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Assessing the benthic quality status of the Bohai Bay (China) with proposed modifications of M-AMBI

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Abstract

Multivariate AZTI's Marine Biotic Index (M-AMBI) was designed to indicate the ecological status of European coastal areas. Based upon samples collected from 2009 to 2012 in the Bohai Bay, we have tested the response of variations of M-AMBI, using biomass (M-BAMBI) in the calculations, with different transformations of the raw data. The results showed that the ecological quality of most areas in the study indicated by M-AMBI was from moderate to bad status with the worse status in the coastal areas, especially around the estuaries, harbors and outfalls, and better status in the offshore areas except the area close to oil platforms or disposal sites. Despite large variations in nature of the input data, all variations of M-AMBI gave similar spatial and temporal distribution patterns of the ecological status within the bay, and showed high correlation between them. The agreement of new ecological status obtained from all M-AMBI variations, which were calculated according to linear regression, was almost perfect. The benthic quality, assessed using different input data, could be related to human pressures in the bay, such as water discharges, land reclamation, dredged sediment and drilling cuts disposal sites. It seems that M-BAMBI were more effective than M-NABMI (M-AMBI calculated using abundance data) in indicating human pressures of the Bay. Finally, indices calculated with more severe transformations, such as presence/absence data, could not indicate the higher density of human pressures in the coastal areas of the north part of our study area, but those calculated using mild transformation (i.e., square root) did.

Key words: macrozoobenthos, M-AMBI, abundance, biomass, data transformation, ecological status

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1 Introduction

It has become a major challenge worldwide to assess the ecological integrity and health status of marine waters and to restore degraded habitats in impaired environments (Borja et al., 2008; Halpern et al., 2012). One of the most widely used ecosystem components to assess the status is soft-bottom macroinvertebrates, for which many assessment methods have been developed (Díaz et al., 2004; Pinto et al., 2009). Among this plethora of indices, the M-AMBI (multivariate AZTI's Marine Biotic Index) is one of the most extensively used worldwide (Borja et al., 2012), as well as in China (Li et al., 2013; Cai et al., 2014; Forde et al., 2013).

M-AMBI was designed primarily to assess the ecological status in European marine waters (Muxika et al., 2007), and it is based upon a Factor Analysis that includes richness, Shannon's diversity and AMBI (AZTI's Marine Biotic Index). AMBI, an index showing the proportion of different ecological groups (i.e., sensitive to disturbance, indifferent, tolerant, opportunists of 2nd and 1st order), is calculated based on the abundance of each species (Borja et al., 2000).

However, other parameters, such as biomass can get more functional ecological relevance than abundance (Warwick et al., 2010). Hence, Warwick et al. (2010) proposed the use of some variations of the original AMBI, based upon abundance, including biomass (BAMBI) and various transformations in calculating these data, increasing in severity: square root, fourth root, log and presence/absence. Warwick et al. (2010) found non-linear significant correlation among the variations of the index, and demonstrated that the ability of AMBI responding to an organic enrichment gradient was enhanced after a mild transformation (square root). As such, Muxika et al. (2012) demonstrated that despite large variations in the form and nature of the input data, all the above variations of AMBI are highly correlated and can be used to assess the status, if boundaries between quality classes are determined for these new variations.

Despite of the attention paid to AMBI transformations, little research has been undertaken in defining the ability of transformed M-AMBI in assessing the status. Thus, Cai et al. (2014) used biomass to calculate M-AMBI (M-BAMBI) for the first time and found good agreement between both versions of the assessment method (using biomass and abundance). Moreover, higher taxonomic level data was also used to calculate M-AMBI values and found that these indices were also sensitive to environment-

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al disturbances calculating the index at the family level (Forde et al., 2013). Since M-AMBI has become a common assessment method across different continents, testing the usefulness of the various transformed M-AMBI could be of importance for benthic assessment research.

Hence, within this context, our objectives are: (1) to analyze the discriminatory power in assessing the ecological status, which is indicated by M-AMBI calculated using abundance or biomass (transformed or not), using the Bohai Bay (China) as the testing area; (2) to describe how the various transformed indices respond to human pressures within this Bay; and (3) to illustrate the variations of transformed indices along selected temporal patterns.

2 Materials and methods

2.1 *Sampling area*

The sampling area was located in the Bohai Sea, the north of China (Fig. 1), a shallow water basin with mostly fine mud sediments. In recent years, plenty of industrial and municipal wastewater from coastal cities has been discharged into the Bohai Bay through rivers and drain channels (Liu et al., 2011). These discharges have produced an increasing concentration of pollutants in waters and sediments (Cai et al., 2014). Furthermore, many land reclamation projects, carried out in Tianjin Binhai New Area (Li et al., 2010; Zheng et al., 2011), have brought geomorphological changes to the Bohai Bay. These changes have produced lower average velocity of residual current (Zheng et al., 2011), and increased deposition of pollutants in coastal areas, especially in estuaries and the vicinity of outfalls (Meng et al., 2008). Besides, the oil extraction activities also produced some negative impacts on the area (Liu et al., 2006; Zhang et al., 2009). In general, macrozoobenthic community in the Bohai Bay had changed remarkably because of the increasing human activities mentioned above (Zhou et al., 2012).

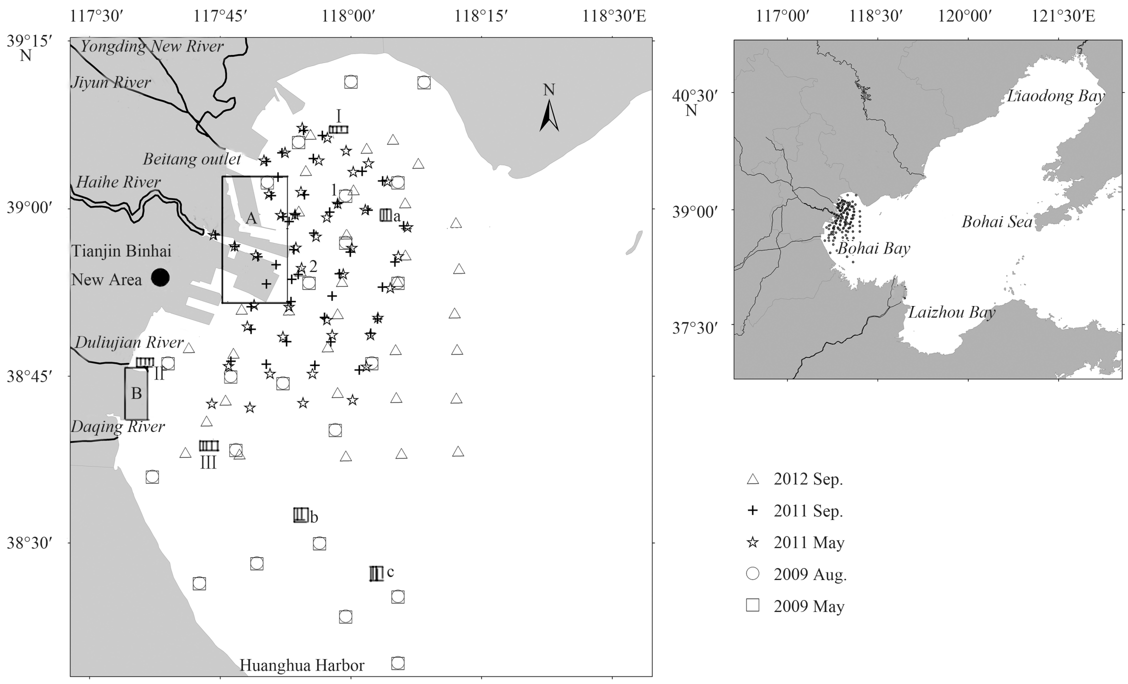

Fig. 1. Sampling sites in the Bohai Bay from 2009 to 2012. 1 and 2 represent selected Sites 1 and 2; I–III oil platforms (refer to Cai et al. (2014)); A Tianjin Harbor; B Southern Harbor of Tianjin; and a–c Dredged sediment and drilling cuts disposal sites in Tianjin Harbor, Zhaodong oil field and Huanghua Harbor (refer to Wu (2013)). The figure was modified from Cai et al. (2013).

2.2 *Data collected*

Both biotic and associated environmental samples were collected in May and August 2009, May and September 2011, and September 2012 (see Fig. 1 for sampling locations). The uneven distribution of sampling sites for each sampling year was addressed according to the actual need of different projects, but the principle for designing them was the same as detailed in Meng (2009) and Cai et al. (2014). Details on sampling methods can be consulted in Table 1. All the samples were taken using a modified van Veen or Ekman-Birge grab, sieved through a 0.5 mm mesh and fixed in 75% anhydrous ethanol in the field. In addition, all the sampling and analyzing methods of environmental variables used in this research were the same as in Cai et al. (2014), including those of September 2012.

Table 1. Sampling dates, grab characteristics, number of stations and replicates sampled and combination rules used

Sampling month/year	Grab type	Sample size/m^2	Station number	Number of grabs	Replicates	Combination
May 2009	modified	0.05	22	2	1	1 of 0.1 m^2
Aug. 2009	modified	0.05	22	2	13	1 of 0.1 m^2
May 2011	modified	0.05	43	6	3	1 of 0.1 m^2
Sep. 2011	modified	0.05	43	3	3	1 of 0.05 m^2
Sep. 2012	modified	0.05	35	3	3	1 of 0.05 m^2

2.3 *Environmental variables analysis*

The water depth, turbidity, salinity, dissolved oxygen (DO), pH and chlorophyll *a* in both surface and bottom water layers were measured using a CTD. Surface and bottom waters were collected using Niskin bottles and then kept at 4°C to analyze nutrients (total nitrogen (TN), total phosphorous (TP), NH_4^+, PO_4^{3-}, NO_2^- and NO_3^-), total organic carbon (TOC), chemical oxygen demand (COD) and metals in the laboratory. In particular, an Agilent 7500a ICP-MS was employed to measure the sediment and water metal concentrations (Zheng et al., 2011). Nutrients in water and other water quality parameters were measured with a Bran+luebbe auto analyzer. Sediment grain size was analyzed by Mastersizer 2000, and classified according to the Wentworth scale (Buchanan, 1984). The detailed information for the sampling and analyzing methods of all the environmental variables were shown in Cai et al. (2014).

2.4 *Data treatment*

All the benthic samples were identified to species level in the laboratory, whenever possible. Then, biomass (wet weight) and abundance were determined. Abundance and biomass data were transformed using a set of transformations of increasing severity: square root, fourth root, log(1+*x*) and presence/absence, and through the use of dispersion weighting (Clarke and Goreley, 2006), which down-weights clustered species. The same methodology was also adopted by Warwick et al. (2010) and Muxika et al. (2012).

At each of the replicates, richness and Shannon's diversity were calculated as well as AMBI. Once the three metrics were calculated, all the variations of M-AMBI values were calculated at the replicate level with AMBI 5.0 software (freely available at http://ambi.azti.es.htm), using the March 2012 species list. Then mean values of the replicates were calculated to determine the station M-AMBI values, which were used to analyze the relationships between this index and environmental variables. This method was used in Cai et al. (2014) and it avoids the problems associated with different number of replicates when using various methods.

The approach for determining reference conditions for the variations of M-AMBI was that recommended by Borja and Tunberg (2011) and Forchino et al. (2011). It consists in increasing 15% upon the highest diversity and richness values of all replicates and decreasing 15% upon the lowest AMBI values. This method has been affirmed by Cai et al. (2014) as the most suitable one for assessing the ecological quality of the Bohai Bay, because it is supposed that this area is impacted by human disturbances. As for the bad status, all references were based upon the azoic situation (diversity and richness equal to 0 and AMBI equal to 6).

The cut-off points and boundaries for ecological status classification of M-AMBI were in accordance with Borja et al. (2007) for the area. As noted by Warwick et al. (2010), these values should be set at suitable points on the scale, depending on which combination of input data (abundance, biomass) and transformations were used. In this context, the linear regression was used to detect the relationships between M-AMBI and the various modified M-AMBI. The regression equations were then used to calculate threshold values of the various indices corresponding to those classification defined by Borja et al. (2007).

The agreement between the different variations of M-AMBI was determined by means of a Kappa analysis (Cohen, 1960; Landis and Koch, 1977), as done during the European intercalibration exercises (Borja et al., 2007). The level of agreement between the indices was established, based upon the equivalence table from Monserud and Leemans (1992). As the importance of misclassification is not the same between close categories as between further categories, Fleiss-Cohen weights were applied to the analysis (Fleiss and Cohen, 1973).

The relationships between the environmental variables and all the indices, from 2009 to 2012, were explored using Pearson correlation with one-tailed significant testing (Cai et al., 2014), which was carried out in SPSS 13.0 software packages. The relationship between the environmental variables in the bottom water layer and sediment was calculated only in 2011, as data for other years were not available.

Two sites (Sites 1 and 2; see the details in Fig. 1) were selected according to the known history of human pressures, as described by different authors (Liu et al., 2007; Meng et al., 2008; Li et al., 2010; Cai et al., 2012; Zhang, 2012; Gao et al., 2014), to detect whether the changes in pressures were correlated with changes in M-AMBI values. Site 1 was affected by a large amount of discharged wastewaters from the Jinyun River and Beitang outfall and land reclamation projects around Tianjin Harbor; Site 2 was mainly affected by the wastewaters from the Haihe River and Dagu outfall as well as shipping activities and land reclamation projects in Tianjin Harbor, as described by the authors mentioned above. The significance of differences between values before and after the change in the pressure was tested by non-parametric Mann-Whitney tests.

3 Results

The mean values of the environmental variables in the surface water layer in the study area are offered (Table 2). No sample was classified at high status, using M-NAMBI, at the replicate level. In turn, bad status was found in the southern part of the sampling area, close to Huanghua Harbor. Most M-NAMBI values ranged between 0.20 and 0.53 indicating poor or moderate status. This status was found in the coastal areas of the southern Bohai Bay, not far away from the estuaries and in outfalls of the northern area.

Comparing with M-NAMBI, there were more sites assigned to poor status in M-BAMBI (Fig. 2), especially in the northern part of the Bohai Bay, around Jiyun River and Haihe River estuaries, Beitang sewage outfall, Tianjin Harbor and the South Harbor of Tianjin. The only exception was the use of the presence/absence transformation (Appendix Fig. A1). In particular, M-BAMBI showed the quite similar spatial distribution regardless of any variation of the input data. Interestingly, the similar spatial distribution pattern was found between all the variations of M-NAMBI (calculated from raw or not) and M-BAMBI, calculated using presence/absence data (Appendix Fig. A1).

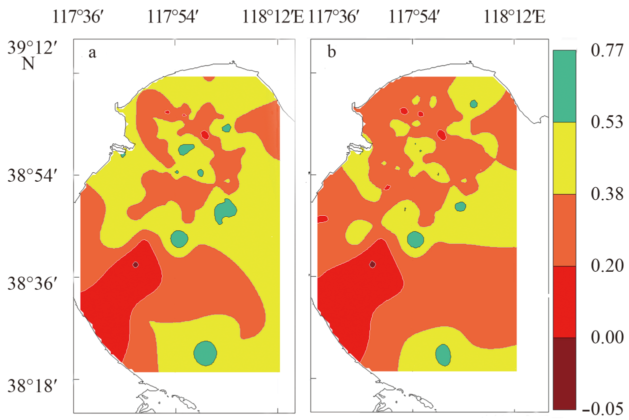

Fig. 2. Spatial distributions of M-NAMBI (a) and M-BAMBI (b) values in the Bohai Bay.

Pearson correlation showed significant (p<0.01) correlations between the various M-AMBI values derived from different input data (Appendix Table A1). The regression equations derived from the analysis were used to determine the new quality boundaries for all the other indices. Once the sampling sites were classified according to these boundaries (Appendix Table A2), a Kappa analysis was carried out to detect the agreement in the quality classification between the indices. The results showed a very good agreement (0.70< kappa coefficients⩽0.85) between the classifications obtained from M-BAMBI calculated with raw data (Table 3). Good agreement (0.55< kappa coefficients⩽0.70) was reached between M-BAMBI calculated with dispersion weighting transformation and M-NAMBI. For the remainder indices, the agreement with M-NAMBI classifications was excellent (0.85< kappa coefficients⩽0.99).

No significant correlation was found between environmental variables measured in the surface water layer and benthic indices, except between TP and M-NAMBI calculated using raw data (R=–0.127, p<0.05) and M-BAMBI calculated using dispersion weighting data (R=–0.129, p<0.05). Otherwise, significant negative correlations were observed between various benthic indices and: (1) total phosphate and ammonium in the bottom water layer; (2) percentage of sand and magnesium in the sediment (Table 4). In turn, significant positive correlations were detected between indices and: (1) pH and temperature in the bottom water layer; and (2) percentage of clay in the sediment (Table 4).

Table 2. Average and standard deviation of environmental parameters, nutrients and metal concentration, in the surface water layer, for all the sites during 2009 to 2012

	Chl a/μg · L^{-1}	pH	S	PO_4^{3-}/μg · L^{-1}	NO_2^-/μg · L^{-1}	NO_3^-/μg · L^{-1}	TP/μg · L^{-1}
Range	1.2–138.34	6.9–9.24	13.43–32.07	0.00–256.00	0.00–269.00	28.00–879.00	0.00–740.20
Average	12.74	8.21	29.05	27.54	59.64	347.02	115.36
Standard deviation	20.58	0.59	2.63	34.51	46.74	120.01	116.18
	TN/μg · L^{-1}	NH_4^+/μg · L^{-1}	Cu/μg · L^{-1}	Pb/μg · L^{-1}	Zn/μg · L^{-1}	Cd/μg · L^{-1}	
Range	430.00–10 224.00	1.00–2 094.10	0.71–19.27	0.06–8.26	0.00–70.32	0.05–0.78	
Average	2 910.42	113.09	5.85	1.62	20.64	0.25	
Standard deviation	1 858.66	211.31	5.00	1.86	15.08	0.15	

Notes: Chl represents chlorophyll, S salinity, PO_4^{3-} phosphate, NO_2^- nitrite, NO_3^- nitrate, NH_4^+ ammonium, TP total phosphate, TN total nitrogen, Cu copper, Pb lead, Zn zinc, and Cd cadmium.

Table 3. Results obtained after linear regression between M-NAMBI and each of the variations (n=164). Kappa coefficients obtained to assess the agreement between M-NAMBI and each of its variations is also shown, as well as the interpretation of the coefficients after Monserud and Leemans (1992)

	Linear regression		Boundaries M-NAMBI				Kappa analysis	
	r^2	b	0.77	0.53	0.38	0.20	Coefficient	Interpretation
M-srAMBI	0.976	0.008	0.79	0.54	0.39	0.21	0.91	excellent
M-frAMBI	0.932	0.022	0.79	0.55	0.40	0.22	0.87	excellent
M-logAMBI	0.922	0.024	0.78	0.55	0.40	0.22	0.88	excellent
M-p/aAMBI	0.907	0.030	0.78	0.55	0.40	0.22	0.87	excellent
M-dwAMBI	0.937	0.029	0.78	0.54	0.39	0.21	0.61	good
M-BAMBI	0.856	0.022	0.68	0.47	0.35	0.19	0.83	very good
M-srBAMBI	0.905	0.014	0.73	0.51	0.37	0.20	0.87	excellent
M-frBAMBI	0.914	0.022	0.77	0.54	0.39	0.22	0.91	excellent
M-logBAMBI	0.878	0.018	0.76	0.53	0.38	0.21	0.87	excellent
M-p/aBAMBI	0.906	0.031	0.75	0.52	0.37	0.20	0.87	excellent
M-dwBAMBI	0.853	0.014	0.70	0.49	0.35	0.19	0.86	excellent

Notes: sr represents square root, fr forth root, log logarithm, dw dispersion weighting, p/a presence/absence, and b the constant of linear regression equation.

The response of various M-AMBI indices to known changes of environmental pressures was studied at two sites (Figs 3 and 4). At Site 1 (located at the extension line of Beitang sewage outfall), all the M-AMBI values (modified or not) showed a significant (Mann-Whitney U=0, P=0.000) improvement from August 2009 to May 2011. In this period, the status improved from bad-poor to moderate-good. After May 2011, the status was stabilized around moderate-good (Fig. 3). In the case of Site 2 (located at the Haihe River Estuary), the status was poor-moderate, until May 2011, with a further improvement (Mann-Whitney U=0, P=0.000) to moderate-good. However, when comparing with 2011, a significant (Mann-Whitney U=348, P=0.002) decrease of the M-AMBI values (modified or not) was observed in 2012.

4 Discussion

Most of the sampling sites where the different M-AMBI values showed a benthic ecological status lower than good, were affected by important human pressures, responding to the important changes of benthic communities in the Bohai Bay (Zheng et al., 2011; Zhou et al., 2012; Cai et al., 2014). In particular, the bad status areas (the values of all the variations of M-AMBI were around zero) distributed in the southern part of the Bohai Bay, close to the Huanghua Harbor. This status, which implied an (near) azoic situation, was detected very close to the dredged sediment disposal site of the Huanghua Harbor, Huanghua canal, and the drilling cuts disposal from the Zhaodong oil platform. It is well-known that sediment disposal (both from dredging or drilling cuts) can impact benthic communities by changing the structure and functioning of the community (Van Dolah et al., 1984; Daan et al., 1996; Bolam et al., 2006; Ware et al., 2010; Ellis et al., 2012). In the case of Yantai coast, Liu et al. (2010) demonstrated changes occur in benthic species composition, and decreasing abundance and richness, close to disposal areas. Moreover, oil platforms introduce pollutants into the Bohai Bay (Liu et al., 2006; Gao and Chen, 2012), such as metals (Middleditch, 1984; Steinhauer et al., 1994), which affect the benthic communities around the platforms (Daan et al., 1994). This seems to be the cause of such low M-AMBI values in the above mentioned two areas, as detected in this investigation.

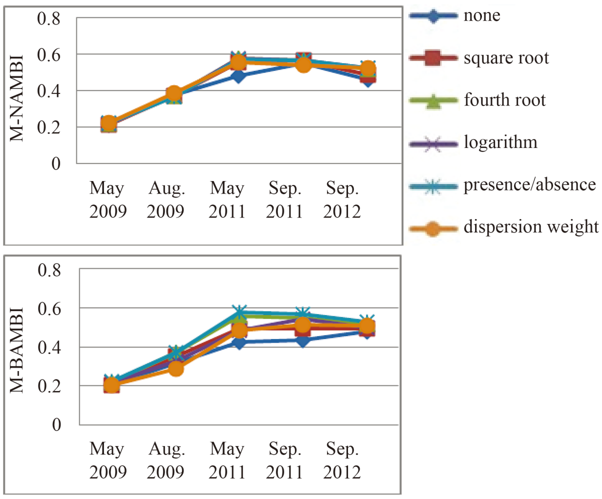

Fig. 3. M-NAMBI and M-BAMBI results calculated using both raw and transformed data for Site 1.

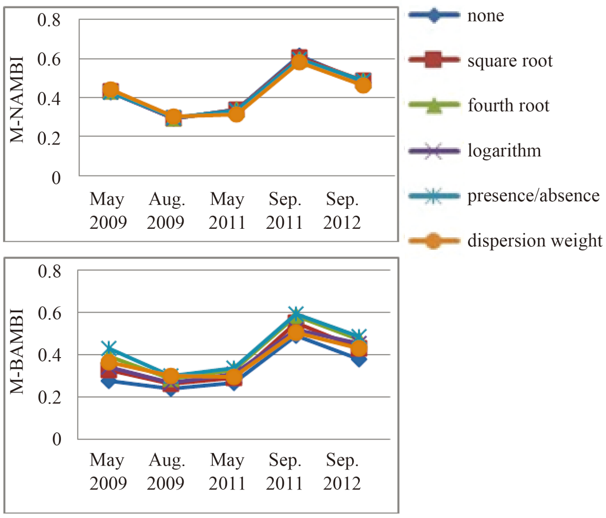

Fig. 4. M-NAMBI and M-BAMBI results calculated using both raw and transformed data for Site 2.

In addition to those offshore sources of pollution, the main sources of human pressures are located at coastal areas of the Bohai Bay. Hence, there are several land reclaimation projects, especially in estuaries, some outfalls (Wang and Wang, 2007; Li et al., 2010; Zheng et al., 2011) and commercial harbors (Zhang, 2012) producing environmental impacts. These pressures, compounded by the poor self-purification of the area (Cai et al., 2014) and low tidal flow renewal in the bay (Tao, 2006), result in higher pollution levels close to the coast, with a gradient of pressure from the coast to offshore (Liu et al., 2007; Wu et al., 2007). In general, M-AMBI, especially M-BAMBI, had succeeded in showing the impacts of this spatial gradient of pressures, with worse benthic quality around the estuaries, outfalls and harbors, and better quality in offshore areas, excepting those close to oil platforms or disposal sites.

However, in the northern part of the Bohai Bay, there are much more land reclamation projects (e.g., around the Tianjin harbor), aquaculture activities and wastewater discharges along the rivers than in the other parts of the bay (Li et al., 2010; Zheng et al., 2011; Meng et al., 2008; Zhou et al., 2011). These pressures resulted in a poor status of benthic quality, as determined by M-BAMBI. This status pattern seems to be in much more agreement with the main current direction and velocity in the Bohai Bay than that of M-NAMBI (Cai et al., 2014). In fact, as some authors state, data transformation prior to calculate indices was very important to the affection of one index based on relative abundance of species (Clarke and Warwick, 2001), especially when the insensitive species tend to be small bodied opportunists in stressed situations (Warwick et al., 2010).

In our investigation, one dominant disturbance tolerant species (*Musculus senhousia*), very abundant, was mainly distributed in the north part of the Bay (Cai et al., 2013), which would affect the sensitivity of M-NAMBI. Hence, M-NAMBI did not indicate the worse status in that area while the indices calculated using biomass did. This might because the biomass could downweight the influence of abundant small species, making M-BAMBI being more sensitive to human pressures in the northern part of the Bohai Bay, especially around the stressed estuaries and outfalls, with the worst status in the northern part and better in the other areas. Moreover, biomass only improves marginally on abundance data (Warwick et al., 2010). Although some inconsistencies were found, no obvious divergence was observed between M-NAMBI and other variations of the index. In general, a mild transformation of the data (square root) could enhance the ability of AMBI responding to organic enrichment gradient; in turn, severe transformation, such as presence/absence, might degrade the relationship between derived indices and the impact detected. This could be because severe transformation adds too much noise from the relatively large weight given to random occurrences of rarer species (Warwick et al., 2010). As in our case, M-AMBI calculated with more severe transformations, such as presence/absence data, could not indicate the higher density of human pressures in the north part of the Bohai Bay, but benthic indices calculated using mild transformation (i.e., square root) did, as observed by Warwick et al. (2010).

Actually, when determining the new class boundaries for M-AMBI from transformed data, the final ecological status classification showed a good level of agreement with the classification using M-NAMBI (raw data). This suggests that all the variations of these indices (raw or transformed) gave basically the same information. This means that the similar results can be obtained independently of the index used or the level of transformation of the input data (from none to presence/absence), as the case found for AMBI (raw or transformed), M-AMBI and M-BAMBI, by Warwick et al. (2010), Muxika et al. (2012) and Cai et al. (2014).

Table 4. Results from Pearson correlation between benthic indices and environmental variables in the bottom water layer and the sediment, for all the sampling sites in 2011

Index	Pre-treatment		Fine sand/%	Clay/%	T/°C	pH	NH_4^+/μg · L^{-1}	PO_4^{3-}/μg · L^{-1}	TP/μg · L^{-1}	Mg/μg · g^{-1}
M-NAMBI	no	*R*	−0.262	0.269	0.291	0.153	−0.220	−0.171	−0.201	−0.326
		p	**0.007**	**0.006**	**0.003**	**0.080**	**0.021**	**0.059**	**0.033**	**0.001**
		n	**86**	**86**	**86**	**86**	**85**	**85**	**85**	**86**
	sr	*R*	−0.283	0.272	0.293	0.171	−0.170	−0.154	−0.181	−0.338
		p	**0.004**	**0.006**	**0.003**	**0.058**	**0.060**	**0.080**	**0.049**	**0.001**
	fr	*R*	−0.296	0.277	0.296	0.188	−0.143	−0.145	−0.174	−0.346
		p	**0.003**	**0.005**	**0.003**	**0.042**	**0.096**	**0.093**	**0.056**	**0.001**
	log	*R*	−0.301	0.281	0.299	0.193	−0.141	−0.145	−0.175	−0.349
		p	**0.002**	**0.004**	**0.003**	**0.038**	**0.099**	**0.093**	**0.054**	**0.000**
	p/a	*R*	−0.304	0.283	0.300	0.195	−0.129	−0.140	−0.171	−0.352
		p	**0.002**	**0.004**	**0.002**	**0.036**	**0.121**	**0.101**	**0.059**	**0.000**
	dw	*R*	−0.317	0.288	0.305	0.196	−0.107	−0.123	−0.142	−0.360
		p	**0.001**	**0.004**	**0.002**	**0.036**	**0.165**	**0.130**	**0.098**	**0.000**
M-BAMBI	no	*R*	−0.295	0.284	0.301	0.192	−0.213	−0.170	−0.224	−0.343
		p	**0.003**	**0.004**	**0.002**	**0.038**	**0.025**	**0.060**	**0.020**	**0.001**
	sr	*R*	−0.298	0.277	0.297	0.187	−0.171	−0.154	−0.191	−0.344
		p	**0.003**	**0.005**	**0.003**	**0.043**	**0.059**	**0.080**	**0.040**	**0.001**
	fr	*R*	−0.296	0.273	0.291	0.185	−0.141	−0.144	−0.175	−0.342
		p	**0.003**	**0.005**	**0.003**	**0.044**	**0.099**	**0.094**	**0.055**	**0.001**
	log	*R*	−0.285	0.248	0.276	0.182	−0.189	−0.162	−0.188	−0.321
		p	**0.004**	**0.011**	**0.005**	**0.047**	**0.041**	**0.070**	**0.043**	**0.001**
	p/a	*R*	−0.303	0.282	0.300	0.195	−0.128	−0.141	−0.171	−0.351
		p	**0.002**	**0.004**	**0.003**	**0.036**	**0.121**	**0.099**	**0.059**	**0.000**
	dw	*R*	−0.311	0.269	0.304	0.180	−0.146	−0.142	−0.163	−0.338
		p	**0.002**	**0.006**	**0.002**	**0.049**	**0.091**	**0.098**	**0.068**	**0.001**

Notes: no represents raw data, dw dispersion weighting, fr forth root, log logarithm, p/a presence/absence, sr square root, Mg magnesium (sediment), NH_4^+ ammonium (bottom water layer, BWL), PO_4^{3-} phosphate (BWL), *T* temperature (BWL), and TP total phosphate (BWL).

In the study area, anthropogenic disturbances were severest in 2009 as many land reclamation projects carried out around the Tianjin Harbor (Li et al., 2010; Wu, 2013), and a large amount of industrial and domestic wastewater discharges from the Beitang outfall and Haihe River (Zheng et al., 2011; Cai et al., 2012). This severe pressure has been detected by M-AMBI, showing the worst status in 2009. In addition, the first stage of land reclamation projects in the Binhai New Area was completed in 2010, combined with a decrease of urban and industrial discharges through the Haihe River and Beitang outfall in 2011 (Cai et al., 2012). As such, benthic community showed some recovery in 2011 (Cai et al., 2012, 2014). However, all these environmental changes resulted in a dominance of the sensitive-tolerant species in the Bohai Bay, as demonstrated by the significant increase of the M-AMBI values at the selected two sites. In spite of this, the new reclaiming projects in the bay started after 2011 and may impact macrozoobenthos communities (Li et al., 2010), as indicated by the significant decrease of the M-AMBI values from 2011 to 2012 at Site 2.

It has been demonstrated that biomass and richness of benthic communities increased significantly along the gradient from fine to coarse sediment (Chester et al., 1983), as it has been observed in the Bohai Bay (Cai et al., 2013), indicated by M-AMBI spatial and temporal gradient patterns with the worse status in the northern part and better in the southern part. In the last 30 years, nutrients in the Bohai Bay have increased gradually, causing eutrophication in the nearshore areas with the Haihe River Estuary being the most eutrophicated area (Wu et al., 2013). Eutrophication has direct effect on the stability of benthic communities structure (Beukema, 1991), which can be confirmed by the worse status in the above areas. Additionally, DIN (Dissolved Inorganic Nitrogen) increased remarkably from 2009 to 2010, and then decreased from 2010 to 2011 (Yi and Yin, 2013), showing a high level of agreement with the temporal variations of M-AMBI at Sites 1 and 2.

In the last 60 years, the macrobenthic community in the Bohai Sea has changed dramatically with the increasingly severe human pressures mentioned above (Cai et al., 2012; Zhou et al., 2012). Compared with the 1950s, there has been a considerable increase in the abundance of small polychaetes, bivalves and crustaceans but a decreased number of echinoderms (Zhou et al., 2007). It meant that sensitive species were disappearing and opportunistic species were becoming dominant, which would increase the AMBI values, and decrease the M-AMBI values (Cai et al., 2014). Moreover, the spatial distribution of macrozoobenthos community in the Bohai Bay in the last 60 years (Zhou et al., 2007; Wang et al., 2010; Zhou et al., 2012) was coincident with M-NAMBI and M-BAMBI (Cai et al., 2014), as the case in the research.

5 Conclusions

In general, the ecological quality of the study area in the Bohai Bay, indicated by M-AMBI, was poor with the worse status in the coastal areas, especially around the estuaries, harbors and outfalls, and better status in the offshore areas except ones close to oil platforms or disposal sites. M-AMBI calculated using abundance and biomass data (raw or transformed) were able to assess the benthic quality in a similar way, with high correlation between the different methods. Once the boundaries of the ecological status classifications are determined, the final assessment provides similar pictures of the status, whatever the method is used. The benthic quality, assessed using abundance and biomass (raw or not), could be related to human pressures affecting the bay, such as water discharges, land reclamation, oil platforms and sediment disposal sites. Finally, it seems that indices calculated with more severe transformations, such as presence/absence data, could not indicate the higher human pressures in the north part of the Bohai Bay (especially around the estuaries, outfalls and harbors in the area), but those calculated using mild transformation (i.e., square root) did.

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Appendix:

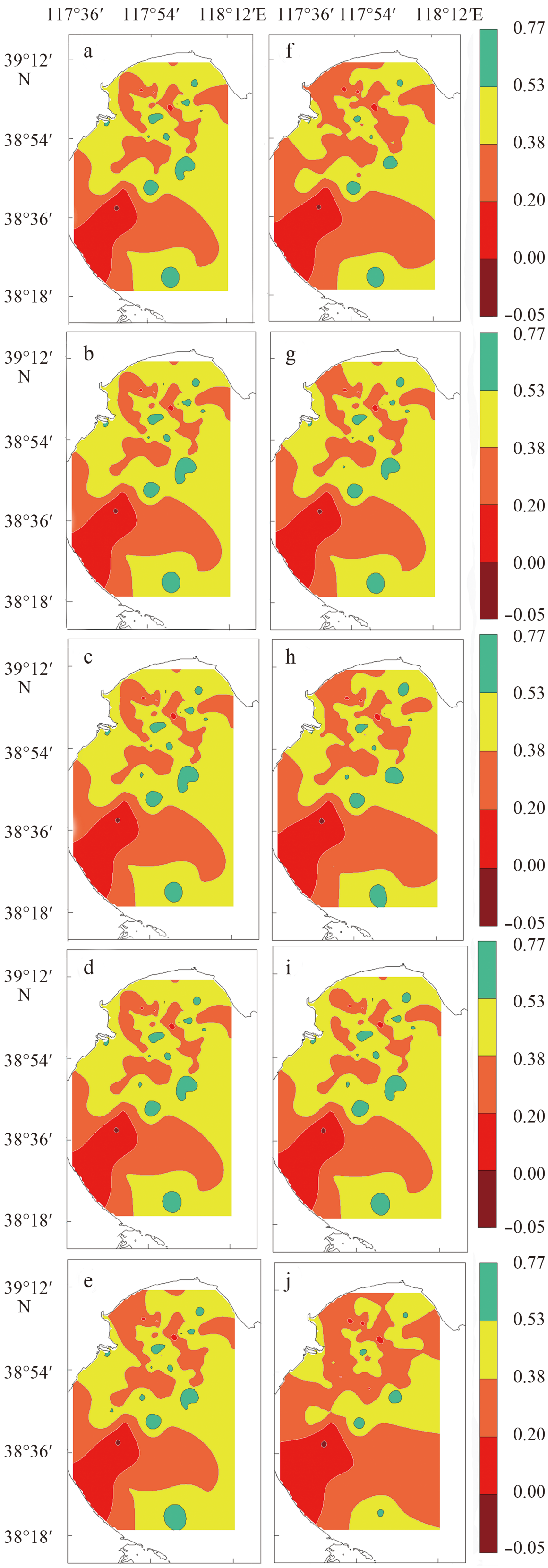

Fig. A1. Spatial distribution of M-AMBI from transformed data. a–e. M-NAMBI from square root, fourth root, logarithm, presence/absence and dispersion weighting, respectively; and f–j. M-BAMBI from square root, fourth root, logarithm, presence/absence and dispersion weighting. Good status close to the green color, moderate to the yellow, poor to the orange and bad to the red for all the variations of M-BAMBI and M-NAMBI.

Table A1. Pearson correlations (R) between all the indices (for names see text), together with the level of significance (P) (n=164)

		M-srNAMBI	M-frNAMBI	M-logNAMBI	M-p/aNAMBI	M-dwNAMBI	M-BAMBI	M-srBAMBI	M-frBAMBI	M-logBAMBI	M-p/aBAMBI	M-dwBAMBI
M-	*R*	0.988	0.965	0.960	0.952	0.968	0.925	0.951	0.956	0.937	0952	0.924
NAMBI	*P*	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
M-sr	*R*		0.992	0.989	0.985	0.987	0.942	0.975	0.985	0.966	0.984	0.952
NAMBI	*P*		**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
M-fr	*R*			1.000	0.998	0.986	0.945	0.982	0.996	0.977	0.997	0.963
NAMBI	*P*			**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
M-log	*R*				0.999	0.985	0.945	0.982	0.996	0.978	0.998	0.964
NAMBI	*P*				**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
M-p/a	*R*					0.981	0.944	0.981	0.996	0.977	0.999	0.964
NAMBI	*P*					**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
M-dw	*R*						0.936	0.971	0.982	0.963	0.980	0.958
NAMBI	*P*						**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
M-	*R*							0.985	0.961	0.976	0.947	0.932
BAMBI	*P*							**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
M-sr	*R*								0.992	0.991	0.983	0.962
BAMBI	*P*								**0.000**	**0.000**	**0.000**	**0.000**
M-fr	*R*									0.988	0.997	0.968
BAMBI	*P*									**0.000**	**0.000**	**0.000**
M-log	*R*										0.980	0.960
BAMBI	*P*										**0.000**	**0.000**
M-p/a	*R*											0.965
BAMBI	*P*											**0.000**

Table A2. Comparison of the quality levels obtained using M-NAMBI and using the remainder of the derived M-AMBI values, as calculated in this investigation. The numbers represent the sampling sites used from 2009 to 2012

Benthic indices	Ecological quality status	M-NAMBI				
		High	Good	Moderate	Poor	Bad
M-BAMBI	High	0	2	2	0	0
	Good	0	16	9	0	0
	Moderate	0	6	45	6	0
	Poor	0	0	10	57	1
	Bad	0	0	0	3	9
M-srNAMBI	High	0	0	0	0	0
	Good	0	20	3	0	0
	Moderate	0	3	58	3	0
	Poor	0	1	3	61	0
	Bad	0	0	0	2	10
M-frNAMBI	High	0	0	0	0	0
	Good	0	22	4	0	1
	Moderate	0	2	52	5	0
	Poor	0	0	8	59	1
	Bad	0	0	0	2	8
M-logNAMBI	High	0	0	0	0	0
	Good	0	21	4	0	0
	Moderate	0	3	52	5	0
	Poor	0	0	8	59	1
	Bad	0	0	0	2	9
M-p/aNAMBI	High	0	0	0	0	0
	Good	0	21	5	1	0
	Moderate	0	3	53	5	0
	Poor	0	0	6	58	1
	Bad	0	0	0	2	9
M-dwNAMBI	High	0	0	0	0	0

to be continued

Continued from Table A2

Benthic indices	Ecological quality status	M-NAMBI				
		High	Good	Moderate	Poor	Bad
	Good	0	23	6	0	0
	Moderate	0	1	52	4	0
	Poor	0	0	6	60	0
	Bad	0	0	0	2	10
M-srBAMBI	High	0	0	0	0	0
	Good	0	20	3	0	0
	Moderate	0	4	53	7	0
	Poor	0	0	8	55	1
	Bad	0	0	0	4	9
M-frBAMBI	High	0	0	0	0	0
	Good	0	21	5	0	0
	Moderate	0	3	56	4	0
	Poor	0	0	3	56	1
	Bad	0	0	0	6	9
M-logBAMBI	High	0	0	0	0	0
	Good	0	19	5	0	0
	Moderate	0	5	53	7	0
	Poor	0	0	6	54	1
	Bad	0	0	0	5	9
M-p/aBAMBI	High	0	0	0	0	0
	Good	0	23	11	0	0
	Moderate	0	1	53	13	0
	Poor	0	0	0	52	1
	Bad	0	0	0	1	9
M-dwBAMBI	High	0	0	0	0	0
	Good	0	21	11	0	0
	Moderate	0	2	45	6	0
	Poor	0	0	8	55	1
	Bad	0	0	0	5	9