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Analysis of macrobenthic assemblages and ecological health of Yellow River Delta, China, using AMBI & M-AMBI assessment method

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ABSTRACT

Yellow River delta (YRD) is a typical example of a valuable coastal ecosystem that is under increasing anthropogenic threat in China. To understand the current health status of this region, three surveys in 2011 for the abiotic conditions and macrobenthic assemblages were performed. The concentration of trace metals were relatively low in the sediment at all sampling stations representing a good sediment quality. A total of 159 macrobenthic species were identified during the three surveys. ABC curves showed that the macrobenthic fauna at 8 sampling stations suffered disturbances from human activities. M-AMBI index indicates that the benthic ecological quality of YRD is currently still not in a good condition. Five trace metals, water temperature and depth were the main environmental variables affecting the distribution pattern of macrobenthic assemblages. Community succession has occurred over the past 60 years, as evidenced by changes of species composition, key species, distribution pattern and range.

1. Introduction

The Yellow River delta (YRD) in China is a typical example of a valuable coastal ecosystem with enormous biological resources, which are under increasing anthropogenic threat. The evolution of the YRD is influenced by changes in river discharge, suspended sediment load, and changes of the river channel. Water flow and sedimentation from the Yellow River (YR) formed an important base for the extension and development of the YRD and its wetland landscape (Li et al. 2009). The current course of the YR has resulted from the artificial change of the river course in 1976, followed by a shift towards the north bank of the Qingshuigou-course in 1996 (Cui, 2002).

Over the last decades, the YRD has experienced major anthropogenic influences due to i) large-scale land reclamation for agriculture and ii) intensification of marine aquaculture (Fang et al., 2005). This has led to the large-scale replacement of wetlands by paddy fields and prawn pools (Zhang and Wang, 2008). Moreover, the pond aquaculture area increased >300 times in area between 1985 and 2005 (Zhang and Wang, 2008). The combination of increased agriculture and marine aquaculture, with at the same time a major decrease in wetland areas, has led to significant eutrophication problems, both in the YR-estuary and the adjacent sea (Zhang et al., 2008). Resulting red tides led to major economic losses. For example, in 2004 alone, a 12,000 ha covering red tide caused economic damage estimated at 366 thousand dollars. Moreover, for phytoplankton and zooplankton it has been shown that biodiversity significantly declined due to such red tides (Ji, 2006). There is lack of in depth analysis of the ecological impact of this long-term anthropogenic developmental trend in the YRD region, and it remains unpredictable how these developments will affect the future of the YRD ecosystems. To maintain the fishery resources, the government implemented a policy since 1979, e.g. the forbidden fishing period, which lasts from June 1 to September 1. During this period, all the fishing activities in Bohai Sea are forbidden. The implementation of the above policy not only benefits for the recovery of economical marine resource, but also for the ecosystem, including the recovery of macrobenthic fauna.

Macrobenthic communities form a critical component of estuarine ecosystems, playing a vital role in maintaining ecosystem functions such as the reworking, breakdown and incorporation of organic matter into sediments and the energy flow in estuarine food webs (Heip et al., 1995; Herman et al., 1999). Macrobenthic invertebrates are an important food resource for many crustaceans, fish and birds. Humans also harvest many species of shellfish and crustaceans. Macrobenthic communities typically shift in species composition in response to changing abiotic conditions and human interferences (Pearson and Rosenberg, 1978; Ysebaert and Herman, 2002; Snelgrove, 1998). The latter makes that macrobenthos is frequently used as a long-term indicator for

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ecosystem health status (Bilyard, 1987; Diaz et al., 2004). As macrobenthic species are relatively sedentary, their species composition and abundance can be used as biological indicators to reflect changes in the marine environment, such as, the deterioration of water and sediment conditions (Pearson and Rosenberg, 1978; Borja et al., 2000; Borja and Muxika, 2005; Borja and Tunberg, 2011).

In this study, we analyzed the sediment trace elements and bottom water nutrient content as well as the macrobenthic fauna of the coastal area of YRD, all as indicators of the environmental status. Moreover, we explored if there was a relationship between environmental variables and macrobenthic assemblages, using Principal Component Analyses (PCA) analyses. We subsequently tried to (1) identify the impacts of different anthropogenic activities on the benthic assemblages by calculating ABC-curves, biodiversity index and richness and (2) to assess the benthic ecological health status of this special coastal zone by using AMBI and M-AMBI (Borja et al., 2000; Borja and Muxika, 2005). The AMBI and M-AMBI were derived for different periods of the year, to assess the effect of the presence and absence of fisheries.

2. Materials and methods

2.1. Field site characterization & sampling stations

The YRD is located in the southern part of the Bohai sea (Fig. 1). It has undergone great changes in environmental conditions over the past 60 years (Table 1). The bottom water temperature was 17.79 °C in 1950s and became 17 °C in 2000s, with slightly downward trend in Laizhou Bay (Comprehensive sea survey department in State Scientific and Technological Commission, 1961; Zhou et al., 2012), while in the Southern Bohai Sea (including survey sites from Bohai Bay, coastal water of Yellow river and Laizhou Bay), it had an average increase of 0.013 °C per year (Ning et al., 2010). The bottom salinity was stable at 28.7 PSU between 1950s and 1980s and then increased to 30 PSU in 2000s with an increasing rate of 0.105 PSU per year (Comprehensive sea survey department in State Scientific and Technological Commission, 1961; Zhou et al., 2012; Ning et al., 2010). Both the meiofaunal and macrofaunal abundance were 2–5 times greater in the 1990s than in the 1980s and the 2000s (Zhou et al., 2012). The sediment grain size generally tended to become coarser in Laizhou Bay (Zhou et al., 2012). The biogenic element in southern Bohai Sea also changed

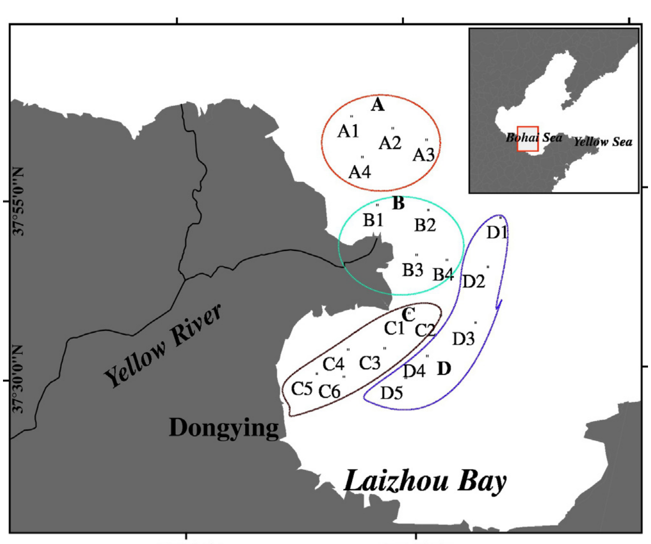

Fig. 1. Sampling sites for macrobenthic assemblages in YRD. Four zones were artificially divided based on different disturbance factors: zone A is an area with drilling platform (stations A1 to A4); zone B represents the new YR entrance since 2007 (stations B1 to B4); zone C represents the old YR entrance before 1996 with aquaculture zone (stations C1 to C6); zone D represents the offshore area (stations D1 to D5).

obviously (Ning et al., 2010), e.g. a significant decrease in DO concentration was observed in the southern Bohai Sea during the period 1978–1991; both bottom P and Si concentrations exhibited decreasing trends in the 1978–1996; the concentration of DIN increased from 2.9 in 1985 to 8.7 μmol L^{-1} in 1996 during 1985–1996, which lead to the increase of N:P and N:Si ratio, then affect the phytoplankton community. The sediment chlorophyll *a* and phaeopigment concentrations were 7 to 9 fold lower in the 2000s than in the 1980s and 1990s (Zhou et al., 2012). However, the organic content in the sediment also increased by 3–4 folds in 1990s and markedly decreased in the later ten years (Zhou et al., 2012; Ning et al., 2010).

To investigate the current status of macrobenthic fauna around YRD, we selected 19 stations to be representative of four zones around the entrance of YR: zone A near shore drilling platform area, with impacts from construction and oil spilling (station A1 to A4); zone B near the new YR entrance since 1996, with the impacts from Yellow River discharge (station B1 to B4); zone C near the old YR entrance from 1976 to 1996, with impacts from the aquaculture (station C1 to C6; zone D representing the offshore area, with relatively less disturbed from human activities (station D1 to D4) (Fig. 1). Distance between two sampling sites was more or less 8 km, due to the fishing nets and sea conditions. Three sampling surveys (i.e., May, August and November 2011) were carried out to collect i) sediment trace metals, ii) nutrient content in bottom water and iii) macrobenthic assemblages in the Yellow River delta (YRD). Due to the weather and sea conditions while sampling, the number of stations sampled during the three surveys differed, e. g., 19 stations in May, 18 stations in August and 15 stations in November.

2.2. Sampling methods and procedure

Macrobenthos samples: Macrobenthos was collected by taking sediment samples ($n = 3$) of 0.05 m^2 by using a van Veen grab at each station. The samples were sieved through a mesh with a 1.0 mm aperture to collect all macrobenthic organisms. All animals were subsequently preserved in 80% ethanol until laboratory identification to the lowest possible taxonomic level, then counted and weighted (wet weight) using a 0.01 g precision electric balance. Near bottom water samples and sediment samples were collected in situ for further analysis on sediment trace elements and bottom water nutrient content.

Abiotic parameters: Near bottom seawater were collected using a Go-Flo bottle (5 L). At each station, three surface sediment samples were collected using a hand corer and the samples were well mixed to form a composite sample. To characterize the abiotic environment, we measured at all stations the water depth, water temperature, salinity, nutrient concentrations (NH_4-N, NO_2-N, NO_3-N, PO_4-P) in the bottom water, and the concentration of trace metal (Cr, Co, Ni, Cu, Zn, Cd, Pb, As) in the surface sediment (i.e., 0–5 cm). The depth, temperature and salinity of ambient seawater were measured onsite by a portable 600QS sensor (YSI Incorporated, USA). Analyses of nutrients (NH_4-N, NO_2-N, NO_3-N, PO_4-P) in bottom water samples were performed on a gas-segmented continuous flow system (AutoAnalyzer 3, SEAL Analytical, Germany). The grain size of collected surface sediments was measured by a laser particle sizer (Mastersizer 2000, Malvern Instruments, UK), which yields the reproducibility within ±5% (from 0.02 to 2000 μm) as revealed by five repeated tests for each sample. Following the digestion process of Li et al. (2013a), the concentrations of potential hazardous metals (Cr, Co, Ni, Cu, Zn, Cd, Pb, As) were measured by an Elan DRC II inductively coupled plasma mass spectrometry (Perkin-Elmer, USA). A combined internal/external standard method was applied in analyses and In^{115} was selected as the internal isotope. The measurement was repeated three times for each element during one ICP-MS test, which demonstrates that the relative standard deviation (RSD) for each element is well restricted within the range of ±2.5% for most samples and ±5% for all samples. For quality control, standard addition tests were further performed by the spike of 0.1 mL mixed standards (5000 μg/L for each element) to five different aliquots of 10 mL digestion

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Table 1
Interannual change of benthic environmental parameters in southern Bohai Sea.

Laizhou Bay					Southern Bohai Sea			
Benthic environmental parameters	1950s① (n = 17)	1980s② (n = 24)	1990② (n = 5)	2000s② (n = 25)	1960–1996③			
					Benthic environmental parameters	Annual rate	Amplitude[a]	Observed period
Depth (m)	15–20	17	16	15	BS(PSU year^{-1})	0.105	3.79	1960–1996
Bottom temp.(BT) (°C)	17.79	19	–	17	BT(°C year^{-1})	0.013	2.573	1960–1996
Bottom salinity (BS) ‰	28.7	28.7	–	30.0	Bottom DO (μmol L^{-1} year^{-1})	−1.596	68	1978–1991
DO (mg L^{-1})	–	–	–	8.53	Bottom P (μmol L^{-1} year^{-1})	−0.011	0.536	1978–1996
Chl-α (mg kg^{-1})	–	6.12	2.92	0.85	Bottom Si (μmol L^{-1} year^{-1})	−0.602	28.671	1978–1996
Pha-α (mg kg^{-1})	–	14.78	4.21	2.08	Bottom DIN (μmol L^{-1} year^{-1})	0.613	5.92	1985–1996
Organic%	–	0.76	1.79	0.59	N:P	1.401	32.7	1985–1996
Silt-clay%	–	93	98	80	Si:N	−0.064	2.6	1985–1996
MDφ	–	3.19	6.86	5.13	–	–	–	–
QDφ	–	0.82	2.4	1.93	–	–	–	–
Meiofauna abundance (ind. 10 cm^{-2})	–	1012	1056	842	–	–	–	–
Macrofauna abundance (ind. m^{-2})	–	1415	1659	1049	–	–	–	–

① Refers to Comprehensive sea survey department in State Scientific and Technological Commission, 1961, ② refers to Zhou et al., 2012, ③ refers to Ning et al., 2010.
"–" means survey data is missing.
[a] The amplitude is the difference between the maximal and the minimal annual mean values during the observed period.

solutions. The recovery rates were finally determined to fall into an overall range of 92.8–106.7%. Sediment samples for trace metals analysis were collected only in May 2011 and bottom water samples were collected only in November 2011. These additional samples were used to evaluate the relationship between environmental variables and macrobenthic assemblages. We did not collect the sediment and water samples in all three surveys as we expected the average values of these parameters to be good indicators of the environmental status, with the seasonal variability being less important.

2.3. Statistical analysis

The Plymouth routines in multivariate ecological research (PRIMER 6.0) software and SPSS 15.0 were used for statistical analysis. The biological properties included are total biomass (*B*; *g. m*$^{-2}$, *wet weight*), abundance (*A*; *ind. m*$^{-2}$), number of species (*S*), Shannon-Wiener diversity index (H') (Shannon and Weaver, 1963), Margalef richness index (*d*), Evenness index (J') (Pielou, 1966) and the dominant index (*Y*). Three biodiversity indices were calculated according to the following formulas (Eq. (1)) and the dominant index was calculated by Eq. (2) (Chen et al., 1995):

$$H' = \sum_{i=1}^{s} P_i \log_2 P_i; d = (S-1)/\log_2 N; J' = H'/\log_2 S \quad (1)$$

$$Y = (ni/N)\,fi \quad (2)$$

where *Pi* is the percentage of the abundance of species *i*; *N* is the abundance of all species at all the stations; *S* is the number of macrofaunal species of each sample; *ni* is the abundance of the species *i* at all the stations; and *fi* is the occurrence frequency of species *i* at all the stations.

Principal component analysis (PCA) was performed to identify the dominant environmental factors. Environmental variables were normalized prior to applying PCA. Biota- Environment Stepwise Analysis (BIOENV/BVSTEP) was conducted to explore the correlation between single environmental factor and benthic community structure. BVSTEP analysis was performed to study the optimal combination of environmental factors impacting the community structure. One –way ANOVA were used to test if there are significantly different or not among four zones, whereas non-parametric Mann–Whitney rank sum test were used in case of unequal variances. Statistical tests were performed with PASW Statistics version 19 at a 0.05 significance level.

Five functional groups of macrofauna was defined according to their food resource by referred to Li et al. (2013b) and Liu et al. (2015), including (1) Planktophagous group (Pl): suspension-feeding on small microzooplankton; (2) Phytophagous group (Ph): feeding on vascular plants and seaweeds; (3) Carnivorous group (C): feeding on meiofauna and larva; (4) Omnivorous group (O): feeding on rotted leaf, small bivalves and crustaceans; (5) Detritivorous group (D): feeding on organic detritus and sediment.

2.4. Health indicators

2.4.1. Abundance/Biomass comparison (ABC method)

The Abundance/Biomass comparison (ABC method) is an internal comparison of abundance and biomass distribution of the species, which can be used to indicate the levels of (pollution induced or other) disturbance on the benthic macrofauna communities (Clarke and Warwick, 2001). Species are ranked in order of their dominance to the community on a logarithmic *x*-axis, and with percentage dominance in terms of abundance and biomass on the *y*-axis (Clarke and Warwick, 2001; Pagola-Carte, 2004). The *W* statistics (Clarke, 1990) were also calculated by applying the equation:

$$W = \sum_{i=1}^{s} (B_i - A_i) \Big/ 50(S-1)$$

where *S* is the number of macrofaunal species of each sample, *Bi* is the biomass of the species *i*, *Ai* is the abundance of the species *i*. The ABC curve and *W* were computed by DOMPLOT program included in the PRIMER 6.0 statistical package. *W* gets a value between −1 and 1. A value of *W* close to +1 indicates unpolluted conditions, whereas a value close to −1 points at disturbed/polluted conditions (Clarke and Warwick, 2001). And, when the macrofaunal abundance curve fall above the biomass curve or the two curves crossover, the benthic environment is considered as disturbed conditions; otherwise it is in a undisturbed conditions (Clarke and Warwick, 2001).

2.4.2. The Azti Marine Biotic Index (AMBI) and Multivariate-AMBI (M-AMBI)

The Azti Marine Biotic Index (AMBI) and Multivariate-AMBI (M-AMBI) were calculated by means of AMBI program (version 5.0, using the species-list of V. March 2012) freely available online at http://ambi.azti.es. By using the species-list of March 2012 and judged by expert opinion, all the species collected during three surveys were assigned to different ecological groups according to their different tolerance of environmental pollutant. Based on the AMBI guidelines (Borja and Muxika, 2005) and Borja and Tunberg (2011), the threshold values for the M-AMBI conditions are as follows: 'high' quality > 0.77; 'good' = 0.53–0.77; 'moderate' = 0.38–0.53; 'poor' = 0.20–0.38; and 'bad' < 0.20. According to the guidelines for the use of AMBI (Borja and Muxika, 2005), all of the non-benthic invertebrate taxa (fish and megafauna) were removed.

According to the AMBI program, the value of AMBI ranges from 0 to 7, with the lower value the better in ecological health; the value of M-AMBI ranges from 0 to 1, with the higher value the better in ecological health. The reference condition is vital important as assessing ecological health of water body using M-AMBI. By this criterion and results of AMBI, the water body can be defined as good or bad in ecological health. To get an appreciate reference condition, we adopted the following method: chose the lowest AMBI value and highest value of diversity H ′, richness S of three surveys in YRD, then increased these value by 15%, as the M-AMBI reference conditions in this area (Li et al. 2013a), e.g., AMBI = 0, H = 5.45, S = 41 and the 'worst' possible values were based on the following values: AMBI = 6, H = 0, and S = 0, representing the conditions resulting from major human activities impact.

3. Results

3.1. Abiotic parameters

3.1.1. Analysis of sediment grain size, trace metal and bottom water nutrient content

The sediment type of YRD was primarily dominated by fine silt at most of the sampling stations, except for station B2. The YR water carries a large amount of silt from the Loess Plateau area to the Laizhou Bay every year and deposits them at the inlet of the YRD, which forms the silty sediment type of this region.

The trace metal content in the sediment of YRD varied across sampling sites (Table 2). Nevertheless, most of the trace metal content at sampling stations was qualified as Class I. The only exception was the Cd content at station A2 and the As content at stations A2, A4, C3, D5, which qualified as class II. Our results thus indicate that the sediment quality of the YRD was generally good according to Chinese Marine Sediment Quality Standard GB 18668-2002 (i.e., National Standard of the People's Republic of China GB 18668-2002, issued by General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China on March 10, 2012). And, the content of trace metals among the four zones were significantly different (Multi-factor variance analysis, F = 3.5, $p < 0.05$).

High values of PO_4-P concentrations were located in an area with offshore drilling platforms, with the highest values of 10.6 μg/L, while lowest values of 3.44 μg/L were located near the old YR entrance. Mean PO_4-P concentrations in the bottom water were around 5.87 ± 2.09 μg/L (Table 3). The highest concentrations of NO_3-N (0.53 μg/L) and NO_2-N (34.22 μg/L) were observed at station C1, indicating that this station may have experienced anthropogenic nitrogen input. The concentration of NH_4-N was high at stations A3, A4, C6 and D5 (Table 3), all located near an offshore drilling platform area (A3 & A4) or at the old YR entrance (C6 & D5). However, these observations relate to trends, as the concentration of PO_4-P, NO_3–N, NO_2–N and NH_4-N among the four zones were not significantly different (Multi-factor variance analysis, F = 0.827, $p > 0.05$). Thus that all zones should be regarded similar in terms of nutrient concentrations.

3.2. Species composition

In total, 159 species of macrobenthos were identified during our three surveys, with 88 species in August, 85 species in Spring and 73 species in Autumn. Polychaeta represented the most abundant taxon with 53 species (33.3%), followed by Crustacea with 46 species (28.9%), Mollusca with 45 species (28.3%), Echinodermata with 5 species (3.1%), other groups with 7 species (4.4%). The species number in zone D in May 2011 was significantly higher than that of the other three zones (F = 3.5, $p < 0.05$, ANOVA Test). A total of 16 dominant species were identified according to the value of dominant index (*Y*). However, the dominant species composition differed among the three surveys (Table 4). The 16 dominant species belong to 4 types of functional groups: 6 species in the detritivorous group (D), 5 species in the planktophagous group (Pl), 4 species in the carnivorous group (C) and 1 specie in omnivorous group (O).

3.3. Biomass and abundance

The average value of biomass was 32.5 ± 16.33 g m^{-2} at 19 sites among three surveys, of which the Mollusca species contributed approximately 44%, the Echinodermata approx. 32%, and the Crustacea and Polychaeta approx. 10%. The spatial distribution patterns of macrobenthic biomass for the 19 stations in May 2011 were uneven, with the higher values found at two stations at the old YR entrance (i.e., C5 and C6) and two stations near the drilling platform area (i.e., A4 and B2) (Fig. 2, left). However, the biomass values for each of the

Table 2
The content of trace metals (g/m^3) and silt content (%) in the sediment of YRD as sampled in May 2011. Zones A, B, C, D were assigned based on different disturbance factors (for details see legend of Fig. 1).

Sites	Cr	Co	Ni	Cu	Zn	Cd	Pb	As	Silt (%)
A1	58,884	10,544	31,569	22,455	110,003	323	21,318	18,341	65.85
A2	77,220	11,936	34,236	24,927	107,175	505	20,308	22,728	63.65
A3	54,702	10,270	30,884	20,033	91,451	293	17,842	16,814	73.78
A4	63,465	11,995	34,953	26,260	106,989	362	24,055	21,473	73.19
B1	62,385	10,156	29,881	20,722	95,717	363	17,638	18,235	86.88
B2	57,560	7440	21,938	13,134	82,038	416	17,722	12,258	17.50
B3	56,054	9986	31,092	21,059	100,704	425	18,214	17,647	73.05
B4	61,138	9862	28,788	18,551	93,397	353	15,615	16,580	59.63
C1	58,573	9714	31,093	20,181	104,260	339	17,626	15,996	67.28
C2	65,340	11,735	34,063	23,908	102,684	368	20,569	18,224	73.84
C3	66,442	11,011	31,512	21,575	99,370	458	19,720	20,182	69.63
C4	65,748	8022	23,393	15,661	71,294	463	15,933	13,042	35.89
C6	60,611	9707	29,346	20,387	107,754	491	18,322	15,555	71.23
D1	55,917	9210	29,137	19,888	93,293	302	17,083	16,878	67.55
D2	53,479	8721	26,923	17,156	81,513	372	16,059	13,846	56.27
D3	66,108	8368	25,209	16,552	91,963	444	15,884	14,098	54.53
D4	67,413	12,534	37,709	27,738	111,942	379	22,262	19,204	75.98
D5	76,265	14,353	40,660	30,312	122,988	403	24,125	24,337	62.16

Table 3
The nutrient content (μg/L) in bottom column water of YRD, as sampled in November 2011. Zones A, B, C, D were assigned based on different disturbance factors (for details see legend of Fig. 1).

Sites	PO_4-P	NO_3-N	NO_2-N	NH_4-N
A1	7.64	261	24.26	37.22
A2	10.60	248	7.27	25.91
A3	9.33	137	8.41	90.55
A4	6.50	309	4.51	49.92
B2	6.19	345	19.44	20.88
B4	5.38	185	0.62	36.86
C1	5.80	525	34.22	35.80
C3	4.74	453	13.69	15.21
C4	4.09	323	7.99	25.12
C6	3.44	540	9.25	57.73
D1	6.21	400	10.98	38.74
D2	6.69	343	22.62	37.43
D3	4.14	403	7.53	25.27
D4	3.96	117	1.19	27.98
D5	3.58	539	24.25	48.30

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Table 4
The dominant species of three surveys in YRD, sorted from high to low dominant value. The dominant value Y was calculated cf. (Eq. (1)). (The + and − values indicates the dominant species to be present or absent during different sampling periods. Pl refers to planktophagous groups; Ph, phytophagos groups; C, camivorous group; O, omnivorous group; D, detritivorous group).

Species	Group	Sampling period			Dominant value (Y)	Functional group
		May	August	November		
Ostracoda sp.[a]	Crustacea	−	−	+	0.219	Pl
Ringicula doliaris[a]	Mollusca	−	+	−	0.067	O
Ophiuroidea sp.[a]	Echinodermata	−	−	+	0.065	D
Alvenius ojianus[a]	Mollusca	−	+	−	0.059	Pl
Heteromastus filiformis[a]	Polychaeta	−	+	−	0.045	D
Amphioplus japonicas[a]	Echinodermata	+	−	−	0.045	D
Glycinde gurjanovae[a]	Polychaeta	+	+	−	0.038	C
Iphinoe tenera	Crustacea	+	−	−	0.037	D
Sthenolepis japonica	Polychaeta	−	+	+	0.033	C
Amaeana occidentalis	Polychaeta	−	+	−	0.032	D
Leptochela gracilis	Crustacea	−	−	+	0.030	Pl
Anaitides papillosa	Polychaeta	−	−	+	0.026	C
Nitidotellina minuta	Mollusca	−	+	−	0.025	Pl
Lumbrineris heteropoda	Polychaeta	+	−	+	0.023	C
Chaetozone setosa	Polychaeta	−	+	−	0.023	D
Moerella iridescens	Mollusca	−	+	−	0.020	Pl

[a] Distinguished dominant species belonging to different Group and functional groups with important roles in the material circulation.

four zones in May 2011 were not significantly different ($\chi^2 = 1.6$, $p > 0.05$, Kruskal Wallis ANOVA Test).

The average abundance value was 226 ± 178 ind. m^{-2} at 19 sites in May 2011. The spatial distribution patterns of macrobenthic abundances were different from those of the biomass, with the higher abundance values distributed in the offshore area and the old YR entrance than other two zones (Fig. 2, right). And, the abundance value in zone D in May 2011 was significantly higher than that of the other three zones ($F = 3.27$, $p < 0.05$, ANOVA Test).

3.4. Biodiversity

In May 2011, the Shannon-Wiener index H' varied from 0.00 to 3.86 (with the average value of $2.84 \pm 0.0.68$) at 19 stations. The highest H'-value of 3.86 was found at station B2; the lowest H'-value of 1.79 at station B3. Margalef richness index D ranged from 0.81 to 3.76 (average value of 2.03 ± 0.75), with the highest value of 3.76 observed at station D3, and the lowest value of 0.81 at station B3. Evenness index J' ranged from 0.55 to 0.94 (average value of 0.82 ± 0.12), with the highest value of 0.94 at station B2, and the lowest value of 0.81 at station D2 (Fig. 3). However, these observations relate to trends, as the three biodiversity indices value for each of the four zones in May 2011 were not significantly different ($F = 0.91$ J', $1.4H'$, D 2.99, $p > 0.05$, ANOVA Test). This indicates that all zones should be regarded similar in terms of biodiversity.

3.5. ABC curves

To assess the "disturbance status" as proxy for the human impact on the benthic assemblages, we analyzed the ABC of the macrobenthos based on the data of the May cruise. ABC curves with corresponding W value were obtained for 19 stations (Fig. 3), showing that the benthic fauna of the 19 stations suffered different disturbance levels. Four stations (A1, A3, C1, D2) were considered as "perturbed" according to the negative W values; four stations (A2, C4, D1, D4) were classified as "moderately perturbed" because of the low W values and the crossover of two curves; and the other 11 stations showed positive W values and were considered to be "less perturbed", with abundance more evenly distributed than biomass (Fig. 4).

3.6. AMBI

The value of AMBI ranges from 0 to 7, with the lower value representing a better ecological health. In May 2011, the mean values of AMBI from three replicates of each sampling stations ranged from 0.64 to 3.25, with only 1 (5.3%) undisturbed station (station B1; AMBI = 0.638), and 18 (94.7%) slightly disturbed stations (i.e., AMBI between 1.23 and 3.25), as only AMBI values over 5.5 are representative for a heavily disturbed environmental quality and over 6 for an extremely disturbed environmental quality. These AMBI values thus imply that the benthic environment only slightly suffered from human activities.

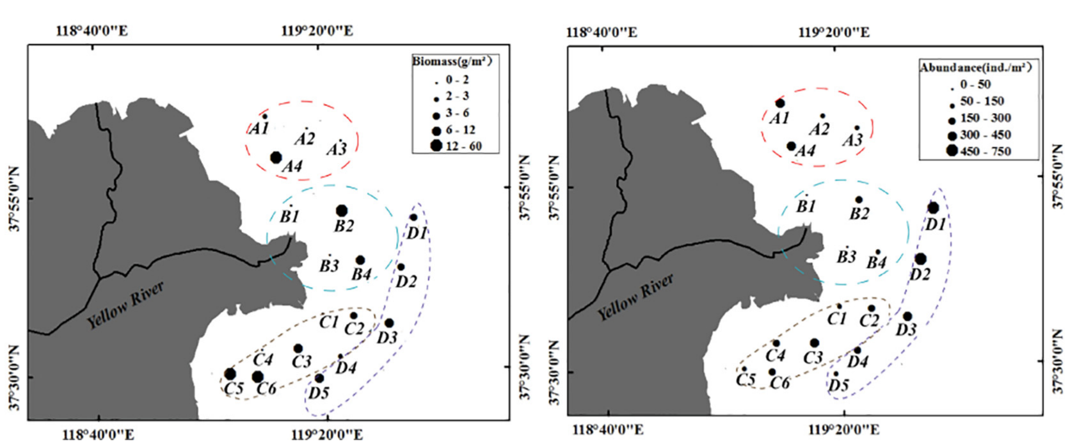

Fig. 2. The spatial distribution of biomass (left) and abundance (right) of macrobenthic fauna in YRD and adjacent waters in May 2011.

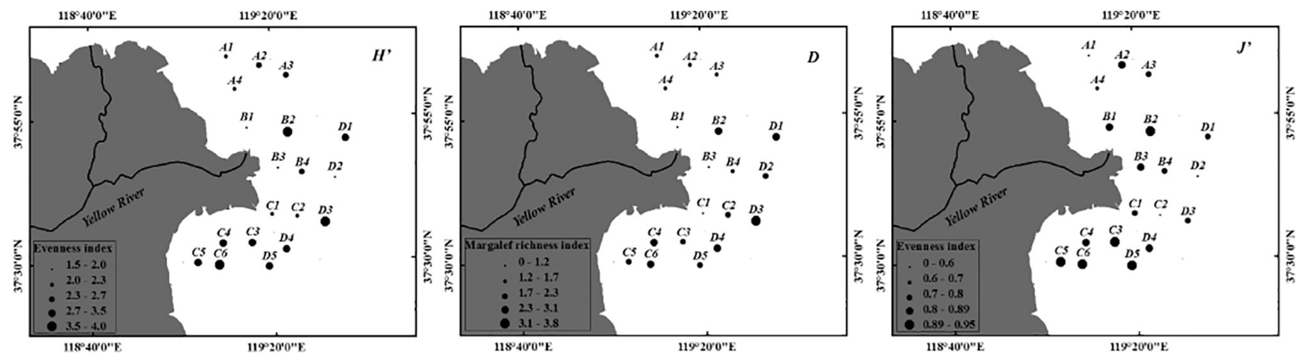

Fig. 3. Three biodiversity indices in YRD and adjacent waters in May 2011.

Apart from four stations with over 20% not assigned species (i.e., stations A2, B2, D4 and D5), the results of AMBI at most of stations were acceptable.

In August 2011, the mean AMBI values of 18 sampling stations varied from 0.37 to 2.24, with 4 (22.2%) undisturbed station (stations A3, C4, D2, D4), and 14 (78%) slightly disturbed stations. This implied that the benthic assemblages had only slightly suffered disturbance either from environmental change or human activities. Apart from the above four stations (i.e., stations A2, B2, D4 and D5) with over 20% not assigned species, the results of AMBI at most of stations were acceptable.

In November 2011, the mean AMBI values of 10 sampling stations ranged from 0.42 to 1.86. However, due to 5 stations (i.e., A1, C1, D2, D4, D5) having over 20% of unassigned species, the AMBI values were only acceptable for the 5 remaining stations: i.e., A2, C3, C6, D1, D3. From these 5 stations, four were regarded undisturbed and one station was regarded slightly disturbed, implying that the benthic assemblages had not or only slightly suffered from environmental change or human activities.

3.7. M-AMBI

In May 2011, the M-AMBI results revealed that 5 stations (i.e., C3, C4, C6, D1, D3) from 15 sampling stations (i.e., 4 stations were exclusive; A2, B2, D4 and D5) with 'good' ecological status (ES), and 7 stations (i.e., A1, A4, B4, C1, C2, D2, D5) stations with 'moderate' ES; the other 3 stations (A3, B1, B3) had a 'poor' ES. The stations with good ES were located outside of the new channel of YR and the south of the old YR channel. In contrast, the stations with poor ES were located in the area close to the new YR channel and the offshore drilling platform. The M-AMBI value of zone C was significantly higher than that of other three zones, which indicates that the ecological status of zone C was better that the other three zones (F $= 10.6$, $p < 0.05$, ANOVA Test).

In August 2011, the benthic ecological health was markedly improved comparing to that of May 2010 based on the M-AMBI of 14 sampling stations: station D3 had 'high' ecological status (ES), 12 stations (80%) had 'good' ES, and station B1 had 'moderate' ES. Compared to May and August 2011, the benthic ecological health improved in November 2011: of all the 5 stations, 2 stations (C6, D4) had 'high' ES, 2 stations (A1, C4) 'good' ES and one station (D1) 'moderate' ES (Fig. 2). The M-AMBI value of the four zones were not significantly different, which indicates that the ecological status of the four zones was similar (F $=$ 0.56, $p > 0.05$, ANOVA Test) (Fig. 5).

When taking into account 11 common stations between surveys of May and August (except of the stations with not assigned species higher than 20%), the M-AMBI values were statistically significantly different between May and August (Krushal-Wallis ANOVA, Chi-sq. $= 9.32$, $p <$ 0.01), which indicates that the ecological status improved from May to August.

3.8. Correlation analysis between biotic and sampling stations

Principal components analysis (PCA) based on environmental variable (including water temperature, salinity, water depth, pH, trace metal concentrations in the sediment and grain size) at the sampling stations in Yellow River Estuary (Fig. 5), showed the first two principal components (PC1 and PC2) explaining 72% of the total variability. On PC1 axis, trace metals Co, Ni, Cu, Zn and As were important variables to differentiate the sampling stations. Whereas on PC2 axis, the important variables changed to temperature and water depth. The above 5 trace metals as well as water temperature and depth were the main environmental variables leading to the difference of sampling stations, and further affect the distribution pattern of macrobenthic assemblages (Fig. 6).

The abundance and biomass of macrobenthic assemblage were significantly related to above measured environment variables ($R =$ 0.299, $p < 0.05$ for abundance; $R = 0.265$, $p < 0.05$ for biomass) (RELATE analysis in Primer software). Analysis of BIOENV showed that abundance were closely related to water temperature, sediment grain size, concentration of Zn and Pb (with the spearson coefficient of 0.44); and biomass was related to water temperature, water depth, sediment grain size, concentration of Ni, Zn and Pb (with the Pearson coefficient of 0.40). The biodiversity indices were also related to environmental variables, of which the evenness index (J') was significantly negatively related to water depth ($R = -0.47$, $p < 0.05$); and richness index (d) was significantly related to the silt content ($R = 0.47$, $p < 0.05$).

The YRD bottom water nutrient content also impacted the distribution pattern of macrobenthic assemblages. The biomass and abundance were closely related to the content of NO_3-N, NO_2-N with the spearson coefficient of 0.41 and 0.34, respectively (BIOENV analysis in Primer); the richness index (d) and Shannon–wiener index (H') was significantly negative related to PO_4-P ($R = -0.84, p < 0.01$).

4. Discussion

In all our YRD sampling stations, the sediment type was primarily dominated by fine sandy mud & mud, with only low trace-metal concentrations (i.e., Class I according to the Chinese Marine Sediment Quality Standard GB 18668-2002). The latter indicates that the area can legally be used for marine fishery, natural reserve areas, natural preservation zones for rare and endangered animals, marine culture zones, bathing beaches, direct body contact marine sports and industrial water area related to marine foods. If sediments are Class II, the area can legally be used for normal industrial water and coastal scenic areas. The nutrients in near-bottom water were not significantly

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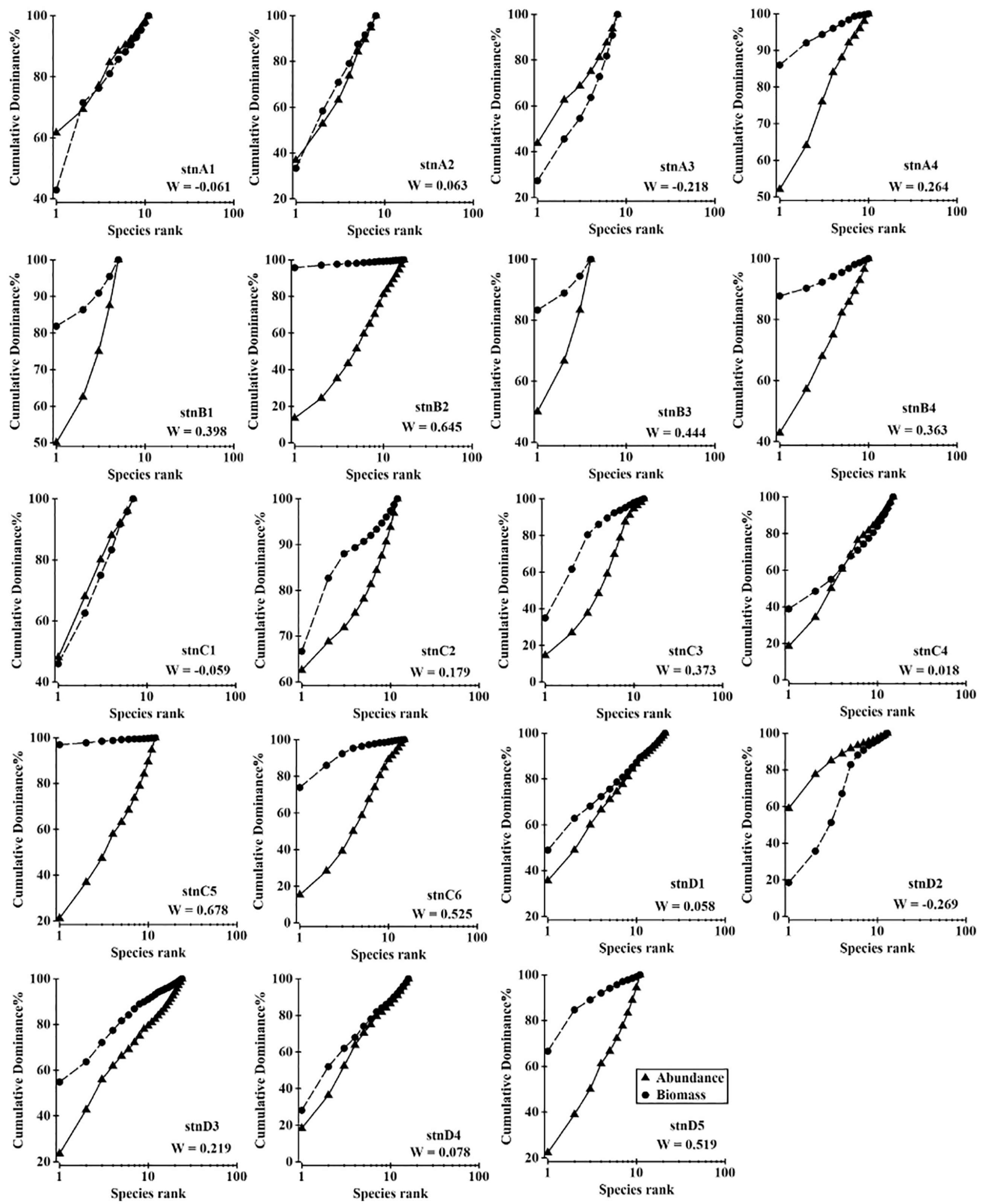

Fig. 4. ABC curves of macrobenthic fauna in YRD in May 2011, as sampled at station 1 to 19 (Stn A1-D5; different colors indicate disturbance status: red = perturbed, yellow = moderate perturbed, green = less perturbed). The *W* statistics in each panels takes (values ranging between −1 to 1) indicates the disturbance/pollution level, with *W* close to +1 indicating pristine conditions and *W* close to −1 indicating disturbed/polluted condition. When the macrofaunal abundance curve fall above the biomass curve or the two curves crossover, the benthic environment is considered as disturbed (perturbed) conditions; if the abundance curve always is below the biomass curve, it is in an undisturbed conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

different among the four zones. However, the content of trace metals in sediment was significantly different among the four zones. The biomass, abundance and biodiversity indices of marcobenthos assemblages were closely related to trace metal contents, which suggest that anthropogenic activities may have impacted the benthic community by increasing the trace metal content. The distribution patterns of macrobenthic

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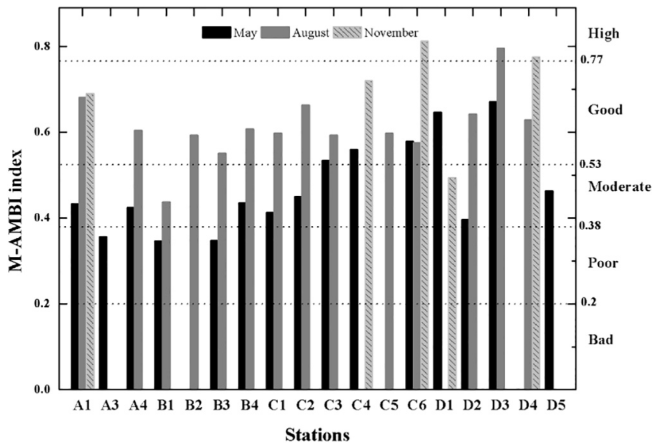

Fig. 5. M-AMBI values of YRD in May, August and November 2011. The label on the right axis, "bad to high" indicates how the ecological status of sampling sites relates to the observed M-AMBI values.

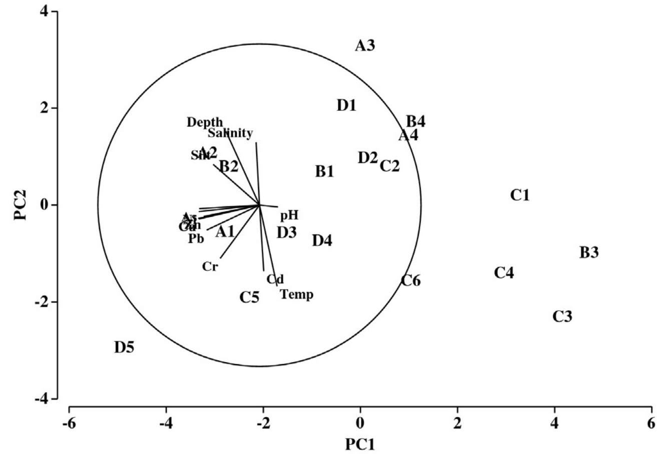

Fig. 6. Two-dimensional PCA ordination of the environmental factors of sampling stations in May 2011 (the trace metals overlapped in the figures were Co, Ni, Cu, Zn, Pb and As).

species and abundance also differed between the four zones. Species number and abundance in zone D were significantly higher than that of other three zones.

4.1. Macrobenthic community succession and functional group in YRD and the driving factors

Comparing the current survey with previous investigations on the benthic macrofauna in YRD and adjacent waters, reveals that some community succession has occurred over the past 60 years, including the temporal and spatial changes of species composition, key species, distribution pattern and range (original data of National Sea Surveys in 1958, China, unpublished; Sun and Tang, 1989; Sun and Liu, 1991; Han et al., 2001, 2003; Zhou et al., 2010; Liu et al., 2014; Wu et al., 2014). Three different temporal stages can be divided based on the changing species composition, biomass and abundance. The first stage was before 1960s, when the community was characterized by a low number of species composition, high biomass and abundance, with commercial molluscs and crustacean as the dominant groups. The second stage was from 1980s to 2006, during which the assemblages had changed by increased species number, combined with decreased biomass and abundance. The dominant groups also changed to small molluscs and echinoderms. The third stage started after 2006, showing community recovery as presented by the increased biomass and dominant station of both molluscs and crustaceans (Chen et al., 2016). Previous investigation also showed similar trend for the macrobenthic community in the Bohai Sea (Zhou et al., 2007, 2012).

Dominant species can characterize a community by the functional groups that they belong to. Functional groups are defined as species with similar effects on the major ecosystem processes (Chapin et al., 1992). Different tropical functional groups play different role in the benthic ecosystem. That is, they can play an import role in the processes of transformation and decomposition of organic and inorganic matter inside the sediment due to their bioturbation, e.g., feeding, burrowing and construction activities (Aller, 1994, 2001). The suspension-feeding functional group may induce facilitative interactions, and enhanced the resource consumption (Cardinale et al., 2002), which will be beneficial to other groups. Other functional group (mainly depositional food habit and burrowing behavior) decompose the detritus and increase the oxygen sediment porewater, and accelerate the decomposition of organic matter (Pearson, 2001). The 16 dominant species in YRD belong to Pl group and D, C, O groups, respectively, also plays crucial role in matter decomposition and nutrient recycling for the benthic ecosystem.

It is well known that anthropogenic activities and environmental factors can strongly influence the spatial and temporal distribution of macrofaunal abundance and biomass (Magni et al., 2005, 2006). Pollution may also cause dramatic reductions in diversity of macrobenthic communities (Snelgrove, 1998). Thus the environmental changes that occurred during the last decades in the different zones of the YRD (Fig.2) may have induced a shift in the macrobenthic community. The disturbance condition and benthic health among the four designed zones showed obvious differences in May 2011. This suggests that the impact from different human activities (pollutant discharge, oil industry and aquaculture) on macrobenthic fauna within our YRD study area is continuous rather than per zone, most likely due to the relatively small scale of sampling area in this region. Actually, in this four zones, the different human activities were mixed just with one primary activities, e.g. in the oil industry zone also having aquaculture activities and in the aquaculture zone also with the pollutant discharge.

The marine culture area (prawn pool) in YRD and adjacent area increased 7713 hm^2 between 1997 and 2004 (Zhang et al., 2008). The combination of increased agriculture and marine aquaculture has led to significant eutrophication problems, both in the YR-estuary and adjacent sea. Marine aquaculture is known to influence the environment and its macrobenthic community in several ways. The impacts of feeding and moving activities of cultured organisms on ecosystem are directly from aquaculture itself (DelValls et al., 1998). The culture effects also associated with the effects of pets, creation of novel habitat, and alteration of the nutrient cycling (Drak and Arias, 1997; Han et al., 2001; Forrest et al., 2009; Tomassetti et al., 2009).

4.2. The benthic ecological health of YRD

Macrobenthos is extensively used for environmental monitoring, due to its long life span, strong response to anthropogenic and natural stresses and there relatively sedentary nature. Different species compositions reflect differences in tolerances to stresses, and may have consequences for the bioturbation and bioirrigation in the benthic ecosystem.

In the survey of May 2011, the M-AMBI index of macrobenthos achieved the ecological status from "poor" to "moderate" at 67% of the sampling stations, and 33% of stations with "good" ecological status, indicating that the benthic ecological quality of YRD was not in a good condition. Both the relatively low biodiversity index and ABC curves also indicated macrobenthic assemblages at some stations have suffered some disturbance. And the stations with "perturbed" and "moderately perturbed" condition indicated by ABC curves mostly coincide with

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the "poor" to "moderate" stations shown in M-AMBI. However, the benthic ecological health was significantly improved in August surveys ($F = 18.8$, $p < 0.05$, ANOVA Test), which most possibly benefited from ongoing prohibition of fishing in summer and less fishing activities in autumn. In general, finishing activities can greatly impact the benthic community, both directly by trawling and indirectly by changing the sea floors, substrate types ((Borja et al., 2000; Schratzberger et al., 2002; Kaiser et al., 2002, 2006; Lohrer et al., 2004; Borja and Tunberg, 2011). Due to high fishing intensity, the fish community structure in the Bohai Sea has greatly changed by declined biomass (mean catch), species richness, diversity and evenness (Jin and Tang, 1998; Jin and Deng, 2000; Jin, 2004). To recover the fishery resources, the forbidden fishing period from June 1 to September 1 carried out in Bohai Sea since 1979, which also benefit for the recovery of macrobenthic fauna. The fishing activities (especially bottom net trawling) seriously disturbed the benthic community in spring. Then the community had a recovery process due to the ongoing forbid fishing in summer and less fishing activities in autumn. The improving health status in three seasons also was reflected by the different value of M-MAMBI.

YRD and adjacent waters has been dramatically influenced by human activities, and suffered from pollution, decreasing fishery catches and macroalgal and jellyfish blooms (Wang et al., 2009; Dong et al., 2010). Our results also found the macrobenthic community succession and the relatively moderate ecological status, which coincides with the previous reports (Cai et al., 2014; Luo et al., 2016). Compared to Xiaoqing River estuary (which is also in Southern Bohai Sea, southward of YRD, and is classified as vulnerable and fragile ecosystem due to pollution and eutrophication), the macrobenthic community in YRD presented by more species number, less polychaetes species composition, and lower abundance and biomass (Luo et al., 2013). Whereas, the ecological status of coastal water around Yantai (located south of the YRD in Shandong province, China) was different to YRD, with the condition of "moderate" to "good" due to the removal of marine raft culture and minimizing the amount of waste water in Yantai, which indicates the coastal management is important to improve the ecological status (Li et al. 2013a).

The use of biotic indices based on species traits to assess ecological quality status (EcoQ) of marine waters has becomes a hot research topic in many estuaries and coastal waters (Khedhri et al., 2016). The use of AMBI and M-AMBI has proved to be efficient in detecting degradation of habitat quality in different kinds of estuary, especially in European countries (Borja et al., 2000, 2007; Bigot et al., 2008; Muxika et al., 2007; Khedhri et al., 2016). These two indexes can thus be used as suitable bio-indicator indices to assess the ecological health in YRD and adjacent waters, particularly M-AMBI (Cai et al., 2014; Luo et al., 2016). However, AMBI is more related to the organic matter content gradient than other physical disturbance (Carvalho et al., 2006; Cai et al., 2014). Comparing with *W*-value and AMBI, we also found the M-AMBI can induce more positive results in the research area. Both AMBI and M-AMBI need further research for their adaption to habitat specificities, especially in a semi-enclosed system seem to be less efficacious (Khedhri et al., 2016).

W-value is based on the abundance biomass comparison (ABC) distribution curves, which have been successfully used in detecting the influence of oil pollution (Gray, 1979; Warwick and Clarke, 1994), and industrial pollution (Pearson and Rosenberg, 1976). However, the *W*-value method seemed to work adequately (Austen et al., 1989; Ritz et al., 1989) or inadequately (Teixeira et al., 2007; Marín-Guirao et al., 2005) at different estuarine environment. We found the EcoQ of YRD reflected by *W*-value did not coincide well with M-AMBI, suggesting the *W*-value worked inadequately in this area. Wetzel et al. (2012) evaluated the performances of eight benthic biotic indices (AMBI, MAMBI, BOPA, BO2A, *W*-value, Shannon diversity, species richness, abundance), and found only the *W*-value did not induce the significant differences between two different communities. But, only *W*-value was significantly correlated to most sediment pollutants, and did not show significant correlations to mean grain size and sediment sorting (Wetzel et al., 2012).

4.3. Species assignment

The species assignment is crucial in calculating the value of AMBI and M-AMBI, then further affecting the assessment of ecological health for some regions. Actually, the species-list of V. March 2012 in program AMBI (version 5.0) was applicable in most European regions. However, due to the different fauna characters compare to other regions worldwide, e.g. Asian, Africa, Australia, etc. some territorial and local species were not included in the species list of V. March, which will affect the application of AMBI in the above region. In the present work, up to 13.7% (about 10 species) of total species remained unassigned even after assignment due to the lack of information, which is almost same to the result of species assignment in another sea water region in Baohai Sea, China (Li et al. 2013a). We adopted the following approach to deal with the species excluded in the name list, e.g., consulting references, same genus and expert opinion (Borja et al., 2008). But, there are two other questions emerging: the accuracy and comparability of the results. Due to the lack of information and different understanding of species assignment, a specific species could be assigned to different ecological group by different ecologist and taxonomist, which would result in a different AMBI and M-AMBI value. The latter makes it difficult to compare the results for further study or management. The feasible way to avoid the above two situations, is to enrich the species name list by experts worldwide, containing more territorial and local species, which will form a unique criterion to calculate the AMBI and M-AMBI value.

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