Reliability of Taxonomic Sufficiency for Simplifying Benthic Index M-AMBI Methodology in A Heavily Polluted Estuary

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Research Article

Keywords: Benthic index, Ecological Quality, Macrofauna, Taxonomic Sufficiency, Eutrophication, Liaohe Estuary

Posted Date: December 7th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-109416/v1>

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Abstract

In order to assess the ecological quality status of coastal environments in Europe, the AZTI's Marine Biotic Index (AMBI) and multivariate-AMBI (M-AMBI) have been developed. However, the applicability and validity of these methods worldwide remains in question, particularly for complex ecosystems such as estuaries. The present study, therefore, is an investigation of the relationship between the M-AMBI and different contamination variables in a eutrophic estuary in three seasons (i.e., spring, summer and autumn). In addition, the reliability of taxonomic sufficiency for simplifying M-AMBI operation was tested. The results showed that genus- and family-level data accurately reproduced the spatial-temporal patterns of species-level community assemblages. The M-AMBI values showed a consistent spatial distribution pattern in all sampling seasons, with a decreasing trend along the increasing distance from the estuary inlet. Furthermore, both genus- and family-level results performed nearly as well as species-level data in detecting the seasonal variations of different contaminants (i.e., nutrients and organic enrichment). The taxonomic sufficiency succeeded in this temperate ecoregion is owing to the high aggregation ratios at different taxonomic levels in all sampling events. In general, these findings suggested that application of taxonomic sufficiency based on the M-AMBI provides a simple and efficient method for evaluating variations of ecological quality in the Liaohe Estuary.

Introduction

Globally, estuaries are among the most valuable and productive ecosystems. Estuaries have been shown to support a variety of essential ecological functions and provide social benefits to humans¹. In recent years, estuarine ecosystems have been increasingly demonstrated habitat degradation due to intensive anthropogenic activities (e.g., urbanization, industrial development, shipping, fishing, and aquaculture), along with the consequent stressors that accompany these activities (e.g., chemical pollution, overfishing, invasive species and climate change)^{2,3,4}. From the perspective of environmental management, proper efforts to understand the anthropogenic impacts on the health status of these critical ecotones are necessary. Traditional ecological assessment programs rely on the identification of taxa to the species level. Considering the increasing anthropogenic disturbances to coastal ecosystems and limited expertise, there has been an urgent need to simplify this process. The concept of taxonomic sufficiency (TS) involves analysis of higher taxa, rather than species, to satisfy the objectives of a program without the costly and time-consuming species identification⁵. The ecological status assessments have developed by several biotic indices, and most of them are based on macrobenthic communities⁶. Macrobenthic invertebrates have the specific physiological characteristics of a wide distribution, limited mobility, and sensitivity to environmental disturbance, making them ideal indicators of ecosystem health⁷.

Benthic indices are especially relevant in environmental management since they reduce complex scientific data, integrate different types of information, and provide information that can be easily understood and implemented by policymakers⁸. Benthic indices have been applied in many countries

worldwide in order to protect coastal areas^{9,10,11,12}. As widely applied benthic indices, AZTI's Marine Biotic Index (AMBI) and multivariate-AMBI (M-AMBI) are based on the proportion of species assigned to five ecological groups according to specific tolerances to organic enrichment^{13,14}. Although it was originally developed for European coastal waters, it has been applied or evaluated in many countries outside Europe. In China, some studies evaluating the potential application of AMBI as a measure of ecological quality have produced promising results (e.g., Yangtze River Estuary, Liaodong Bay, Bohai Bay, Yellow River Delta, etc.)^{12,15,16}. Until now, most of the benthic indices currently used to assess ecological quality (EcoQ) were developed, applied, and validated for coastal marine ecosystems that generally have much less seasonal variations than estuarine ecosystems. Seasonal variability strongly influences the abiotic and biotic factors in the estuarine ecosystems. The seasonal variations in macrofaunal assemblages are controlled by changes in salinity, temperature, dissolved oxygen, and sediment types, of which most are closely related to riverine freshwater input¹⁷. Therefore, the natural stressors that impact the species composition may influence the performance of the benthic index. However, few studies have tested the AMBI index against seasonality, particularly in China.

Some preliminary verification needs to be recommended in the context of biomonitoring programs¹⁸. The taxonomic level of identification may have a great influence on biotic indices based on species information, since sensitivity to pollution in the same taxonomic group may differ from one species to another. Previous studies indicated that the performance of higher taxa as surrogates for species is highly variable, making it difficult to predict whether the method will be suitable for a particular objective¹⁹. Despite its great potential to simplify the ecological assessment, the TS approach should be tested and not merely assumed. In order to provide background information to achieve a better use of M-AMBI in a distinct area under severe eutrophication pressure, we investigated the benthic EcoQ using M-AMBI at three seasons (summer, autumn and spring) in the Liaohe Estuary. The aims of this study were: (1) to describe the spatial-temporal distribution of EcoQ in the studied area by the M-AMBI classification; (2) to test the effects of using higher taxonomic data on the performance of M-AMBI; and (3) to relate the benthic ecological quality based on M-AMBI with the contents of different pollutants. Furthermore, we discussed the results with regard to implications for the applications of M-AMBI along the China coast.

Results

The Liaohe Estuary (lat. 40°20'–41°N and Lon. 121°20'–122°E) is located in the Liaodong Bay, a part of the Bohai Sea, Northeastern China (Fig. 1). With the rapid industrialization and urbanization of upstream cities, the Liaohe Estuary is exposed to considerable anthropogenic pressures including industrial and agricultural effluent, domestic sewage discharge, oil mining, and physical disturbance by trawling and dredging. The surrounding sea-going rivers (i.e., Liaohe River, Shuangtaizi River, Daling River, and Xiaoling River) carry large amounts of terrigenous pollutants, which significantly deteriorate the habitat quality of Liaohe Estuary²⁰. With the massive expansion of high-density aquaculture practice in the Liaohe delta wetland, crab aquaculture pollution has also become a severe environmental problem²¹. Liaohe Oilfield, the third-largest oilfield in China, also poses high pollution pressures on the surrounding environment.

Furthermore, Liaohe Estuary is serving as a vital fishery ground, and commercial bottom trawling may alter the structure and function of ecosystems. According to the “Bulletin of Marine Ecology and Environment Status of China in 2018” (Ministry of Ecology and Environment of the People’s Republic of China), Liaohe Estuary is one of the most contaminated area in China, with the primary dominant pollutants being inorganic nitrogen and active phosphate. In present study, a total of 25 sublittoral sampling stations were set up in the Liaohe Estuary, which were divided into six categories based on the distance from the station to the river inlet (A, No. 1–2; B, No. 3–6; C, No. 7–10; D, No. 11–15; E, No. 16–20; and F, No. 21–25; A to F meant closest to farthest from the river inlet). After data collection in spring, summer, and autumn seasons, the spatial-temporal variations of environmental conditions and benthic indices were investigated.

Environmental variables

The spatial-temporal variability of abiotic parameters in the studied estuary is presented in Fig. 2. Significantly higher Chl-a, $\text{NO}_2\text{-N}$, and COD concentrations were recorded in summer compared to spring and autumn (ANOVA, $p < 0.05$). In contrast, the water salinity increased in spring and decreased in summer compared to autumn (ANOVA, $p < 0.05$). The $\text{NO}_3\text{-N}$ and NH_4 contents in the water were higher in summer and lower in autumn compared to spring (ANOVA, $p < 0.05$). Moreover, the TOC concentrations in summer and autumn were significantly higher than those in spring. however, the $\text{PO}_4\text{-P}$ in spring and summer were significantly lower than autumn (ANOVA, $p < 0.05$). In addition, no significant differences were observed in the sediment compositions and PHc content among the different seasons (ANOVA, $p > 0.05$).

Changes in environmental quality indicators at different distances within the same season were also analyzed. Salinity rose in all three seasons as the distance from the inlet increased. In summer, Chl-a concentrations in water samples from the A, D, E, and F regions were significantly higher than in the other samples. In summer and spring, the $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, and COD concentrations significantly decreased as the distance from the inlet increased. In contrast, the values of these indicators showed no significant spatial variance in autumn. As the distance from the estuary increased, the proportion of sand and clay in sediments decreased at first, and then increased. No obvious change in the pattern of PHc content was found in the sediments at different distances within the same season.

Macrobenthic community structure and seasonal variation

We sampled 15,680 individuals from 105 species during three seasons, representing 87 Genera, 70 Families, and 27 Orders. Polychaete was the dominant taxa, with the largest number of species. Mollusca, Crustacea, and Echinodermata were also common, in contrast, Bryozoa, Nemertinea, Sipuncula, and Plathyelminthes were far less common. Mollusca (56.7%) was the dominant taxa in terms of abundance, followed by Polychaeta (23.7%), Crustacea (13.8%), Echinodermata (3.3%), and others (2.5%). The number of each taxonomic unit and the dominant species in each season with aggregation ratio (λ) (Bevilacqua et al. 2012) (higher rank taxon count to species count) are shown in Table 1. Species

richness slightly decreased from summer to autumn and spring, with 59, 57, and 57 taxa, respectively. Only 20 species (19%) were common among all three seasons, while 57 species (54%) were season specific. Moreover, 31 species were common in both summer and autumn, whereas 28 were common in spring and autumn (Fig. 3).

Table 1

Summary of orders, families, genus and species counts, aggregation ratio (α) for higher taxonomic resolutions and dominant species in three seasons. Ecological groups assigned to dominant species are provided in parenthesis. NA indicates “not assigned”.

Season	Orders	Families	Genera	Species	Dominant Species ($\geq 2\%$)
Spring	18	37	47	59	<i>Potamocorbula ustulata</i> (NA)
Aggregation ratio (α)	0.42	0.83	0.87		<i>Ampelisca</i> sp. (I)
Summer	19	39	58	57	<i>Potamocorbula laevis</i> (V)
Aggregation ratio (α)	0.37	0.80	0.98		<i>Aonides oxycephala</i> (III)
					<i>Capitella capitata</i> (V)
Autumn	20	36	50	57	<i>Corophium acherusicum</i> (III)
Aggregation ratio (α)	0.47	0.85	0.93		<i>Grandidierella</i> sp. (I)
					<i>Amphioplus</i> sp. (I)
					<i>Capitella capitata</i> (V)
					<i>Potamocorbula laevis</i> (V)
					<i>Glossaulax didyma</i> (I)

All the identified taxa were assigned to 5 EGs based on the AZTI's classification (Table S1 in Appendix. A), 33 taxa (31.4%) were classified as EG I, 36 taxa (34.3%) assigned to EG II, 16 taxa (15.2%) were assigned to EG III, 7 taxa (6.7%) assigned to EG IV, 2 taxa (1.9%) assigned to EG V, and 11 species (10.5%) were unassigned. In terms of abundance, EG VI and EG I were dominant, which were due to the higher densities of *Potamocorbula laevis* and *Amphioplus* sp., respectively. Aggregating the species data to genus and family taxonomic levels produced 93 and 74 taxa, respectively (Table S1).

Calculation and inter-calibration of M-AMBI across taxonomic levels

Among all 70 M-AMBI values for sampling stations in all three seasons, the ecological status derived from species-level data indicated that the majority of the stations were categorized as Good, Moderate, and Poor status (40%, 18.6%, and 20%, respectively). A minority of 8.6% stations were classified as High, and 12.8% were ranked as Bad. In terms of seasonal variability, 20 of 25 (75%) showed variations in their ecological status over the sampling periods (Fig. 4). Most of the variations occurred among the adjacent categories (e.g., High to Good, Good to Moderate), however, S6 and S14 fluctuated from High to Bad. The

stations with consistent ecological classification belonged to the Good (S12, S16, S21 and S22) and Moderate (S3) categories. Overall, the summer showed the highest M-AMBI values among the three seasons, which indicated a decreased ecological status. Moreover, M-AMBI values showed consistent spatial distribution during all three seasons, with a clear distribution pattern related to the distance from the estuary and coast (Fig. 4). The inshore sites were more disturbed than the offshore sites. It is noted that the inshore sites (e.g., S1 to S6) generally had the highest temporal variations in the ecological status. Conversely, offshore sites showed a relatively consistent increased ecological status.

Kappa analysis indicated “Almost perfect” agreement existing between species- and genus-level M-AMBI EcoQ classifications using the standard class boundaries (Table 2) (Kappa value = 96.2%, Asymp. Std. Error = 2.7%). However, similar analysis for species- and family-level outputs resulted in “Substantial” agreement (Kappa value = 68.8%, Asymp. Std. Error = 6.6%) between EcoQ classifications. Inter-calibrating the class boundaries (High/Good 0.78, Good/Moderate 0.56, Moderate/Poor 0.44 and Poor/Bad 0.24) for family-level EcoQ outputs produced a positive effect on the agreement between species- and family-level EcoQ classifications, which resulted in an “Almost perfect” agreement (Kappa value = 82.7%, Asymp. Std. Error = 5.4%).

Table 2

Number of stations for each ecological quality (EcoR) classification using the M-AMBI calculated with aggregated datasets.

EcoR Classification	Aggregation level		
	Species ^a	Genus ^a	Family ^b
High	6	6	6
Good	28	26	28
Moderate	13	15	12
Poor	14	14	15
Bad	9	9	9
Total	70	70	70
^a Standard EcoQ classification boundaries used; High/Good 0.77, Good/Moderate 0.53, Moderate/Poor 0.39 and Poor/Bad 0.2.			
^b Inter-calibrated EcoQ classification boundaries used: High/Good 0.78, Good/Moderate 0.56, Moderate/Poor 0.44 and Poor/Bad 0.24.			

Relationships between M-AMBI, ecological group distribution, and abiotic factors

The assemblage-environment relationships were explored by DistLM analysis (Table S2). The sequential tests using forward procedure revealed that seven environmental variables collectively contributing power

of explanatory model for macrofauna assemblage composition in three taxonomic levels. The increased prediction power (adjusted R^2) was observed as taxonomic levels increased, with the family level showing the best predictive power, followed by the genus and species levels. The db-RDA routine also showed that environmental variables of importance differed among three seasons within the three taxonomic levels (Fig. 5). M-AMBI scores obtained from three taxonomic-level datasets (i.e., species, genus, and family) indicated a significant relationship with abiotic factors. M-AMBI scores showed strong correlation with salinity, EI, and TOC (Fig. 6). The highest correlations with salinity, EI, and TOC were all obtained from the family-level data.

Discussion

Benthic indices are useful metrics for environmental quality, but their use require specialized taxonomic expertise with a labor intensive and time-consuming process. TS applied in environmental monitoring programs significantly reduces the expertise, cost, and time needed for species identification. According to several studies, using a high taxonomic level may not produce a substantial loss of information for discriminating the effects of anthropogenic activities on benthic fauna^{3,21,22}. According to the “hierarchical-response-to-stress” principle, the taxonomic level on the variation of benthic assemblages depends on the intensity of anthropogenic stress²³. On the other hand, analysis at high taxonomic levels could alleviate the confounding effects of species-level responses to natural environmental variability. This allows the detection of anthropogenic disturbance more accurately. Studies have shown that high taxonomic groups in marine soft-bottom benthic habitat, especially the family-level data, are sufficient to portray the response of benthic assemblages under strong anthropogenic disturbances^{23,24}. Furthermore, natural variables are thought to change the benthic community structure by the approach of species replacement, while intensive anthropogenic disturbances force changes by altering the proportion of higher taxa²⁵. This judgment contributes to the rationality of using higher taxa levels to analyze the effects of anthropogenic influences. However, it has been suggested that the use of taxonomic levels in biotic indices should be only restricted to areas where a homogeneous community is represented, with families across sampling stations having similar species compositions²¹.

In this study, the suitability of family-level M-AMBI for EcoQ assessment in the Liaohe Estuary was tested based on its ability to reproduce the spatial-temporal patterns in species-level communities. Some researcher²⁶ suggested that high aggregation ratios ($\alpha > 0.4$) indicated minor differences among the studied taxonomic resolutions, and in turn, supported the robust application of TS. Compared with references²⁶ therein, the aggregation ratios for genus (0.93) and family (0.83) were high in the geographical region of this study. Thus, little information is lost if higher taxonomic levels were used instead of species for this geographical region. In the present study, the highest predicted power was observed in the family-level model for studying the assemblage-environment relationships. This result indicates that most congeneric species responded to the environmental gradient in the same way. A concern regarding the application of TS is the loss of ecologically important information provided by the species-level data that may be helpful to reflect particular disturbances^{27,28}. This is troublesome in

freshwater ecosystems where congeners give diversified responses to various disturbances. However, it is suggested that marine macrofaunal congeners exhibit a similar response to pollution^{29,30}. On the other hand, the diversity of high taxon may influence the consistency of responses to environmental gradients, since a species-rich high taxon may be more likely to show internal ecological heterogeneity. In regions characterized by low degree of radiation, coarser taxonomies (e.g., family or even order) are suitable to study assemblage-environment relationships³¹.

The current study found temporal variations in most stations' ecological status, especially in areas with poor environmental conditions (i.e. sampling stations near the mouth of the river). Several studies have shown that AMBI/M-AMBI are impervious to natural abiotic fluctuations^{12,32,33}. However, other studies have suggested the temporal variations of AMBI in an area subject to anthropogenic activities^{10,34,35}. In the present study, the area at the mouth of the river is the closest to land-based pollution sources. Therefore, this region may receive more constant and direct human disturbances, which could partly explain the energetic temporal variation patterns. In the Liaohe Estuary, the summer presents the most stressed scenario due to the accumulation of contaminants, whereas better environmental conditions prevail during the autumn and spring due to the flushing out of contaminants after summer. In terms of macrofaunal assemblages, the mortality and recruitment of macroinvertebrates during different seasons result in seasonal variations in composition and abundance. We recommend that determining the ecological quality of marine benthic environments should not be based on only one sampling event. A seasonal sampling strategy should be considered to give a comprehensive view of temporal variations in environmental quality.

Inconsistency in methodology during biomonitoring programs, such as discrepancies in the literature or changes in personnel collecting data, could contribute to significant inaccuracies. For example, it has been shown that inconsistencies in findings arise when there is a change in personnel analyzing samples during the course of a long-term program. Major changes in biological community structure arose from changes in the expert staff or in the laboratory responsible for data collection in time-series monitoring programs^{36,37}. Therefore, formulating quick and efficient monitoring strategies like TS is necessary. It has been shown that TS tends to increase the accuracy and trustworthiness of the results obtained, especially for large-scale and long-term programs²⁶. China is a large country with a correspondingly high variety of coastal habitats (e.g. estuary, bay, mangroves, coral reefs, sea grass and lagoon). Given its extensive marine biodiversity, China has been reported to contain 22,629 species belonging to 46 different Phyla³⁸. However, considering the rapid decline of marine biodiversity, it is urgent to implement routine biomonitoring programs to understand the process of global climate change and human activities that are having an impact in China. A big challenge for biomonitoring programs is the lack of taxonomists who provide information on biodiversity, provide species names for communication, and are at the forefront of documenting biological community structure. The use of coarser taxonomic resolution may be a more efficient way to ensure temporal and geographical comparability, especially for countries with a heterogeneous coastal line with high biodiversity.

In China, researchers have argued that the feasibility of AMBI might depend on the extent of sediment pollution in the studied area. Wu *et al.*³⁹ suggested that AMBI would not be suitable for the ecological quality assessment in Fujian Province, since the TOC content of sediment is low in that area. The results of the present study corroborate that M-AMBI are good indicators of organic and nutrient enrichment, since they respond predictably to those pressures, as indicated by Spearman correlations. Considering the effects of monitoring periods and/or stations on the performance of benthic indices as showed in this study, it is recommended that a long-term monitoring program with consistent sampling periods/stations should be implemented on the national level for a comparative assessment of Chinese estuaries.

In summary, this study tested the reliability of using high taxonomic levels for simplifying benthic index M-AMBI operations in a heavily polluted estuary. The results indicated that family-level data provides a sufficient degree of confidence for application with M-AMBI, since this approach produces a good classification of sampling stations affected by freshwater discharges with seasonal variability. The ability of M-AMBI to classify EcoR consistently at increasingly higher taxonomic levels supports a TS approach to monitoring using M-AMBI. The high temporal variability in both the biotic and abiotic parameters of the study area suggested the importance of setting identical sampling periods for different regions in national biomonitoring programs, since only one seasonal sampling each year is currently recommended in China. Considering that some local species are not assigned by the M-AMBI database, the application of AMBI requires further expertise to recognize species tolerance and improve ecological group classifications in China.

Methods

Field sampling

Field sampling was carried out in three surveys representing three seasons i.e., summer (August 2013), autumn (November 2013), and spring (May 2014). We set a total of 25 sublittoral sampling stations in the Liaohe Estuary (S1 to S25) (Fig. 1) with the depth of 2–11 m. The stations were divided into six categories based on the distance from the station to the river inlet (A, No. 1–2; B, No. 3–6; C, No. 7–10; D, No. 11–15; E, No. 16–20; and F, No. 21–25; A to F meant closest to farthest from the river inlet). It is unappeasable to collect the fieldwork sediment samples for S1 and S3 in summer, S2 and S4 in spring, and S23 in autumn probably due to nearby sand dredging project. Sediment samples were collected using a 0.05 m² van Veen grab. Three replicates were collected for each station and pooled together before further treatments. The samples were sieved over 0.5 mm mesh to separate the macrofaunal community. The organisms were then fixed in 5% formaldehyde on board and then taken back to the laboratory for further analysis. Meanwhile, about 100 g sediment from one of the grab samples of each station was taken for the measure of grain size, total organic content (TOC), and petroleum hydrocarbons (PHC). Surface seawater samples in each station were collected simultaneously with 5 L Niskin bottles. The salinity and pH were measured in situ with multiparameter sensor (YSI 6600).

Laboratory analysis

In the laboratory, specimens in each sediment were isolated, counted, and identified to the lowest feasible taxonomic level, using a dissecting microscope and an optical microscope where necessary. We checked the validity and synonyms of taxa names with the database of the World Register of Marine Species (<http://www.marinespecies.org/>). For sediment samples, grain size was determined using a Mastersizer 2000 laser particle size analyzer (Malvern, UK). Approximately 0.2 g of powdered samples were totally digested in a Teflon vessel with a mixed solution of $\text{HNO}_3 + \text{HCl} + \text{HF}$ (5:4:1) on a heating plate and heated ($< 150^\circ\text{C}$) to dryness. Afterward, the residue was extracted with HNO_3 and diluted to a specific volume. The total organic carbon (TOC) was measured with an elemental analyzer. For seawater samples, dissolved oxygen (DO) for seawater samples was determined using the Winkler titration method. The seawater sample was filtered through a $0.45\ \mu\text{M}$ microporous filter membrane, and the concentrations of chlorophyll a (Chl-a), chemical oxygen demand (COD), $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and $\text{PO}_4\text{-P}$ in seawater were determined colorimetrically according to the methods described by Grasshoff *et al.*⁴⁰.

Data analysis

Difference in the environmental variables among the three seasons or the six different distances from the inlet with the same season were compared with PAST 3.0 software using a one-way ANOVA with the Turkey's honestly significant difference HSD test. Before the ANOVAs, Hellinger transformation was applied to the environmental variables making them suitable for parametric tests. Distance-based linear models (DistLM) were used to examine the relationship between macrofaunal community composition and environmental variables. A forward stepwise selection procedure was applied to select the significant explanatory environmental variables, with the best model being selected Adjusted R^2 information criterion method. Distance-based redundancy analysis (db-RDA) was performed by PRIMER v7.0 to visualize the results of DistLM⁴¹.

The AMBI was calculated using the software provided at the AZTI's website (<http://ambi.azti.es>) with the updated species list (June 2017). Calculation of M-AMBI includes a factor analysis (FA) of AMBI score, species richness, and Shannon-Wiener diversity. At "high" status, the M-AMBI value reaches one, and at "bad" status, the M-AMBI reaches zero. In the present study, reference conditions for M-AMBI calculation were set using the highest and lowest values in the datasets for each metric⁴². The M-AMBI scale is divided into five EcoQ categories (i.e., High, Good, Moderate, Poor, and Bad) by assigning a numerical value to each of the class boundaries.

The M-AMBI EcoQ of each station were determined and compared with species-, genus- and family-level database. On species level, taxa were ascribed an ecological group (EG) based on AMBI library. When species were not assigned in the database, they were assigned to the most common group found for the genus. If there were absent hitting, species were classified as "not assigned"⁴³. On the genus and family level, taxa that were not included in AMBI library were assigned to EGs based on median values of all AMBI entries in the parental taxa, as described by Forde *et al.*²⁹. We used a Kappa analysis^{44,45} to test the agreement between the number assigned to each of the five EcoQ categories based on the species-, genus-, and family-level data. To inter-calibrate EcoQ outputs to maximize the agreement across different

taxonomic levels, the standard boundaries (i.e., High status > 0.77; Good, 0.53–0.77; Moderate, 0.39–0.53; Poor, 0.20–0.39; and Bad, < 0.20) were modified using the approach suggested by Borja *et al.*⁴⁶.

The Spearman's Rank correlation coefficient (*r*) was estimated between abiotic variables and M-AMBI values obtained from different taxonomic levels to test if this index performed better with coarse taxonomy using SPSS 18.0. To relate M-AMBI values with eutrophication, the eutrophication index (EI) was calculated using the following formula: $EI = (COD \times DIN \times PO_3^{4-} \times 10^6) / 4500$, where $DIN = NO_3-N + NO_2-N + NH_4-N$, and COD, DIN, and PO_3^{4-} are in mg/L. EI > 1 indicates eutrophication status⁴⁷.

Declarations

Acknowledgements

This work was supported by the National Marine Public Welfare Research Project (201305030) and National Natural Science Foundation of China (31572595).

Conflicts of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Conceptualization: Hongjun Li; Methodology: Yi Sun; Formal analysis and investigation: Yanbin Gu, Pengfei Xie, Yuan Liu; Writing - original draft preparation: Hongjun Li; Writing - review and editing: Yi Sun; Funding acquisition: Jingfen Fan; Resources: Hongjun Li; Supervision: Hao Guo.

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

Map showing the sampling stations of Liaohe Estuary. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

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Figure 2

Spatial-temporal distribution of environmental variables (Sal: salinity; Chl-a: Chlorophyll a; COD: chemical oxygen demand; TOC: total organic carbon; PHc: petroleum hydrocarbons) in the Liaohe Estuary. The stations were divided into six categories based on the distance from the station to the river inlet (A, No. 1–2; B, No. 3–6; C, No. 7–10; D, No. 11–15; E, No. 16–20; and F, No. 21–25).

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Figure 3

The seasonal succession (Venn diagram) of microbenthic assemblages in the Liaohe Estuary. The numbers indicate the taxa number detected, and the percentage in the brackets suggest the proportion of species in the pool of three seasons.

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Figure 4

AMBI ecological classification across stations and seasons. S1-S25: sampling stations 1 to 25 indicates the increased distance from river inlet.

Ordination plots of Distance-based Redundancy Analysis (db-RDA) of species-, genus- and family-level assemblages using Bray-Curtis similarity for abundance data.

Figure 5

Ordination plots of Distance-based Redundancy Analysis (db-RDA) of species-, genus- and family-level assemblages using Bray-Curtis similarity for abundance data.

Figure 6

Spearman's correlations between AMBI scores derived from species-, genus- and family-level datasets and environmental variables. Sal: salinity; EI: eutrophication index; TOC: total organic carbon.

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Spearman's correlations between AMBI scores derived from species-, genus- and family-level datasets and environmental variables. Sal: salinity; EI: eutrophication index; TOC: total organic carbon.

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