References

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