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Benthic Habitat Quality Assessment in Estuarine Intertidal Flats Based on Long-Term Data with Focus on Responses to Eco-Restoration Activity

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Abstract: A long-term assessment of the benthic habitat quality of intertidal flats in Liaohe Estuary was conducted by three integrating ecological indices, AZTI's Marine Biotic Index (AMBI), Multivariate-AMBI (M-AMBI), and Shannon–Wiener diversity index (H') based on macrobenthos data from 2013 to 2020. The results showed that the macrobenthic communities were characterized by indifferent and sensitive species of AMBI ecological groups. The annual ranges of H' , AMBI, and M-AMBI were 0.77–1.56, 1.44–3.73 and 0.36–0.54, respectively. Noticeable differences were found among assessment obtained by these biotic indices. Approximately 100%, 24%, and 78% sampling sites had “moderate”, “poor”, and “bad” statuses as assessed by H' , AMBI, and M-AMBI, respectively. Compared with H' and AMBI, M-AMBI may be more applicable to evaluate the benthic habitat quality of intertidal flats in Liaohe Estuary. Results suggest that the benthic habitat quality in the middle parts of intertidal flats still had an unacceptable status and has not improved radically to date after large-scale “mariculture ponds restored to intertidal flats”.

Keywords: benthic habitat quality; macrobenthos; biotic indices; intertidal flats; human activity; Liaohe Estuary

1. Introduction

An estuary is one of the most productive ecosystems, and the intertidal flat is one of the most distinctive habitats in estuarine areas [1]. Terrigenous freshwater that carries large amounts of particulate matters, such as mud and sand, flows into the sea and forms large areas of mud-sand flats, and this region is the most distinct habitat in estuarine ecosystems. Terrigenous freshwater that contains high levels of nutrients and organic materials provides abundant food sources for many marine organisms. Therefore, intertidal flats are highly productive and serve as breeding and survival habitats for many macrobenthic species [2–5]. Taking into account the importance of the intertidal flats to estuary ecosystems and economic development, assessing the benthic habitat quality of an intertidal flat is necessary for resource protection and fishery production.

Macrobenthos play an important role in the material cycle and energy flow of estuarine benthic ecosystems [6–8]. Most macrobenthos are characteristic of easy collection, sedentary habitation, and relatively longer lifespan [9]. Therefore, they can be applied as indicators for benthic habitat quality assessment [10–12]. Several biotic indices have been used widely for benthic quality assessment [13,14]. For instance, the Shannon–Wiener diversity index

(H') has been applied widely for assessing various marine benthic environments and was proven to be effective [15–17]. Moreover, AZTI's Marine Biotic Index (AMBI) and Multivariate-AMBI (M-AMBI) have been applied to evaluate the benthic habitat quality of estuaries and coastal systems [18–26].

The studies above indicated that H' , AMBI, and M-AMBI have been successfully used alone or in combination as indicators for benthic habitat quality in marine monitoring and assessment. Meanwhile, their study areas are mostly limited to the subtidal zones of estuaries and coastal areas [27–29]. However, the intertidal flats of estuarine areas where intensive anthropogenic activities (e.g., urbanization and large-scale reclamation projects in the high- and middle-tidal habitats) occur are rarely applied to [19]. To our knowledge, a simultaneous test of these indices to evaluate the benthic habitat quality of intertidal flats in estuaries has not been reported. Moreover, most studies were implemented with short-term monitoring (quarterly, one year or two years) [28,30,31]. Continuous monitoring and using sufficient sampling sites were seldom conducted. In addition, long-term environmental change and intensive anthropogenic activities are likely to influence both the macrobenthic community and benthic habitat of intertidal flats. Therefore, long-term continuous monitoring is necessary for the benthic habitat quality assessment of intertidal flats in estuaries.

The Liaohe Estuary at the top of Bohai Sea in China is an important spawn and grow area for marine organisms, such as mollusk *Meretrix meretrix* and polychaete *Perinereis aibuhitensis* [32]. The estuary was exposed to considerable anthropogenic pressures by the rapid economy development of upstream cities, such as industrial and agricultural effluents, oil exploration, and trawling and dredging disturbance, which considerably deteriorate the habitat quality of Liaohe Estuary [33,34]. Consequently, monitoring and evaluating the benthic habitat quality in Liaohe Estuary based on long-term and multiple sampling surveys are essential.

The aims of the present study based on survey data from 2013 to 2020 in the highest latitude estuary of China were to (i) evaluate the benthic habitat quality of intertidal flats by using H' , AMBI, and M-AMBI; and (ii) explore the suitability of these three indices in the intertidal flats and provide recommendations for similar estuary ecosystems. Moreover, the study could provide advice about an ecologically sustainable estuarine management program.

2. Materials and Methods

2.1. Survey Area

The Liaohe Estuary is an estuary with the highest latitude in China and has a long-time (up to four months) ice cover period in winter. The investigation area in the western part of Liaohe Estuary covers a total area of 65.0 km² (Figure 1), an important unreclaimed intertidal flat for various macrobenthos.

2.2. Samples Collection and Measurements

The macrobenthos was collected in 46 sites, allocated in seven sections 2.2 km apart (A-G, perpendicular to the coastline) in spring (May), summer (August), and autumn (October) from 2013 to 2020 (Figure 1). Sites within the same section were 0.5 km distant from each other. Samples were not collected in winter due to the long period of ice cover in the study area. Eight subsamples were collected at each site by a sample frame (25 cm × 25 cm) down to 20 cm depth, and then sieved through 500 µm iron mesh and preserved in 5% formalin solution before macrofauna identification. In addition, sediment samples in sections were captured by a stainless-steel spade every year, and then were stored under −20 °C condition to analyze sediment grain size and organic matter content. The sediment samples were directly measured by the Mastersizer 2000 laser particle analyzer for grain size analysis. The allowed measurements range is between 0.02 µm and 2 mm with a repeatability error of <3%. Sediment samples were dried at 60 °C for 72 h, subsequently burned at 550 °C for 2 h prior to the final weighing for organic matter calculation [35].

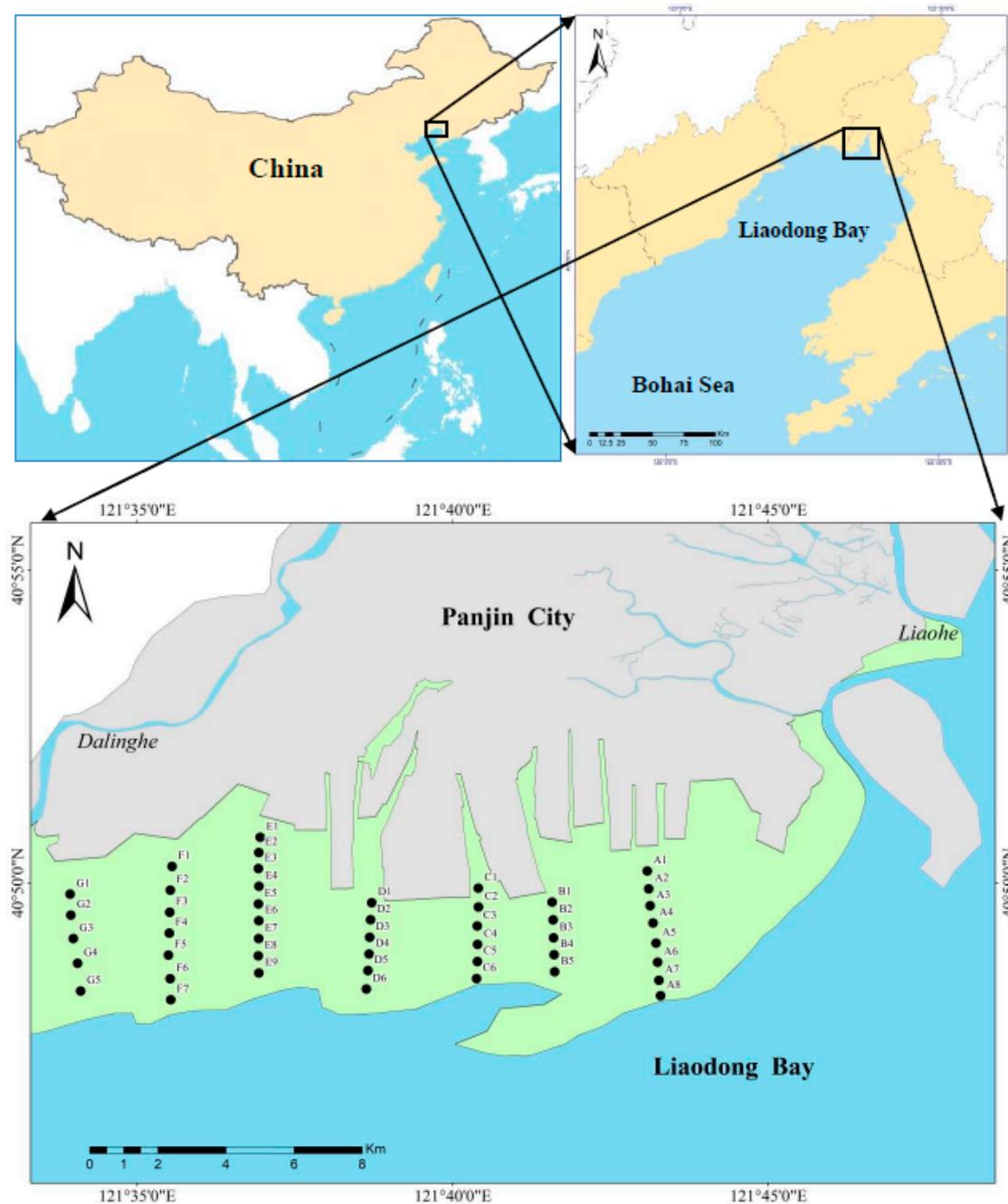


Figure 1. Sampling sites for macrobenthos and sediments in the intertidal flat of Liaohe Estuary. Note: grey indicates areas of the land and mariculture pond; green indicates the tidal flats areas; and blue indicates the rivers and sea areas.

2.3. Biotic Indicators

2.3.1. Shannon–Wiener Diversity Index (H')

H' was analyzed as follows:

$$H' = - \sum_{i=1}^S (n_i/N) \log_2(n_i/N)$$

where n_i is the number of species i , N is the total number of collected macrobenthos individuals in a unit area, and S is the total number of collected macrobenthos species.

The threshold values for the H' conditions are shown in Table 1.

Table 1. The threshold levels of Shannon–Wiener index (H'), and AMBI and M-AMBI indices for benthic habit quality assessment.

H'	AMBI	M-AMBI	Benthic Habitat Quality	Benthic Community Health/Site Disturbance Classification
$H' > 4$	$AMBI \leq 1.2$	$M-AMBI > 0.77$	High	normal/undisturbed
$3 < H' \leq 4$	$1.2 < AMBI \leq 3.3$	$0.53 < M-AMBI \leq 0.77$	Good	unbalanced/slightly disturbed
$2 < H' \leq 3$	$3.3 < AMBI \leq 5.0$	$0.38 < M-AMBI \leq 0.53$	Moderate	transitional to pollution/meanly disturbed
$1 < H' \leq 2$	$5.0 < AMBI \leq 6.0$	$0.20 < M-AMBI \leq 0.38$	Poor	transitional to heavy pollution/heavily disturbed
$H' \leq 1$	$AMBI > 6.0$	$M-AMBI \leq 0.20$	Bad	heavy polluted/extremely disturbed

2.3.2. AMBI and M-AMBI

AMBI was analyzed on the base of abundance of each ecological group and as follows [36]:

$$AMBI = [(0 \times \%EGI) + (1.5 \times \%EGII) (3 \times \%EGIII) (4.5 \times \%EGIV) + (6\% \times EGV)]/100$$

where EGI indicates the species sensitive to environmental interference, EGII indicates the species indifferent to environmental interference, EGIII indicates the species tolerant to environmental interference, EGIV indicates the second-order opportunistic species, and EGV indicates the first-order opportunistic species.

M-AMBI is based on AMBI and along with species richness and H' . AMBI and M-AMBI were analyzed by the AMBI software (version 5.0, AZTI-Tecnalia, <http://ambi.azti.es>). The macrobenthos of intertidal flats were assigned to different ecological groups (EGs) based on the AMBI species list. The undefined species was temporarily defined to the same ecological group when the references about the sensitivity of that was not found, whereas the same genus is shown in the list (Tables S1 and S2) [14]. Based on the AMBI calculation, ranges of the AMBI and M-AMBI are shown in Table 1. A suitable reference condition for M-AMBI was set to evaluate the benthic habitat quality. In order to ensure the reliability of the assessment, AMBI values with >20% unassigned individuals or fewer than 5 species were removed from the AMBI analyses.

Benthic habitat quality was divided into two categories, “unacceptable” and “acceptable”, to facilitate the comparative analysis among these indices. The “high” or “good” status is identified as the acceptable condition, which suggested that the benthic habitat was not significantly impacted. “Moderate”, “poor”, and “bad” statuses were defined as unacceptable conditions, which indicated that the benthic habitat was moderately or severely impacted, and the macrobenthic community in the intertidal flat was transitioning to an unhealthy condition.

2.4. Data Analyze

Pearson correlation was carried out by R software (Version 4.0.2 for Windows, www.r-project.org/) [37] to investigate the significant relationships of H' , AMBI, and M-AMBI with sediment factors (sediment median diameter, and organic matter content). One-way ANOVA was performed using SPSS software (SPSS for Windows, Version 19.0., SPSS Inc., Chicago, IL, USA) to analyze the differences in sediment parameters and three integrating ecological indices among years and different sections, followed by a post hoc comparison with the Tukey HSD method. Spatial distribution maps of H' , AMBI and M-AMBI values of the intertidal flats in Liaohe Estuary were made with the Surfer software 12.0 by the inverse distance to a power method.

3. Results

3.1. Macrobenthic Grouping in Intertidal Flats of Liaohe Estuary

A total of 116 macrobenthic species were quantitatively identified, and consisted mainly of three groups (i.e., Mollusca, Annelida, and Arthropoda) in Liaohe Estuary from 2013 to 2020. The species in ecological groups were displayed in Table S1 and S2. In the current study, 35 species were defined to EGI (30.17%), 52 species were defined to EGII (44.83%), 18 species were defined to EGIII (15.52%), 5 species were defined to EG IV (4.31%), 1 species were defined to EGV (0.86%), and the remaining 5 species were not defined (4.31%). In sum, most of the macrobenthic communities were dominated by indifferent species and followed by sensitive and tolerant species in the intertidal flats of the Liaohe Estuary.

3.2. Variations in Biotic Indices

The range of H' values was between 0 and 2.60, and the lowest value was found in 2013 (0.77), and the highest value was found in 2018 (1.56). Moreover, H' presented a stable trend in 2019 and 2020. The results indicated that the benthic habitat quality of the intertidal flat was the worst in 2013 and 2019 (96% of sites had poor and bad statuses), and the best in 2018 (26% of sites had moderate status, Figure 2a). The benthic habitat quality of all sample sites in the intertidal flats of Liaohe Estuary was assessed as “moderate” “poor” or “bad” by H' from 2013 to 2020 (Figure 2a).

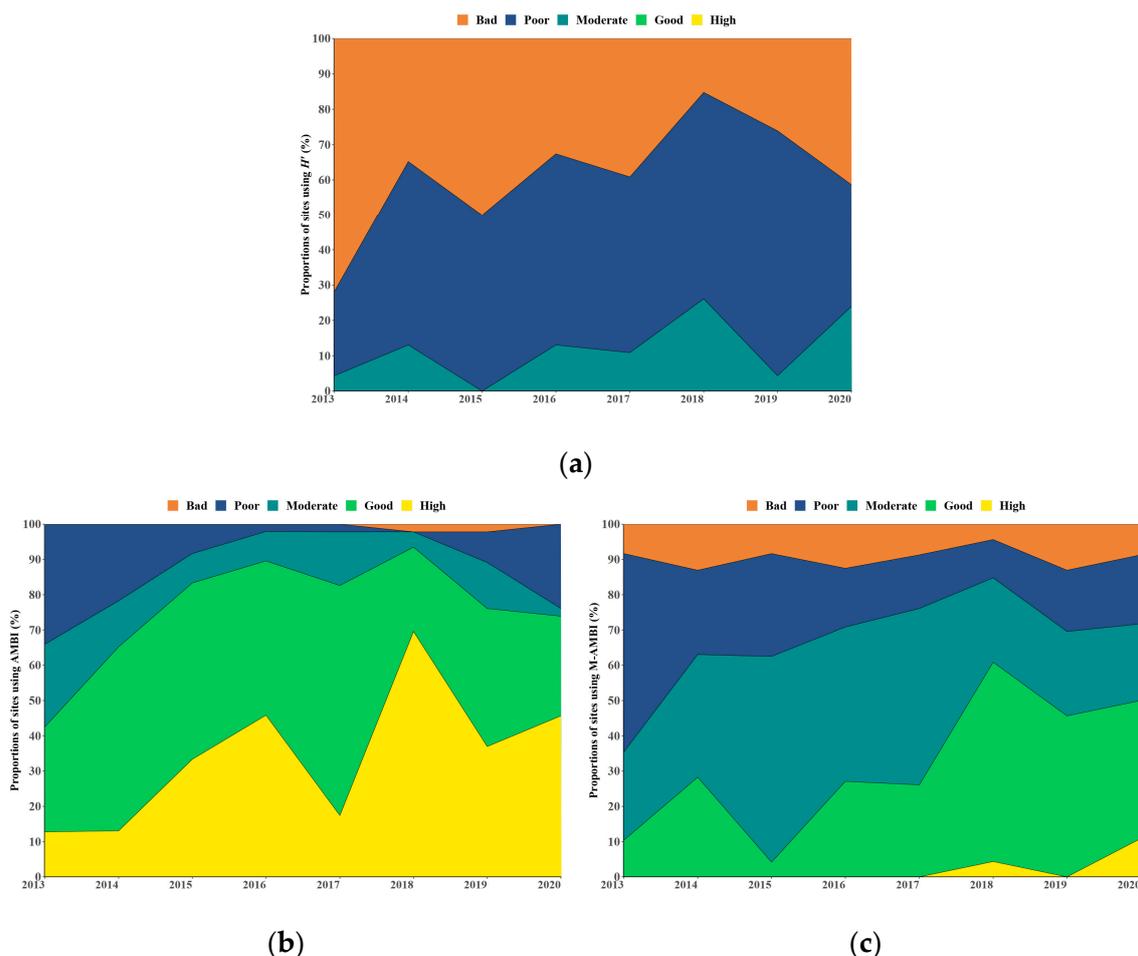


Figure 2. Proportions of sites using H' (a), AMBI (b), and M-AMBI (c) from 2013 to 2020. Note: “high” or “good” indicates the acceptable condition, and the benthic habitat was not significantly impacted; “moderate” “poor” and “bad” indicates unacceptable conditions, and the benthic habitat was moderately or severely impacted.

The range of AMBI values for Liaohe Estuary from 2013 to 2020 was 1.44–3.73. The values indicated that the benthic habitat quality of the intertidal flat was the worst in 2013 (more than 57% of sites had poor and moderate statuses) and the best in 2018 (more than 94% of sites had high and good statuses, Figure 2b). The benthic habitat quality of approximately 76% of sample sites (average value) in the intertidal flats of Liaohe Estuary from 2013 to 2020 was evaluated as “high” and “good” by AMBI (Figure 2b).

The range of M-AMBI values for Liaohe Estuary from 2013 to 2020 was 0.36–0.54. The values indicated that the benthic habitat quality was the worst (more than 80% of sites had bad, poor, and moderate statuses) in 2013 and the best in 2018 (more than 61% of sites had high and good statuses). The benthic habitat quality of approximately 68% of sample sites (average value) in the intertidal flats of Liaohe Estuary was evaluated as “moderate” “poor” or “bad” by M-AMBI from 2013 to 2020 (Figure 2c).

The M-AMBI values of section A and section D fluctuated significantly respectively (Table S4) and showed a similar interannual variation tendency from 2013 to 2020 (Figure 3b). However, that of section B, section C, section E, section F and section G varied insignificantly among these years (Table S4). The benthic habitat quality of most sections was evaluated as “moderate” by M-AMBI from 2013 to 2020 (Figure 3b).

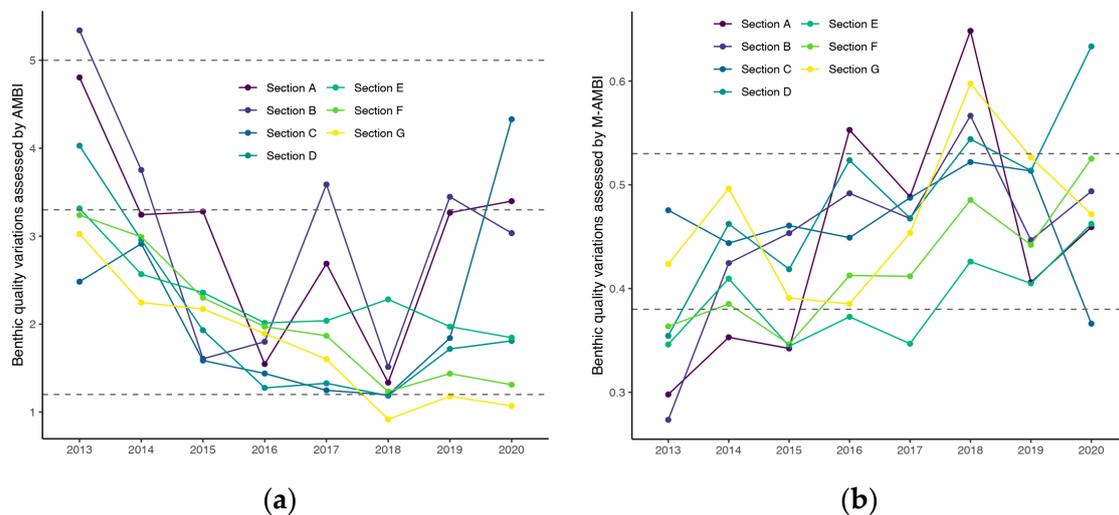


Figure 3. Benthic habitat quality variations assessed by AMBI (a) and M-AMBI (b) in seven sections of the intertidal flats in Liaohe Estuary from 2013 to 2020. Note: the threshold values for AMBI status are as follows: high = 5.0–6.0, good = 3.3–5.0, moderate = 1.2–3.3; the threshold values for M-AMBI status are as follows: good = 0.53–0.77, moderate = 0.38–0.53, poor = 0.20–0.38.

3.3. Sediment Factors in the Intertidal Flats of Liaohe Estuary

The surface sediments in the intertidal flat of the Liaohe Estuary consisted mainly of clay, silt sand, and sand. Sediment median grain size and sediment content (sand and silt sand) did not vary considerably from 2013 to 2020 (Figure 4b,d,e, Table S5). However, the clay and organic matter content of sediment varied significantly and increased from 2013 to 2020, respectively (Figure 4a,c, Table S5). Meanwhile, the organic matter of sediment in section A and section D varied significantly, respectively, and that in section B, section C, section E, section F, and section G all had no evident change (Table S4). Pearson correlation indicated that H' and M-AMBI were remarkably negatively correlated with the sediment median diameter and organic matter, whereas AMBI was remarkably positively correlated with these two sediment factors (Figure 5).

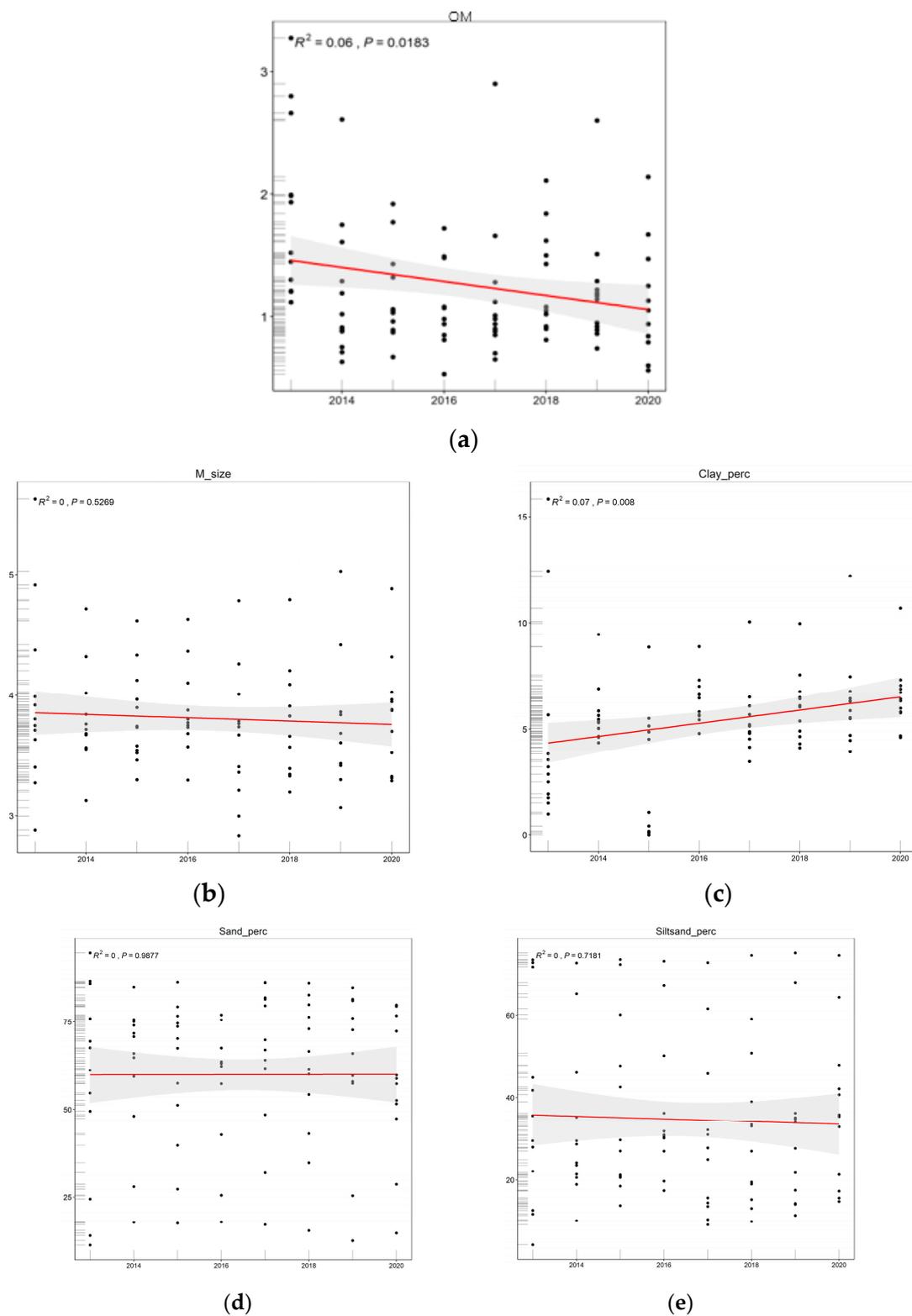


Figure 4. The organic matter (a), median grain size (b), clay (c), sand (d) and silt sand (e) of sediment in the intertidal flat of Liaohe Estuary from 2013 to 2020. Note: OM, M_size, Clay_perc, Sand_perc, and Siltsand_perc indicates the organic matter content, median grain size, clay content, sand content and siltsand content of sediment, respectively. R^2 and P indicate the fitting degrees and credibility of the linear regression equations, respectively.

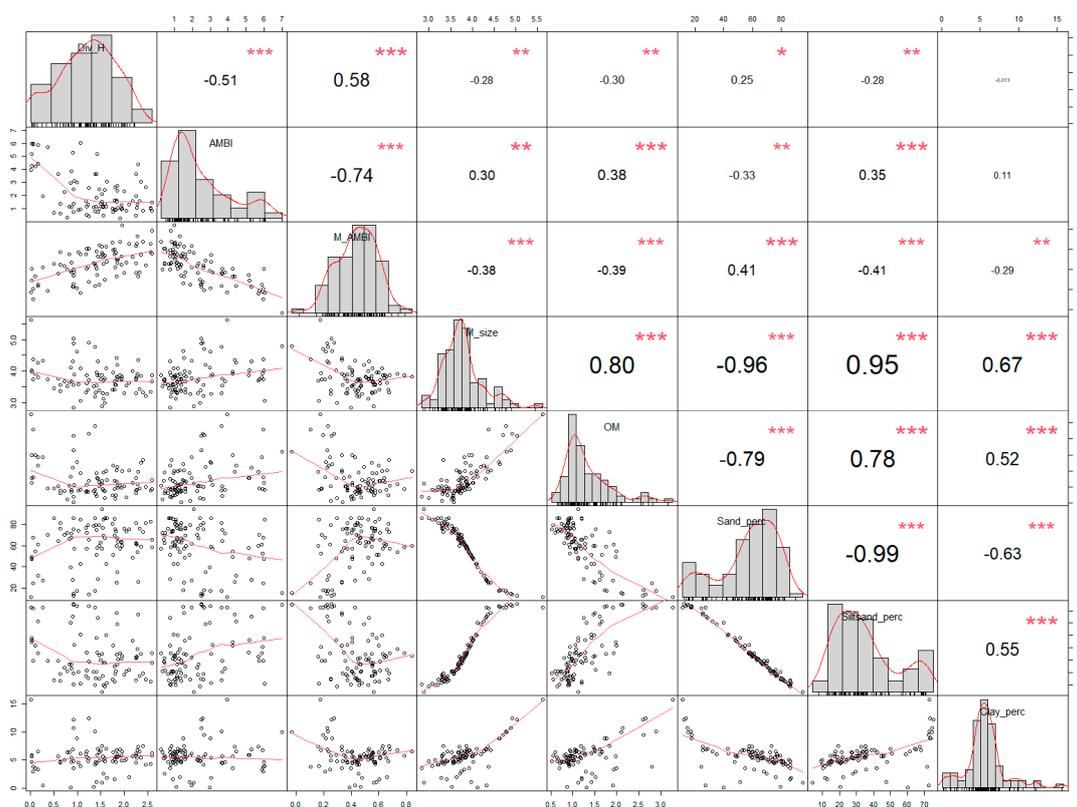


Figure 5. Correlation analysis between H' , AMBI, and M-AMBI with environmental parameters in the intertidal flats of the Liaohe Estuary. Note: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Div_ H' , AMBI, and M-AMBI indicate the Shannon–Wiener diversity index, AZTI’s Marine Biotic Index and Multivariate-AMBI Index, respectively; OM, M_size, Clay_perc, Sand_perc, and Siltsand_perc indicates the organic matter content, median grain size, clay content, sand content and siltsand content of sediment, respectively.

4. Discussion

4.1. Comparisons among Biotic Indices

Determining the effective indicators is a crucial point in assessing benthic habitat quality. Both the difference between indices and their sensitivity to estuarine benthic habitat could be evaluated to determine the availability of the biotic indices. If the stronger correlation was found between biotic index and benthic habitat factors, it is more sensitive to an environmental interference and more reflective to the benthic habitat status [28]. Noticeable differences were found among the assessment results of H' , AMBI, and M-AMBI in the present study. The H' results suggested that the benthic habit quality of the intertidal flats in Liaohe Estuary was unacceptable, especially from 2013 to 2015, and was mostly poor from 2016 to 2020. The AMBI results suggested that a large part of the sites (with an average value of 76%) had an acceptable status (high or good) from 2013 to 2020. However, the M-AMBI results suggested that a large portion of the investigation area (78% sites) were unacceptable from 2013 to 2020. In summary, the benthic habitat quality assessed by AMBI was better than those by M-AMBI and H' , which was consistent with other studies [16,17,27].

Differences of the benthic habitat quality in the same investigation area evaluated by different biotic indices are always emphasized in estuaries [18,23,26,38,39]. In the current study, most sites had few species, that is, approximately 98% of total sampling sites had fewer than ten species, and even 61% of total sampling sites had fewer than five species. Consequently, 40% of the sampling sites had low H' values with less than 1.0, and even 9% sites with the values of 0. These results suggested that the macrobenthic community

structure was very fragile, and the benthic habitat was disturbed to some extent. These results were inconsistent with the acceptable status (high or good) based on AMBI, but consistent with the assessment results based on M-AMBI to some extent. This outcome is because AMBI was almost based on the species assignment and the different ecological group ratios. The macrobenthic species richness was relatively low, but the abundance of single species was high. In the present study, the abundance values of *Potamocorbula laevis* were 9063, 5676, and 7594 ind/m² in 2013, 2015 and 2020, respectively (unpublished data). Meanwhile, the abundance values of *Macraa veneriformis* were 214, 609, and 201 ind/m² in 2017, 2018 and 2019, respectively (unpublished data). In addition, the robustness of AMBI could be reduced when only a very low number of taxa (1–3) was found in a sample [40]. In the current study, there are about 29% of total sampling sites that had fewer than three species from 2013 to 2020. Therefore, the indication sensitivity of AMBI might reduce, and AMBI may overestimate the habitat quality. However, these assessment results could be improved by M-AMBI due to integrating the species number, diversity, and AMBI together [41].

In the current study, AMBI was remarkably positive correlated with sediment median diameter and organic matter, whereas M-AMBI was considerably negatively correlated with these two sediment factors (Figure 5). The results are inconsistent with that in Bohai Sea and Mediterranean coast. There was a negative correlation between AMBI/M-AMBI and sediment median diameter, but the difference was unremarkable in Bohai Sea [16]. Meanwhile, it showed that AMBI is more applicable than M-AMBI because M-AMBI was related poorly with organic matter content in the Mediterranean coast [42]. Moreover, our results indicated that the M-AMBI of section A and section D fluctuated significantly, while that of other sections varied insignificantly among these years (Table S4). Coincidentally, the organic matter of section A and section D also correspondingly fluctuated significantly, and that of other sections varied insignificantly (Table S4). This outcome may be due to the fact that the Liaohe mouth is near the eastern part of section A, and there was a big tidal creek (Laobeihe) close to the eastern part of section D. Consequently, the frequency and intensity of the sediment discharge of these tidal creeks and hydrodynamic may increase organic matter loading in intertidal areas and could affect benthic habitat status. In general, based on the best correlation between M-AMBI and benthic habitat factor variables, M-AMBI may be more applicable to assess the benthic habitat quality of the intertidal flats in Liaohe Estuary. Moreover, different indices should be chosen carefully depending on the features of the studied areas [22].

4.2. Benthic Habitat Quality of Intertidal Flats in Liaohe Estuary

It was reported that the benthic quality of the coastal areas of Liaohe Estuary was remarkably disturbed in 2007 as analyzed by H' and biotic index integrity [43]. However, the benthic habitat quality in the intertidal flats of the Liaohe Estuary was first comprehensively assessed according to three frequently employed benthic indices (i.e., H' , AMBI, and M-AMBI) with long-term monitoring. The H' , AMBI, and M-AMBI results all suggested that benthic habitat quality of intertidal flats in Liaohe Estuary improved to some extent after large-scale mariculture ponds were restored to intertidal flat projects (from 2016 to 2020). However, the results also suggest that the benthic habitat quality of some sites in the middle parts of the intertidal flat was still unacceptable and has not improved radically to date (Figures S1–S3).

In the present study, the benthic habitat quality of most sections was evaluated as “moderate”, which indicated a polluted environment according to the M-AMBI. Meanwhile, the results also suggested that the benthic habitat quality of the intertidal flats was improved to some extent after eco-restoration activities. The reasons may be mainly that the Liaohe Estuary has been distinctly affected by anthropogenic activities such as pollution, fishery catches (such as bottom fish trawling) and eco-restoration activity [34]. On the one hand, large amounts of major pollutants, such as nutrients, metals, organic pollutants, from Liaohe were discharged into the sea from 2013 to 2017. The pollutant

fluxes of ammonia, total phosphorus, petroleum and heavy metals were 695, 133, 122, and 22 t in 2013, respectively [44]. More seriously, the major pollutant fluxes increased in 2017, and the pollutant fluxes of ammonia, total phosphorus, petroleum and heavy metals were 820, 412, 160, and 33 t in 2017, respectively [45]. Fortunately, on December 2018, the action plan for the environmental comprehensive management of Bohai Sea was jointly issued by the Chinese government to improve marine environmental quality and protect the marine ecosystems of the Bohai Sea [46]. The proportion of first-category seawater in Panjin coast area increased by 17.34% from 2017 to 2020 (unpublished data). Therefore, it suggested that the coastal environment management is important for improving benthic habitat quality [47].

Secondly, the sediment discharges may change the species composition and benthic habitat [48]. The data of Liaohe in Liujianfang hydrology station (Table S3) showed that the sediment discharges varied intensely from 2013 to 2020. Moreover, sediment discharges decreased abruptly in 2015 (with the lowest values of 157,000 t) and 2018 (with the lower values 269,000 t), respectively. The annual runoff volume and sediment discharge as a whole take on a synchronously decreasing trend, and the evolution process is characterized by obvious stages. The sediment of Liaohe into the sea mainly includes fine particulate matter; therefore, the sediment concentration reduced with the sediment discharges. Consequently, the sediment concentration had the lowest value (0.172 kg/m³) in 2015 and a lower value (0.287 kg/m³) in 2018 (Table S3). Therefore, the turbidity of seawater and sedimentation of sediment may also change and adjust with the decrease in sediment discharges.

Thirdly, large areas (about 5000 km²) of reclaimed marine culture ponds (especially sea cucumber pond) in the tidal flats were removed from 2015 to 2020 [49]. The species composition and biomass will change with the influencing factors after being directly affected by human activities [50,51]. Biological connectivity and hydrological connectivity could be changed by the ecological conservation and restoration projects [52]. Consequently, the formation and development of new intertidal flat and tidal creek system were beneficial to habitat self-restoration and macrobenthic community recovery. It is a theoretical possibility that the high- and middle-tidal habitats that had been enclosed by reclamation projects may be in the formation process of the original intertidal flat after restoration. Moreover, the self-restoration of the high- and middle-tidal habitats may take a long time [53].

5. Conclusions

A long-term evaluation of benthic habitat quality in the intertidal flats of Liaohe Estuary was conducted using H' , AMBI, and M-AMBI. The macrobenthic communities were characterized by indifferent and sensitive species of AMBI ecological groups. Differences were found among assessment obtained by these biotic indices. M-AMBI may be more suitable to evaluate the benthic habitat quality of intertidal flats in Liaohe Estuary. The benthic habitat quality of some sites in the middle parts of the intertidal flat is still unacceptable and has not improved radically to date after the large-scale mariculture ponds were restored to intertidal flats. The conservation and restoration of intertidal flats and their ecosystem services should be continued to achieve the sustainability of estuarine flats.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w14233846/s1>, Figure S1: Spatial distribution maps of the H' values of the intertidal flats in Liaohe Estuary from 2013 to 2020; Figure S2: Spatial distribution maps of the AMBI values of the intertidal flats in Liaohe Estuary from 2013 to 2020; Figure S3: Spatial distribution maps of the M-AMBI values of the intertidal flats in Liaohe Estuary from 2013 to 2020; Table S1: List of macrobenthos species and grouping in the intertidal flats of Liaohe Estuary. Table S2: List of undefined macrobenthos species when the references about the sensitivity of that are not found. Table S3: Annual run off, sediment discharges and sediment concentration of Liaohe in Liujianfang hydrology station. Table S4: One-way ANOVA results of M-AMBI and organic matter content for different sections in the intertidal flats of Liaohe Estuary from 2013 to 2020. Table S5: One-way ANOVA results for organic matter content, median grain size, clay, sand, and silt sand of sediments in the intertidal flats of Liaohe Estuary from 2013 to 2020.

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