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Macrobenthic assemblage characteristics under stressed waters and ecological health assessment using AMBI and M-AMBI: a case study at the Xin'an River Estuary, Yantai, China

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Abstract

To understand the ecological status and macrobenthic assemblages of the Xin'an River Estuary and its adjacent waters, a survey was conducted for environmental variables and macrobenthic assemblage structure in September 2012 (Yantai, China). Several methods are adopted in the data analysis process: dominance index, diversity indices, cluster analysis, non-metric multi-dimentional scaling ordination, AMBI and M-AMBI. The dissolved inorganic nitrogen and soluble reactive phosphorus of six out of eight sampling stations were in a good condition with low concentration. The average value of DO ((2.89 ± 0.60) mg/L) and pH (4.28 ± 0.43) indicated that the research area faced with the risk of ocean acidification and underlying hypoxia. A total of 62 species were identified, of which the dominant species group was polychaetes. The average abundance and biomass was 577.50 ind./m^2 and 6.01 g/m^2, respectively. Compared with historical data, the macrobenthic assemblage structure at waters around the Xin'an River Estuary was in a relatively stable status from 2009 to 2012. Contaminant indicator species *Capitella capitata* appeared at Sta. Y1, indicating the animals here suffered from hypoxia and acidification. AMBI and M-AMBI results showed that most sampling stations were slightly disturbed, which were coincided with the abiotic measurement on evaluating the health conditions. Macrobenthic communities suffered pressures from ocean acidification and hypoxia at the research waters, particularly those at Stas Y1, Y2 and Y5, which displays negative results in benthic health evaluation.

Key words: macrobenthos, Xin'an River Estuary, ocean acidification, hypoxia, AMBI, M-AMBI

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1 Introduction

Estuarine areas are one of the most productive ecosystems in nature, and have been widely concerned because of their multiple ecological function (Dolbeth et al., 2007). Massive nutrients and organics are transited by terrigenous freshwater, providing abundant nourishments and appropriate habitats for estuarine organisms (Zhang et al., 2016a, b). However, excessive nutriments aroused by anthropogenic activities and inappropriate discharge of effluents would stimulate environmental issues like water contamination and sediment deterioration leading to ocean acidification and underlying hypoxia. These deteriorated environment bring about diverse distortions on the local ecotopes (Marques et al., 2003; Lillebø et al., 2005; Zhu et al., 2014; Piló et al., 2016; Zhang et al., 2016a). Many estuary areas are encountering the same ecological problem, especially those in waters with weak circulation, which is also a balance between environmental protection and economy development (Dolbeth et al., 2007; Cai et al., 2016; Briggs et al., 2017).

The Xin'an River is about 40 km in length with a drainage area of around 315 km^2, which flows through the Muping District, Laishan District and High-tech District in Yantai City and ends up in the Yellow Sea (Ma et al., 2012). Widespread as it is, the river function and environmental conditions were not in a positive status for the past few years. Yantai government have made great efforts to bring the contamination into control since 2009, including performing afforestation, removing aquatic farms, set-

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ting up sewage treatment plant and establishing 24-hour pollution monitoring systems. The research estuarine waters are located in the west of Yangma Island National AAAA Tourist Attraction. The south is the Xin'an River and Yuniao River. A sewage treatment plant is about 400 m west from the Xin'an River Estuary, of which the outlet is 3 232 m to the north of the estuary (Jia et al., 2007). The hydrodynamic water exchange in the research waters is limited due to the breakwater built to the west of Yangma Island harbor. Excessive pollutants (1 800 t per year) are produced by the surface runoff of the Xin'an River. According to Ma et al. (2012), the study areas mainly suffered perturbations from the sewage discharge plant and the rainy season runoff. Moreover, bivalve aquaculture zones scattered around the study water regions, which also impact the benthic assemblages by changing the circulation of substances, introducing physical anchoring structure and causing disturbances to benthic habitats (Dumbauld et al., 2009).

Biota indicators have been proved to be an effective method for marine ecosystem health assessment and widely applied under various water conditions (Peng et al., 2013; Rombouts et al., 2013). Macrobenthos are key components of estuarine ecosystems, playing important roles in the process of trophodynamics as well as in food chains (Herman et al., 1999). Macrobenthos facilitate the decomposition of organic materials in the process of ingestion and defaecation. Thus, the flux rates of nutrient particles could be balanced across the sediment-water interface; upper nourishment could be replenished to supply for the phytoplankton subsistence as well (Ekeroth et al., 2016). In virtue of their sedentary biotope and sensitiveness to environmental changes, macrobenthic organisms are generally adopted as biological indicators to evaluate the sediment health condition (Anderson, 2008; Borja and Tunberg, 2011; Li, 2011).

However, it could be intricate for policy makers and laymen to understand sophisticated information of scientific research data which displays the nexus of macrobenthos and environment. Various biotic indices therefore are wielded to evaluate the environment condition in a more intuitionistic way (Borja et al., 2008). Based on the commission of the water framework directive (WFD) and marine strategy framework directives (MSFD), AZTI's marine biotic index (AMBI and M-AMBI) (Muxika et al., 2007) are of two efficient indices to assess the ecological health status by macrobenthic community characteristics and confined reference conditions (Borja et al., 2008; Cai et al., 2015; Sigamani et al., 2015). Although discrepancies of the evaluation may exist due to variable environment conditions and anthropogenic perturbations, the AMBI and M-AMBI indices are still extensively accepted as well as applied in Europe, North America, South America, and East Asia (Luo et al., 2014).

Changes of certain environmental factors may induce fluctuation in both macrobenthic community structure and dominant species (Dauvin et al., 2010; Dolbeth et al., 2011; Xie et al., 2016). Sewage input is one of the disturbances in estuarine waters, which may change the macrobenthic community structure by providing appropriate conditions for tolerant or opportunistic species (Borja et al., 2000; Gusmao et al., 2016). Ma et al. (2012) found that the chemical oxygen demand (COD) and phosphates were at a high level in waters around the Xin'an River Sewage Treatment Plant. It indicated that the adjacent waters suffered from moderate eutrophication with relatively high inorganic nitrogen and restricted phosphates. COD reveals the content of the consumed oxygen when the organic matters and the inorganic substances were decomposed and oxidized (Kawai et al., 2016). In normal condition, DO values are negatively correlated with COD values in waters (Ahmed, 2014; Zhang et al., 2017). The higher the COD values, the lower the DO values (Zhang et al., 2017). Phosphorus eutrophication could be induced by marine acidification in the way that alters the form of phosphorus in aerobic sediments (Ge et al., 2017). According to aforementioned facts, high level of COD and phosphates in sea waters are closely related with marine hypoxia and acidification. Whether there are hypoxia and acidification at the Xin'an River estuarine waters remains to be discovered. To what extent the local macrobenthic communities are affected by environmental factors also need to be researched. However, so far, few studies had been reported on the macrobenthic assemblages and its relationships with environmental variables at waters around the Xin'an River Estuary.

The objectives of our study was to investigate the macrobenthic community characteristics as responding to small-scale hypoxia and acidification as well as the benthic ecological health by using AMBI and M-AMBI indices. This study also aims to ascertain whether there are divergences between biotic indices and abiotic indices on evaluating the health conditions of interfered waters. We intend to provide a dataset for the evaluation of the effects of contamination control and coastal management from the local government.

2 Materials and methods

2.1 *Sampling area and procedure*

Eight stations at the Xin'an River Estuary were investigated in September 2012, among which Y1 was located at the Xiaoyuniao River Estuary; Y2 and Y4 were located at the Xin'an River Estuary; Y3, Y5 and Y8 were around the Yangma Island; Y9 and Y10 were located at scallop aquaculture zone (Fig. 1). Sediment samples were collected by three separate replicates in each station using a 0.05 m^2 box-corer grab, then sieved through a 0.5 mm aperture mesh to obtain macrobenthos. The macrobenthic organisms were preserved in 80% ethanol and identified to the lowest possible taxonomic level in laboratory, and then counted and weighted using a 0.001 g precision electric balance. All sampling process followed with the *National Oceanography Census Regulation Methods* (State Quality and Technical Supervision Administration, 1992).

2.2 *Environmental factors*

Environmental variables, including water depth (WD), water temperature (WT), dissolved oxygen (DO), salinity (SAL), soluble reactive phosphorus (SRP, including PO_4^{3-}-P) and pH were measured *in situ* by YSI environmental monitoring system (600QS-M-O, US). Dissolved inorganic nitrogen (DIN, including NH_4^+-N, NO_2^--N and NO_3^--N), SiO_3^{2-}-Si, total nitrogen (TN) and total phosphorus (TP) were measured by the nutrient auto-analyzer using gas-segmented continuous flow analysis (AutoAnalyzer 3, Bran Luebbe, Germany).

2.3 *Statistical analysis*

2.3.1 *Environmental data processing*

Single factor evaluation was applied to classify the sea water nutrients in accordance with National Marine Industry Standards, HY/T 086–2005, China. The standard index of pollutants (P_i) was calculated by the following method:

$$P_i = M_i / S_i, \tag{1}$$

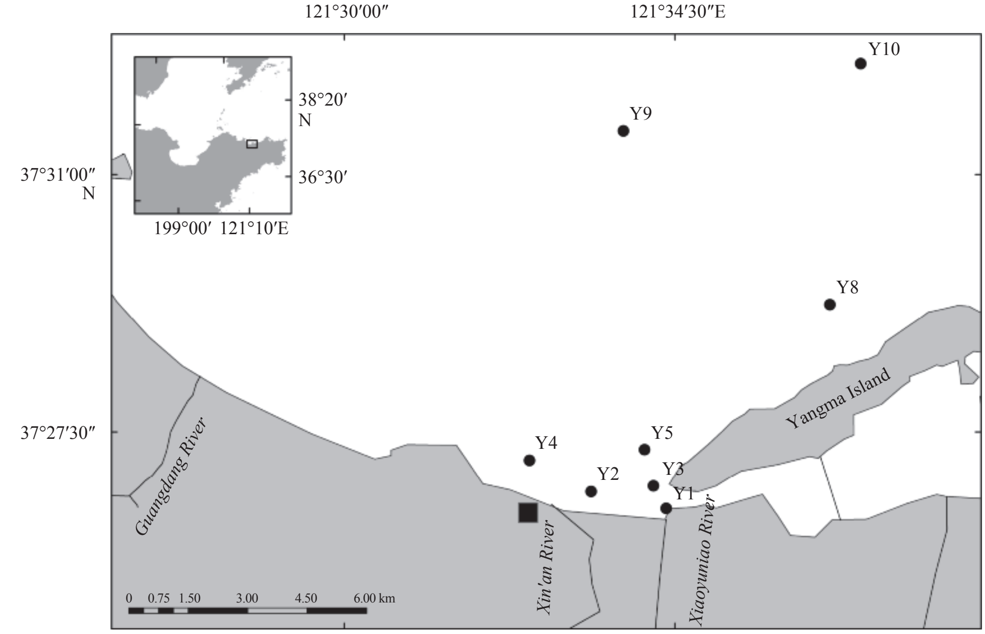

Fig. 1. Sampling stations of macrobenthos in the Xin'an River Estuary and its adjacent waters. The position of sewage treatment plant is marked with the black square.

where M_i is the average measured concentration of pollutant *i*; S_i is the standard concentration for pollutants *i*. Here, GB Class I water quality standards was used here as S_i data according to National Sea Water Quality Standard (GB 3097–1997).

The principal component analysis (PCA) was conducted based on lg (x+1) transformation of environmental data to analyze the characteristic of sampling environment data. The Pearson correlation analysis was conducted to explore the correlation between single environmental factor and benthic community structure. The bio-environment (BIOENV) analysis was performed to study the optimal combination of environmental factors to describe the community structure.

2.3.2 *Biological data processing*

The biological properties were analyzed by PRIMER software package (Version 7.0.11, PRIMER-E Ltd., 2016), including the total biomass (B), abundance (A), number of species (S), Shannon-Wiener diversity index (H_0), Margalef richness index (D), and the Pielou's evenness index (J). The dominant index (Y) (Chen et al., 1995) of species was calculated by the following formula:

$$Y = (n_i/N)f_i, \quad (2)$$

where N is the total abundance of all the stations, n_i is the abundance of the species *i* of all the stations, and f_i is the occurrence frequency of the species *i* of all the stations. Species *i* can be defined as the dominant species when Y>0.02.

The multivariate analysis of the macrobenthic community was also conducted by PREMER 7. Cluster and non-metric multidimensional scaling (n-MDS) plot was used to display the relationship of the species abundance of different macrobenthic communities on the basis of Bray-Curtis similarities. Analysis of similarities (ANOSIM) was used to determine if significant differences existed between samples, and similarity percentages (SIMPER) was used to calculate the contribution of the species which determined different clusters.

2.3.3 *Benthic quality assessment*

AZTI's marine biotic index (AMBI) (Borja et al., 2000) and multivariate AZTI's marine biotic index (M-AMBI) (Muxika et al., 2007) were adopted to assess the benthic quality at waters around the Xin'an River Estuary. The AMBI and M-AMBI value were calculated by the AMBI program (Version 5.0, http://ambi.azti.es). All species data were processed in accordance with the AMBI guidelines (Borja and Muxika, 2005), the non-benthic invertebrate taxa (fish and megafauna) were removed and ecological groups (EG I: disturbance-sensitive species; EG II: disturbance-indifferent species; EG III: disturbance-tolerant species; EG IV: the second-order opportunistic species; EG V: the first-order opportunistic species) were classified based on the species list made at AZTI Laboratory in November 2014. Due to the fact that the research waters suffered from different levels of anthropogenic activities, the threshold values for the M-AMBI conditions were increased by 15% on the basis of original biodiversity index and species number (Borja et al., 2008; Li et al., 2013). Under high quality status, AMBI=0, Diversity=4.24, Richness=25.30, M-AMBI=1; under bad quality status, AMBI=6, Diversity=0, Richness=0, M-AMBI=0.

3 Results

3.1 *Abiotic parameters*

According to Chinese Sea Water Quality Standard GB 3097–1997 (National Standard of the People's Republic of China GB 3097–1997 was issued by the National Environmental Protection Agency of the People's Republic of China on July 1, 1998), the GB Class I sea water quality can be used for marine fishery, natural reserve areas and natural preservation zones for rare and endangered animals; the GB Class II quality can be used for marine culture zones, bathing beaches, direct body contact marine sports and industrial water area related to marine foods; the GB Class III quality can be used for normal industrial water and coastal scenic areas; the GB Class IV quality can be used for port waters and marine development zone for specific application.

The average content of DIN and SRP of all sampling stations were (0.15±0.07) mg/L and (0.011±0.006) mg/L, respectively, which can be defined as GB Class I quality, except that Y2 and Y4 belonged to GB Class II quality, for the DIN values of which are 0.286 mg/L and 0.239 mg/L, respectively; the SRP values of which are 0.0231 mg/L and 0.0183 mg/L, respectively (Table 1). However, the average value of DO ((2.89±0.60) mg/L) and pH (4.28±0.43) were ranked as superior GB Class IV with the declining trend from open sea to the river mouth, which indicated a bad condition of DO and pH values. The pollutant standard index P_{DIN} and P_{SRP} were less than 1 at all sampling stations except for Y2 and Y4, which indicated the nutrients at most stations met with the standards for marine fishery and natural preservation zones, yet Y2 and Y4 still reached the standard for aquaculture and body contact usages (GB Class II).

3.2 *Species composition*

A total of 62 species were identified, among which Polychaeta was the most abundant taxon with 34 species (54.84%), followed by Crustacea with 12 species (19.35%), Mollusca with 10 species (16.13%), Echinodermata with 3 species (4.84%), and the others with 3 species (1 Nemertea, 1 Sipuncula and 1 Vertebrata) (4.84%). In terms of species composition at each station, Y1 (8 species) was observed with the least species, while Y10 possessed the most with 32 species. Moreover, Polychaeta took up most of the species proportions at all sampling stations (Fig. 2).

Table 1. Data and evaluation results of DIN, SRP, DO and pH in the Xin'an River Estuary waters

Station	DIN/mg·L^{-1}	DO/mg·L^{-1}	pH	SRP/mg·L^{-1}	P_{DIN}	P_{SRP}
Y1	0.143	1.60	4.03	0.0063	0.72	0.42
Y2	0.286	2.58	3.51	0.0231	1.43	1.54
Y3	0.109	3.19	4.41	0.0058	0.55	0.39
Y4	0.239	3.37	3.87	0.0183	1.20	1.22
Y5	0.118	2.88	4.5	0.0086	0.59	0.57
Y8	0.097	2.81	4.58	0.0097	0.49	0.65
Y9	0.090	3.31	4.67	0.0071	0.45	0.47
Y10	0.118	3.38	4.68	0.0088	0.59	0.59
Mean±SD	0.15±0.07	2.89±0.60	4.28±0.43	0.011±0.006	0.30±0.14	0.24±0.14

Note: DIN means dissolved inorganic nitrogen, DO dissolved oxygen, SRP soluble reactive phosphorus, P_{DIN} the pollutant standard index of dissolved inorganic nitrogen, and P_{SRP} the pollutant standard index of soluble reactive phosphorus.

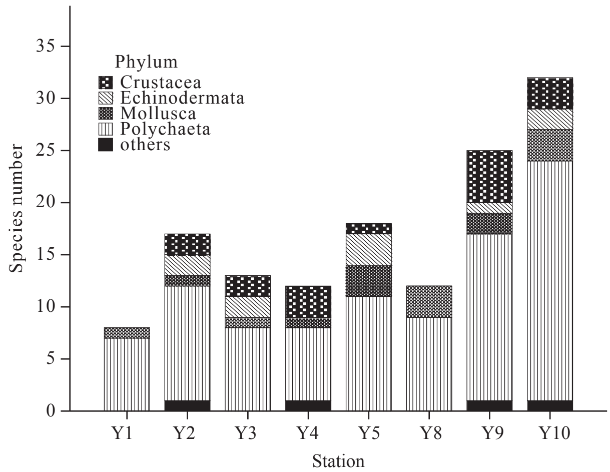

Fig. 2. Macrobenthic species composition of each station in Xin'an River Estuary and its adjacent waters.

Table 2. Spatial distribution of dominant species in each sampling station

Station	*Lumbrineris latreilli*	*Heteromastus filiformis*	*Chaetozone setosa*	*Sigambra bassi*
Y	0.337	0.104	0.023	0.021
Y1	+	+	+	
Y2		+	+	
Y3	+	+	+	+
Y4	+	+	+	
Y5	+	+		+
Y8	+	+	+	+
Y9	+	+	+	+
Y10	+	+		+

Note: + means the dominant species were detected.

Four Polychaeta species were identified as dominant species according to their dominant values (*Y*>0.02). Moreover, contaminant indicator species *Capitella capitata* (Sun and Chen, 1978; James and Gibson, 1980) was only obtained at Sta. Y1, with the abundance of 46.67 ind./m^2 (Table 2).

3.3 *Abundance and biomass*

The distribution of macrobenthic abundances showed the descending trend from the offshore to the estuary areas (Fig. 3). The average species abundance per station was 577.50 ind./m^2, among which Polychaeta contributed the most with average abundance of 470.83 ind./m^2 (81.53% of average species abundance), followed by Mollusca species 42.50 ind./m^2 (7.36%), Crustacea species 32.50 ind./m^2 (5.63%) and Echinodermata species 22.50 ind./m^2 (3.90%). Other species (1 Nemertea, 1 Sipuncula and 1 Vertebrata) contributed 1.59% with average abundance of 9.17 ind./m^2.

The spatial distribution of macrobenthic biomass was presented with lower value in estuary area and higher value in offshore area (Fig. 4). The average species biomass per station was 6.01 g/m^2, among which Polychaeta contributed the most with average biomass of 2.76 g/m^2 (45.90%), followed by Mollusca with 0.73 g/m^2 (12.20%), Crustacea with 0.18 g/m^2 (2.91%). Echinodermata possessed the least with 0.13 g/m^2 (2.22%). Other species (1 Nemertea, 1 Sipuncula, and 1 Vertebrata) possessed 36.74% of the total biomass with a kind of vertebrate *Chaeturichthys stigmatias* obtained at Sta. Y9, which increased the average biomass to 2.21 g/m^2.

3.4 *Biodiversity*

The Shannon-Wiener diversity index (H_0) varied from 1.57

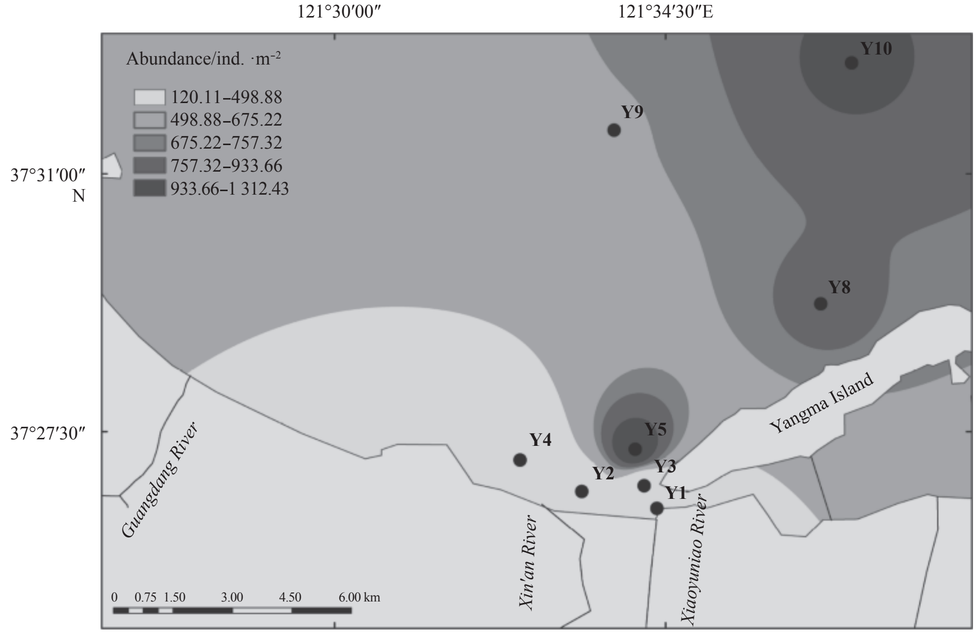

Fig. 3. Spatial distribution of macrobenthic abundance in sampling stations.

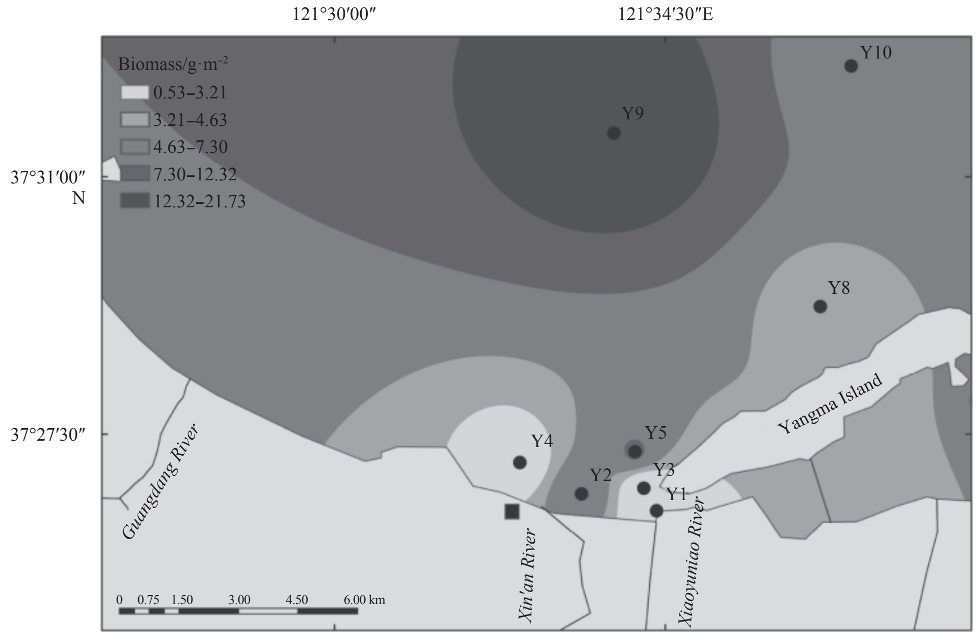

Fig. 4. Spatial distribution of macrobenthic biomass in sampling stations.

(Y5) to 4.04 (Y10), with the average value of 2.96±0.82. The Margalef richness index (*D*) varied from 1.46 (Y1) to 4.50 (Y10), with the average value of 2.60±1.04. The Pielou's evenness index (*J*) varied from 0.38 (Y5) to 0.87 (Y3), with the average value of 0.75±0.17 (Fig. 5).

3.5 *Community structure*

Cluster and n-MDS analysis showed that the similarity between macrobenthic assemblages in different stations ranged from 24.56%-55.99%. According to the similarity values, all the sampling stations were divided into four groups at an arbitrary similarity level of 36.14%. Group I included two stations, Y2 and Y4, with the similarity value of 48.95%. Group II consisted of only Sta. Y1, which was separated from Group I at similarity value of 32.25%. Group III consisted of Y3, Y5 and Y8, with the similarity value of 48.95%. Group IV included Y9 and Y10, with the similarity value of 54.95%. Group IV was separated from Group III at the similarity value of 36.14% (Fig. 6). The same results were obtained by n-MDS ordination plots, with the stress value lower than 0.1, and the four groups showed significant differences on ANOSIM global test (global R=1, significance level=0.1%<0.05, permutations=999).

The results of SIMPER analysis were as follows. Group I consisted of Y2 and Y4, and the average similarity coefficient was 48.95%. The polychaetes *Orbinia dicrochaeta* (Wu, 1962) and *Chaetozone setosa* (Malmgren, 1867) were dominant species with

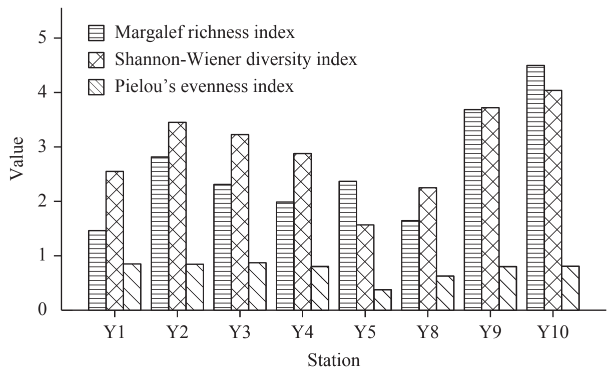

Fig. 5. Margalef richness index (*D*), Pielou's evenness index (*J*) and Shannon-Wiener diversity index (H_0) of each sampling station.

contribution rate of similarity were 22.47% and 15.36%, respectively. Other species which cumulatively contributed higher than 70% were polychaetes *Heteromastus filiformis* (Claparede) (10.8%), *Glycera rouxi* (Audouin et Milne Edwards, 1833) (10.8%) and crustacea *Paraphoxus oculatus* (10.8%). The average similarity and species contributions of Group II (only Y1, located at the estuary of Yuniao River) could not be calculated. The pollution indicator species, *Capitella capitata* (Fabricius, 1780), was only detected with a high abundance (46.67 ind./m^2, maximum in all stations) at Sta. Y1, which indicated a polluted benthic environment condition. Group III consisted of Y3, Y5 and Y8, which showed an average similarity coefficient of 51.30%. The dominant species of Group III were polychaetes *Lumbrineris latreilli* (Audouin et Edwards, 1834), *Heteromastus filiformis* (Claparede) and *Sigambra bassi* (Hartman), whose contribution rate were 33.55%, 25.42% and 11.18%, respectively. Moreover, the abundance in Sta. Y5 reached the maximal value of 1 313.33 ind./m^2. Group IV consisted of Y9 and Y10, whose average similarity coefficient was 54.95%. The species with contribution rate above 10% were *Lumbrineris latreilli* (Audouin et Edwards, 1834) (12.98%) and *Moerella iridescens* (Benson) (12.10%).

3.6 *Relationships between macrobenthic community and environmental factors*

The PCA results showed that the accumulative contribution rate of the first three principal components to total variation accounted for over 85%, maintaining most of information of the 12

Table 3. Eigenvectors of each environmental variable on the first three principal components

Environmental variable	PC1	PC2	PC3
PO_4^{3-}-P	0.314	−0.301	0.147
TP	0.236	0.337	−0.026
NH_4^+-N	0.353	−0.065	0.076
NO_2^--N	0.260	−0.385	0.259
NO_3^--N	0.361	−0.122	0.067
SiO_3^{2-}-Si	0.341	−0.230	0.144
TN	0.268	0.286	−0.175
WT	0.255	0.349	−0.389
SAL	−0.166	0.268	0.703
WD	−0.321	−0.241	0.079
DO	−0.082	−0.489	−0.426
pH	−0.364	−0.059	−0.126

Note: PO_4^{3-}-P represents phosphoric acid-phosphorus; TP total phosphorus; NH^{4+}-N ammonium salt-nitrogen; NO_2^--N nitrous acid-nitrogen; NO_3^--N nitric acid-nitrogen; SiO_3^{2-}-Si silicic acid-silicon; TN total nitrogen; WT water temperature; SAL salinity; WD water depth; DO dissolved oxygen; and PC1, PC2 and PC3 the first, second and third principal components.

environmental factors. The coefficients of eigenvectors were displayed in Table 3, among which pH, NO_3^--N and NH_4^+-N contributed most of the PC1 with coefficients of −0.364, 0.361 and 0.353, respectively; DO and NO_2^--N contributed most of the PC2 with coefficients of −0.489 and −0.385, respectively; SAL, DO and WT contributed most of the PC3 with coefficients of 0.703, −0.426 and −0.389, respectively.

The result of BIOENV analysis (Spearman) showed that the characteristics of spatial distribution of community biomass could be mainly explained by the combination of TP, NO_2^--N and pH, and the Spearman coefficient was 0.747. Pearson correlation analysis showed that only TN and TP were significantly correlative with community abundance among 12 environmental factors, and the Pearson correlation coefficient were −0.745 (sig. level=0.034) and −0.754 (sig. level=0.031), respectively.

3.7 *AMBI and M-AMBI*

Among all 62 species identified in this sea area, 29 species were found to have an abundance lower than 3, which could not be defined to any ecological group based on AMBI guidelines. Moreover, one species remained unassigned in accordance with the species list (November 2014, v 5), which was *Apseudes* sp..

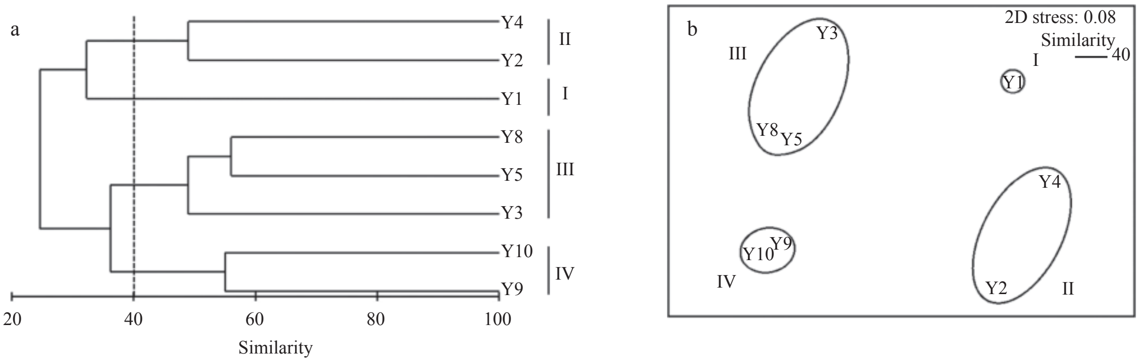

Fig. 6. Analysis of cluster (a) and MDS (b) on macrobenthos of sampling stations, September 2012. The abundance data was standardized, and then overall transformed by lg (x+1) method. The group-average linking was conducted in accordance with S17 Bray-Curtis species similarities.

Table 4. Results of AMBI and M-AMBI for each station in waters around the Xin'an River Estuary

Station	Ⅰ/%	Ⅱ/%	Ⅲ/%	Ⅳ/%	Ⅴ/%	NA/%	AMBI	DC	M-AMBI	Status
Y1	0.0	31.3	0.0	25.0	43.8	0.0	4.219	MD	0.35	poor
Y2	20.0	35.0	5.0	40.0	0.0	0.0	2.475	SD	0.63	good
Y3	8.7	39.1	8.7	43.5	0.0	0.0	2.804	SD	0.51	moderate
Y4	48.6	16.2	27.0	8.1	0.0	0.0	1.419	SD	0.62	good
Y5	2.6	82.9	0.5	14.0	0.0	0.0	1.889	SD	0.53	moderate
Y8	6.7	65.0	2.5	25.8	0.0	0.0	2.212	SD	0.55	good
Y9	16.7	56.7	13.3	13.3	0.0	2.2	1.850	SD	0.73	good
Y10	31.3	28.4	30.6	9.7	0.0	2.2	1.780	SD	0.81	high

Note: I, II, III, IV and V represent for ecological Group I, II, III, IV and V, respectively; NA not assigned; DC disturbance classification; MD moderately disturbed; and SD slightly disturbed.

The unassigned species proportion of all sampling stations was below 20%, which indicated a credible judgement on AMBI and M-AMBI results (Table 4).

The AMBI values of eight sampling stations ranged from 1.419 to 4.219, with seven slightly disturbed stations and one medium disturbed station (Y1), which indicated that the benthic environment suffered from slightly anthropogenic interference. The minimal AMBI value was captured at Sta. Y10 (1.419), where most of the species were disturbance-sensitive species (Group I: 48.6%); the maximum value was detected at Sta. Y1 (4.219), with the first-order opportunistic species as main dominant species (Group V: 43.8%).

The M-AMBI values ranged between 0.35 and 0.81, with Sta. Y10 defined as high quality status, Y1 as poor status, Y3 and Y5 as moderate status, and the rest of the stations as good status. The minimal M-AMBI value (0.35) appeared at Sta. Y1, with the least species number (six species) amongst all sampling stations; the maximum value was obtained at Sta. Y10, with the highest biodiversity index (H_0=3.69) as well as species number (20 species) among all sampling stations.

4 Discussion

The Xin'an River is located at a special geographical position with widespread drainage area. The river is under multiple anthropogenic pressures: industrial and domestic sewage discharge, aquaculture activities, and tourists impacts. Human activities could trigger prompt nutrient eutrophication, which is far more rapid than the natural process (Serrano et al., 2017). The abiotic parameters showed that the pollutant standard index (P_i) of DIN and SRP at most sampling stations belonged to GB Class I. The good condition of nutrients testified the effects that the local government had achieved on eutrophication control. However, the sampling waters still faced with the risk of hypoxia and acidification. The DO and pH values at sampling waters were at a low level with descending trend from open sea waters to the river mouth.

The results of biotic assessments including correlation analysis, AMBI and M-AMBI, which matched well with that of environmental measurements. We found that the pattern of macrobenthic abundance was closely related to the TN and TP in the research waters. Macrobenthos, together with microbes and other physical factors, continually participated in the complex nutrient circulating process across sediment-water interface (Ekeroth et al., 2016). The nutrients in turn influence the behavior of benthic organisms (Shen et al., 2016). Moreover, feeding activities of polychaetes are closely associated with TN and TP in the sediment they inhabit (Webb and Eyre, 2004; Volkenborn et al., 2007; Shen et al., 2016). Polychaetes are also identified as dominant groups in research area.

Previous research on macrobenthos had been conducted in Yantai offshores since 1985 (Wu and Zhang, 1994; Wang et al., 1995; Tang, 2011; Leng et al., 2013; Wang and Li, 2013). However, few surveys focused on the topic of biological response to environmental stressors at the Xin'an River Estuary and its adjacent waters. A precise analysis and comparison of community shift was difficult to achieve. We chose three previous surveys with same sampling methods and seasons to compare the changing of species composition in recent years (Table 5).

From 2009 to 2012, the disturbance-indifferent polychaeta species *Lumbrineris latreilli* was absolutely identified as dominant species, and Polychaeta was also the dominant taxonomic group in waters around the Xin'an River Estuary. In October 2009, 68 species were obtained in the Sishili Bay, among which *Lumbrineris latreilli*, *Musculus senhousia*, *Styela clava*, *Notomastus* sp. and *Asychis* sp. were dominant species (Tang, 2011). In September 2010, 60 species were captured in waters around the Yangma Island, among which *Lumbrinereis longiforlia*, *Mediomastus* sp. and *Sigambra* sp. were dominant species (Leng et al., 2013). In August 2010, 84 species were obtained in Yantai coastal waters, among which *Lumbrineris latreilli*, *Chaetozone setosa*, *Haploscoloplos elongatus*, and *Sternaspis scutata* were dominant species (Wang and Li, 2013). Most of the dominant species are disturbance-indifferent species in October 2009 and September 2010, while more second-order opportunistic species are defined as dominant species in November 2010 and September 2012. The macrobenthic characteristics at the research waters were in a relatively stable condition with polychaetes as the dominant species group from 2009 to 2012.

The macrobenthic communities directly or indirectly suffered from anthropogenic activities, which involve in the interactions across the sediment-water interface (Smith et al., 2000). The macrobenthic communities at waters around the Xin'an River

Table 5. Comparison of species composition with previous surveys in the Xin'an River Estuary

Sampling time	Total species	Polychaeta	Mollusca	Crustacea	Echinodermata	Others	Reference
Oct. 2009	68	54.42%	25.00%	10.29%	4.41%	5.88%	Tang (2011)
Sep. 2010	60	68.33%	10.00%	18.33%	1.67%	1.67%	Leng et al. (2013)
Aug. 2010	84	50.00%	19.05%	21.43%	3.57%	5.95%	Wang et al. (2013)
Sep. 2012	62	54.84%	16.13%	19.35%	4.84%	4.84%	this study

Estuary scattered in a geographical pattern with respective environmental stressors, which was also expressed in the CLUSTER, MDS and PCA analysis. Interfered by anthropogenic activities and environmental stress, the benthic communities in the Bohai Sea also show a trend of simplification in species composition since the 1980s, with increasing abundance of small body sized polychaete, bivalve and crustacean species but decreasing biodiversity (Zhou et al., 2007; Cai et al., 2012; Jin et al., 2015; Hu and Zhang, 2016).

AMBI and M-AMBI have proven to be operative indices on evaluating the benthic ecological conditions under various pressures of sea waters, estuaries, and coastal waters around Europe, North America, Indian and China (Muxika et al., 2005; Borja and Tunberg, 2011; Cai et al., 2013, 2015; Li et al., 2013; Liu et al., 2014; Luo et al., 2014; Sigamani et al., 2015). The establishment of reference condition for the research waters is crucial for calculating the M-AMBI value (Muxika et al., 2007). Four methods can be used to determine the reference conditions: (1) comparison with an existing undisturbed site, (2) historical data and information, (3) numerical models, and (4) best professional judgments (Borja et al., 2004; Muxika et al., 2007; Forchino et al., 2011; Borja et al., 2012). However, pristine sites as reference condition were hardly to find due to multiple disturbances at the Xin'an River Estuary. Historical data and numerical models were also unavailable. We set the reference condition with the highest richness and diversity values observed in this study and increased them by 15%, in accordance with the previous studies (Borja and Tunberg, 2011; Forchino et al., 2011; Paganelli et al., 2011). The M-AMBI value of most stations ranked as "good", which was coincided with the former research (Li et al., 2013).

Extreme conditions of pH and DO values often occurred on the same temporal and spatial scale (Wallace et al., 2014). The threshold DO value of hypoxic waters used to be below 2.0 mg/L. However, the ability of hypoxia tolerance varies with different benthic organisms. The average median sublethal DO thresholds of crustaceans, molluscs, annelids and echinoderms are (3.21±0.28) mg/L, (1.99±0.16) mg/L, (1.20±0.25) mg/L, (1.22±0.22) mg/L, respectively (Vaquer-Sunyer and Duarte, 2008). The DO value at Sta. Y1 was 1.60 mg/L, which was lower than the average median sublethal DO thresholds of crustacean and mollusc species, yet higher than that of annelid and echinoderm species. Long-term or periodic exposure to hypoxia may alter the macrobenthic community structure and thus affect the species biomass, abundance, movement and feeding activities, even the upper trophic structure (Rakocinski and Menke, 2016; Briggs et al., 2017). The species composition of Sta. Y1 indicated the macrobenthic community was exposed to hypoxia, e.g., 6 out of 7 obtained polychaete species belonged to the first and second-order opportunistic species. It is also evidenced by the minimum value of abundance (120 ind./m^2) and biomass (0.53 g/m^2) as well as the appearance of high abundance (46.67 ind./m^2) of typical pollution indicator *Capitella capitate* at Sta. Y1. Station Y5 was in moderate status, yet the macrobenthic abundance and biomass reached a quite high level. A quantity of *Lumbrineris latreilli* and *Heteromastus filiformis* was captured at Y5, whose abundance were 980 ind./m^2 and 160 ind./m^2, respectively. For this reason, the biodiversity index (H_0) at Y5 was the lowest (H_0=1.57) among sampling stations. Moreover, *Heteromastus filiformis* belongs to the second-order opportunistic species (EG IV). High abundance of *Heteromastus filiformis* resulting in a low level status of AMBI and M-AMBI at Y5, which could also be reflected by environmental factors as low DO and pH values.

Ascending level of atmospheric CO_2 result in global warming and ocean acidification, while acidic waters in coastal zones could be mainly attributed to excessive loading of nutrients and production of organisms (Fabry et al., 2008; Wallace et al., 2014). Ocean acidification would affect the growth and development process of some benthic invertebrates via meddling in the mechanisms of mineralogy and calcification, especially for crustaceans, molluscs and echinoderms (Fabry et al., 2008). Moreover, large body sized crustaceans and echinoderms species were rarely found in the research waters.

The M-AMBI results indicated the poor health status of Y1, moderate status of Y3 and Y5, which coincides well with the low DO and pH values of Y1, Y3 and Y5. The research waters suffered from hypoxia and seawater acidification due to its geographical position and aforementioned findings. These environmental and anthropogenic stressors remarkably reshaped the macrobenthic community structure.

5 Conclusions

This is the first time that AMBI and M-AMBI method were used to evaluate the benthic conditions at the Xin'an River Estuary and its adjacent waters, which is expected to be a guideline for improving monitoring measures and protective pathways. The conclusions were made as follows:

(1) The macrobenthic assemblage at waters around the Xin'an River Estuary was not shift from 2009 to 2012. Polychaetes were always presented as the dominant taxonomic group.

(2) The macrobenthic communities suffered pressures from surface runoffs and anthropogenic activities, particularly Stas Y1, Y2 and Y4 at the river mouth interfered with ocean acidification and hypoxia.

(3) AMBI and M-AMBI results showed that most sampling stations were in good to high ecological health conditions, which coincided well with the abiotic measurements.

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