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Assessing benthic ecological status in coastal area near Changjiang River estuary using AMBI and M-AMBI*

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Abstract The Changjiang (Yangtze) River estuary has been subject to a variety of anthropogenic pressures in recent decades. To assess the ecological health of the coastal benthic ecosystem adjacent to the estuary, three surveys were conducted in 2005, 2009, and 2010. The AZTI's Marine Biotic Index (AMBI) and multivariate-AMBI (M-AMBI) were used to analyse the benthic ecological status of this coast. The AMBI indicate that the ecological status of the coast adjacent to the Changjiang River estuary was only slightly degraded in all 3 years. In contrast, the M-AMBI indicated that the ecological status was seriously degraded, a result that is most likely due to pollution and eutrophication induced by human activities. The assessment of the coast's ecological status by the AMBI was not in agreement with that of the M-AMBI at some stations because of lower biodiversity values at those sites. The analysis of the two indices integrated with abiotic parameters showed that the M-AMBI could be used as a suitable bio-indicator index to assess the benthic ecological status of the coast adjacent to the Changjiang River estuary. The reference conditions proposed for the coast of the Changjiang River estuary should be further evaluated in future studies. Designation of local species could also provide an important reference for Chinese waters. To improve the reliability of AMBI and M-AMBI, further research into the ecology of local species is required to understand their arrangement in ecological groups.

Keyword: benthic ecological status; biotic indices; macrobenthos; AZTI's Marine Biotic Index (AMBI); multivariate-AMBI (M-AMBI); Changjiang River estuary

1 INTRODUCTION

During recent decades, marine and estuarine environments have become degraded under increasing human pressures (Halpern et al., 2008). To protect the aquatic environment and provide safe, clean, healthy and productive habitats, several forms of legislation have been implemented worldwide related to monitoring, assessing, and managing ecological integrity. Examples include the Oceans Act in the United States; the Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD) in Europe (Borja et al., 2011); Oceans Policy Acts in Australia and Canada; the National Water Act in South Africa (Borja et al., 2008a); and the Environmental Impact Assessment and Marine Protected Areas Act in China (Fan, 1989; Lau, 2005; Cao and Wong, 2007). The aim of the WFD in Europe is to achieve good ecological status in all waters by 2015, and the MSFD aims to achieve good environmental status in offshore waters by 2020 (Borja et al., 2010, 2011). The requirements of the WFD and MSFD include using an integrative method of assessing ecological status. This assessment involves using several biological elements

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(phytoplankton, benthos, algae, phanerogams, fishes) linked with physicochemical elements (including pollutants). The assessment also involves several tools in the evaluation of water bodies based on chemical elements (Borja et al., 2004b; Maggi et al., 2008; Tueros et al., 2008), physicochemical elements (Bald et al., 2005; Devlin et al., 2007; Giordani et al., 2009; Caruso et al., 2010; García et al., 2010) and biological elements (Borja et al., 2009a; Birk et al., 2012).

One of the abovementioned elements that is more extensively used for assessment is benthic invertebrate communities (Borja et al., 2009b). These communities respond relatively rapidly to anthropogenic and natural stresses with their unique community characteristics. An advantage of using benthic invertebrates is their relatively sedentary habits, which means that they cannot avoid deteriorated water/sediment quality conditions. A second advantage is their relatively long lifespan, which can be used to indicate and integrate water/sediment quality conditions over time. Other advantages are the diversity of different species with different tolerances to stress and their important role in cycling nutrients and materials between sediments and the overlying water column (referred to as bioturbation and bioirrigation) (Pearson and Rosenberg, 1978; Dauer, 1993; Borja et al., 2000).

AZTI's Marine Biotic Index (AMBI) was proposed by Borja et al. (2000) to evaluate the ecological quality of European coasts, especially in areas of anthropogenic disturbance. AMBI is based on the fact that different species of macrobenthic communities have different tolerances to stress. After linking the different sensitivity levels to an anthropogenic stress gradient, the macrobenthic species were classified into five ecological groups (EG). The proportions of individual abundance in the macrobenthic fauna were combined with the weighting of the EG to produce a continuous value (Borja et al., 2000). AMBI is the most commonly used biotic index within the WFD (Borja et al., 2009b). It is efficient in detecting the degradation of habitat quality caused by different human pressures in different parts of the Atlantic Ocean, Baltic Sea, Mediterranean Sea, North Sea and Norwegian Sea and water bodies in China, Uruguay and Brazil (Muxika et al., 2005; Borja et al., 2012; Cai et al., 2013). However, because of the complexity of benthic communities and diversity of benthic gradients, it is unrealistic to use a universal index in all systems (Engle and Summers, 1999). In fact, under some conditions, AMBI was inconsistent with some environmental parameters related to physical disturbance (Muxika et al., 2005). Borja et al. (2003, 2004a) and Muxika et al. (2005) also recommended that the use of AMBI should be complemented with other structural parameters (such as diversity and richness) to minimise some misclassification problems in the assessment of ecological status. M-AMBI, which is AMBI's multivariate extension, integrates the Shannon diversity index, richness and AMBI into a factor analysis multivariate approach to assess ecological status, which overcomes this potential weakness (Muxika et al., 2007).

At present, the assessment of benthic ecological status is critically important in China. China's rapid development in recent decades has led to a drastic increase in pressure on coastal and estuarine waters, with a progressive degradation of coastal water quality, habitat loss and ecosystem health problems (Zhao et al., 2004; Li et al., 2007; Wu et al., 2010; Liu et al., 2011). There is an urgent need to restore ecosystem health in the coastal zone of China. Results of this type of assessment are an indispensable tool for guiding decisions on restoration.

Here, we use the coastal area of the Changjiang (Yangtze) River estuary as a case study. The aims of the present study are as follows: (i) to assess the benthic ecological health of this coast at the ecosystem level using AMBI and M-AMBI; (ii) to test the usefulness of these two indices in Chinese coastal waters; and (iii) to establish a suitable reference condition for the sandy sediment biotype in this area of coast and to assign Chinese species to EG.

2 MATERIAL AND METHOD

2.1 Study area and sampling strategy

The Changjiang River estuary is one of the largest estuaries in the world, and it is located at 121°–124°E, 29°30′–32°N. Its width measures approximately 90 km at the river's mouth (Fig.1). The mean annual temperature in this area is 15.2°C to 15.7°C. The Changjiang River transports approximately 933×10^9 m^3 of fresh water into the sea every year and carries approximately 486×10^6 t of sediment to the coastal area (unpublished data). Because of the rapid development of the economy in this area, the stress from human activities is significant. Over the past 30 years, the population of Shanghai has doubled and reached 23 470 000 in 2011 (Shanghai Municipal Statistical Bureau, 2012). According to official

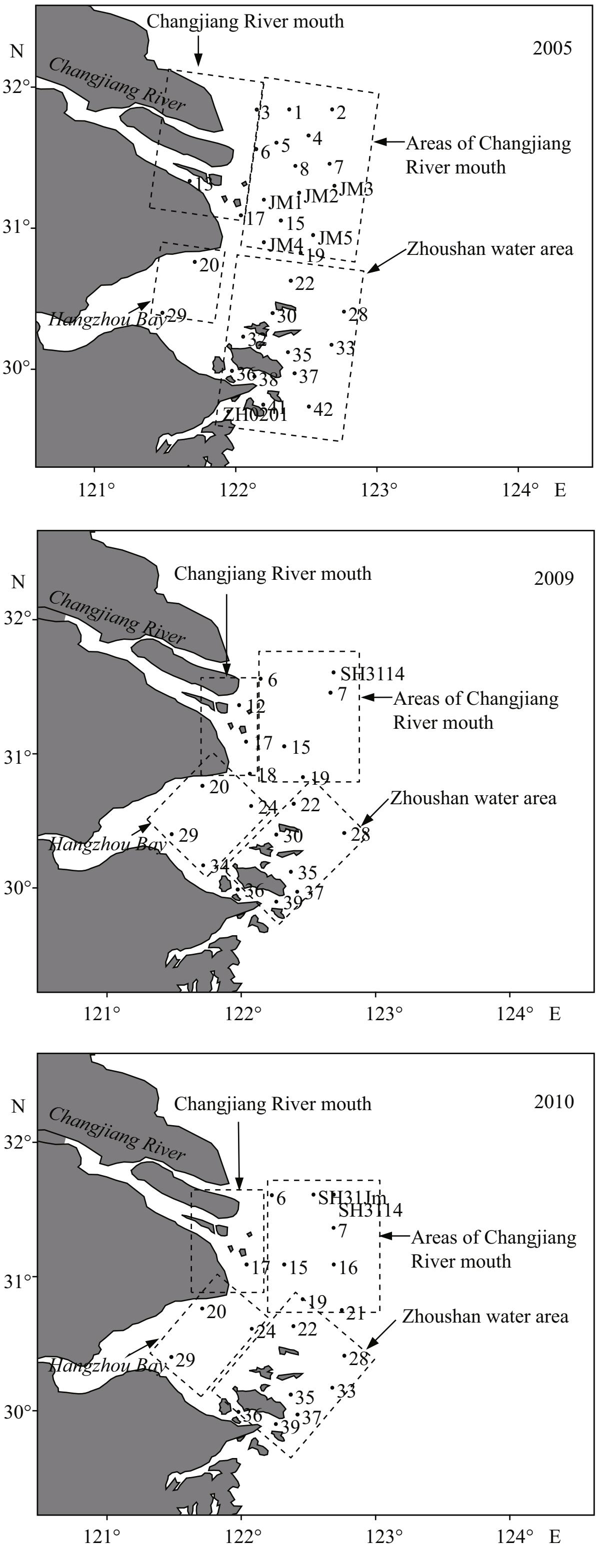

Fig.1 Study area and locations of macrobenthos sampling stations in the coast adjacent to the Changjiang River estuary in 2005, 2009, and 2010

figures, in the past 15 years, the total sewage water discharged into ambient waters from Shanghai alone was 1.8×10^9 t annually (Shanghai Municipal Statistical Bureau, 2012). In 2006, the annual fluxes of chemical oxygen demand (COD), nitrate and total phosphorus (TP) were 1.97×10^6 t, 1.11×10^6 t and 70.5×10^3 t, respectively, in the Changjiang River estuary (Chen et al., 2011a). These loads of nutrients into the water body of this area have resulted in serious environmental problems such as eutrophication and pollution.

Sampling sites were set in the Changjiang River estuary and its adjacent waters, which can be divided into four zones: the mouth of the Changjiang River (MCR), the area off the mouth of the Changjiang River (OMCR), Hangzhou Bay (HB), and the Zhoushan area (ZA). To investigate the macrobenthos, 31 stations were sampled in 2005, 19 stations were sampled in 2009 and 2010, with 12 coincident stations sampled in all 3 years (Fig.1). Water quality data, including COD, dissolved inorganic nitrogen (DIN) and dissolved inorganic phosphorus (DIP), were also collected at the same stations. With regard to sediment physicochemical data collection, 35 stations were sampled in 2005 and 23 stations in 2009 (Supplementary material A).

All field sampling was conducted with an oceanographic research vessel. The macrobenthic samples were collected using a 0.1-m^2 box-corer grab with two separate replicates for every station. The sediments were then sieved through a 0.5-mm aperture mesh to separate the macrobenthic organisms. The macrobenthic samples were preserved in 80% ethanol until laboratory identification.

For sediment chemical analyses, a grab of sediment was collected separately. A 500-g subsample was covered in silver paper and put in cold storage for future analysis. Bottom water samples were collected with Niskin bottle and preserved in refrigerated conditions.

2.2 Water and sediment analysis

The analysis of abiotic parameters was undertaken in the following ways. The organic carbon (OC) content was measured using a Perkin-Elmer Model 240 Elemental Analyser. The organic matter (OM) in the sediments was measured based on loss before and after ignition at 550°C for 3 h. Total sulfide (TS) was analysed by the potentiometric titration of sediments suspended in sulphide anti-oxidant buffer. The concentration of crude oil in sediment was taken by excitation at 310 nm and emission at 360 nm. The total nitrogen (TN) and DIN were measured using the UV spectrophotometric method, and the TP and DIP were measured using the acidic molybdate-ascorbic acid spectrophotometric method. Last, the COD was taken using dichromate method. For metals, sediments were dried at room temperature, and sieved through a 100-mesh nylon sieve. Sediment aliquots of 0.5 g were digested in closed Teflon beakers by ultrapure HNO_3/HF mixtures at 120°C and evaporated to dryness. The residue was then dissolved in HNO_3/H_2O_2, evaporated to dryness again, and finally dissolved in 1% HNO_3. The metal content was analysed using inductively coupled plasma mass spectrometry (ICP-MS) (Perkin-Elmer, USA).

To compare abiotic and biotic variables, a eutrophication index was calculated using the following formula: E=COD×DIN×DIP×10^6/4 500 (Zhou et al., 1983), where COD, DIP and DIN are in mg/L and $E \geq 1$, indicating eutrophication.

The Chinese Marine Quality Standard (GB 18668-2002) was used to check the status of sediments. Marine fisheries, natural reserve areas, nature preservation zones for rare and endangered animals, marine culture zones, bathing beaches, direct body contact marine sports and industrial water areas related to marine food should be rated as "Class I". Normal industrial water areas and coastal scenic areas should be rated as at least "Class II".

2.3 Biological analysis

Once at laboratory, benthic macrofauna were sorted and identified to the lowest possible taxonomic level, then counted and weighed using an electric balance with a precision of 0.01 g to obtain the abundance and wet weight. Macrofauna data were analysed using several univariate indices: species richness, Shannon index (H), AMBI (Borja et al., 2000), and M-AMBI (Muxika et al., 2007), which were all computed using the AMBI program (version 5.0) freely available online at http://ambi.azti.es, and on the basis of the AMBI guidelines (Borja and Muxika, 2005).

Most of the species were assigned using the AMBI species list of V. Mar 2012. Some local species, such as *Arenicola cristata*, *Moerella iridescens*, and *Acetes chinensis*, were assigned based on expert opinion and a literature review (Dr. Ángel Borja, AZTI-Tecnalia/Marine Research Division, together with local expertise and literature).

To set the appropriate reference conditions for the coast adjacent to the Changjiang River estuary, three approaches were tested and their results were calculated. The methods considered were M-AMBI default, the precaution method (Borja et al., 2008b, 2012) and box-plot analysis (Paganelli et al., 2011). After analysing three results integrated with the current environmental status, we chose the precaution method used by Borja et al. (2012). This method used the highest recorded value of H and S increased by 15%, and the lowest AMBI value to calculate the M-AMBI values of the 2005, 2009, and 2010 data (Borja et al., 2012). The values were AMBI=0, H=3.5, S=28. Bad status values were AMBI=6, H=0 and S=0.

Based on Borja and Tunberg (2011), the threshold values for the M-AMBI are as following: 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. For those stations with abundance of unassigned taxa greater than 50%, we only show the AMBI and M-AMBI calculation results. They were not included in further analysis.

2.4 Data analysis

To evaluate significant differences ($P<0.05$) between the sampled stations and different surveys, the results were analysed using analysis of variance (ANOVA). The Kruskal-Wallis and Mann-Whitney test were also adopted where there was heterogeneity of variance. Multiple Regression and the Principal Component Analysis (PCA) method were applied to clarify the relationship between AMBI and M-AMBI and the sediment parameters using the Euclidean distance. All statistical analyses were performed using SPSS and Matlab (R2010 b) software.

3 RESULT

3.1 Abiotic parameters

Sediment-heavy metals and nutrient data were compared for four zones on the coast adjacent to the Changjiang River estuary between 2005 and 2009. Some heavy metals and nutrients in the sediment at some stations differed (Mann-Whitney U-test, $P<0.05$) between 2005 and 2009 (Table 1 and Supplementary material A). When comparing the four zones between 2005 and 2009, most of the measured parameters did not differ except for Pb, Hg, and TN (Mann-Whitney U-test, $P>0.05$) (Table 1).

According to the Chinese Marine Sediment Quality Standard (GB 18668-2002), most of the heavy metals, oil and OC in the sediments of all sampling stations qualified as superior Class I. The exceptions were the values of Cu at stations 17, 20, 28, 35, 39, and 41 in 2009, which qualified as Class II.

Table 1 Mean values of sediment chemical and physical parameters in the different sampling areas of the coast adjacent to the Changjiang River estuary in 2005 and 2009

	Year	Oil	As	Cu	Zn	Cd	Pb	Hg	OC	TS	TN	TP	Class (GB 18668-2002)
All stations	2005	4.11	10.13*	22.91**	71.79**	0.12**	20.39**	0.06**	0.42	7.18**	150.11**	13.05**	I
	2009	4.74	11.90*	29.57**	99.78**	0.19**	24.60**	0.03 **	0.47	10.47**	224.88**	17.53**	I
Mouth of Changjiang River	2005	5.25	8.35	19.50	63.48	0.16	18.20	0.06	0.34	4.37	89.65	9.90	I
	2009	3.00	10.48	26.35	98.30	0.27	24.15	0.04	0.34	64.15	141.75	18.85	I
Areas off mouth of Changjiang River	2005	3.50	10.05	20.31	68.21	0.12	23.56	0.04*	0.39	15.46*	136.58**	13.92	I
	2009	4.17	10.43	22.97	85.02	0.17	22.25	0.02*	0.45	11.87*	246.00**	17.32	I
Hangzhou Bay	2005	6.33	10.80	29.40	86.50	0.15	22.20	0.07	0.56	2.57	160.33	14.07	I
	2009	4.67	12.17	38.07	105.33	0.25	27.87	0.03	0.51	0.53	200.33	18.51	I to II (Cu)
Zhoushan water area	2005	3.93	10.63	25.09**	74.60**	0.11**	17.45**	0.07**	0.47	5.81	183.50	12.84**	I
	2009	5.33	12.81	31.28**	106.02**	0.17**	25.03**	0.03**	0.49	3.31	234.32	17.17**	I
Difference comparisons among four zones (*P*)	2005	0.19	0.23	0.10	0.06	0.07	0.05*	0.03*	0.13	0.57	0.01**	0.54	
	2009	0.53	0.56	0.23	0.28	0.23	0.42	0.24	0.92	0.16	0.78	0.20	

* (P<0.05), ** (P≤0.01) indicate significantly different.

3.2 AMBI

Among the 41 taxa identified in 2005, 19 (representing 58.6% of the total abundance) were not initially listed in the AMBI list or assigned to any ecological group. After assignment, seven (42.3% of the total abundance) remained unassigned. In 2009, 15 (31.6% of the total abundance) out of the 55 taxa identified were not initially assigned to any ecological group, and only two (0.07% of the total abundance) remained unassigned. In 2010, six (16.5% of the total abundance) of the 29 taxa identified were not initially assigned to any ecological group, and three (3.9% of the total abundance) remained unassigned.

In 2005, the proportion of unassigned taxa was over 50% at nine stations (Table 2). These stations were excluded in further analysis. The mean AMBI values of the remaining 22 stations ranged from 0 to 3.75, including three (13.6%) undisturbed stations, 17 (77.3%) slightly disturbed stations and two (9.1%) moderately disturbed stations. These results implied that the benthic environment was in relatively good condition and was subject to slight-to-moderate impacts from human activities in the study area (Table 2). The H value was not high (range, 0–3.09), and the S value ranged from 1 to 11, which indicated that the macrobenthos community had been disturbed by environmental change and human activities. However, because of the relatively high abundance of certain taxa that were not assigned at some stations, the AMBI results for some stations should be evaluated with care (over 20% of individuals remained unassigned).

In 2009, the mean AMBI values for the 19 sampling stations ranged from 0 to 6.0, including six (31.6%) undisturbed stations, 10 (52.6%) slightly disturbed stations, two (10.5%) moderately disturbed stations and one (5.3%) heavily disturbed station (Table 3). The H value ranged from 0 to 3.07, and the S value ranged from 1 to 21, indicating that the macrobenthos community was disturbed by some environmental changes and human activities. Because the percentages of unassigned species were less than 20% in all the 19 stations, the AMBI results should be acceptable.

In 2010, the mean AMBI values of the 19 sampling stations ranged from 0 to 7, with one (5.3%) undisturbed station, 13 (68.4%) slightly disturbed stations, four (21.1%) moderately disturbed stations and one (5.3%) extremely disturbed station. These figures imply that the benthic environment suffered greater disturbance in the study area in 2010 than in previous years (Table 4).

AMBI values at all sampling stations did not differ significantly among the years 2005, 2009, and 2010 (ANOVA, F=2.37, P>0.05). The AMBI values of the 10 sampling stations common to all 3 years also did not differ (ANOVA, F=2.95, P>0.05).

3.3 Reference condition setting

Three approaches were tested to identify appropriate reference conditions for the coastal area of the Changjiang River estuary using the relatively

Table 2 Results of AMBI and biodiversity of macrobenthos from the coast adjacent to the Changjiang River estuary in 2005

Stations	I (%)	II (%)	III (%)	IV (%)	V (%)	Mean AMBI	Disturbance classification	Richness	Diversity	Not assigned (%)	M-AMBI	Status
1*	33.3	0	55.6	11.1	0	2.17	Slightly disturbed	6	2.32	57.1	0.46	Moderate
2*	45.5	18.2	27.3	9.1	0	1.5	Slightly disturbed	9	1.83	73.2	0.496	Moderate
3*	0	33.3	33.3	33.3	0	3	Slightly disturbed	5	2.13	57.1	0.39	Poor
4*	8.3	8.3	58.3	25	0	3	Slightly disturbed	7	1.37	79.3	0.36	Poor
5	0	100	0	0	0	1.5	Slightly disturbed	1	0	0	0.21	Poor
6	0	100	0	0	0	1.5	Slightly disturbed	1	0	0	0.21	Poor
7	16	56	20	8	0	1.8	Slightly disturbed	11	3.09	26.5	0.61	Good
8	11.1	22.2	0	66.7	0	3.33	Moderately disturbed	4	1.69	18.2	0.32	Poor
13	0	50	50	0	0	2.25	Slightly disturbed	2	1	0	0.28	Poor
15	50	0	50	0	0	1.5	Slightly disturbed	2	1	0	0.31	Poor
17*	0	0	0	0	0	7	Extremely disturbed	1	0	100	-0.028	Bad
19	0	40	20	40	0	3	Slightly disturbed	4	1.76	50	0.34	Poor
20	0	0	100	0	0	3	Slightly disturbed	2	1	50	0.24	Poor
22	0	50	50	0	0	2.25	Slightly disturbed	3	1.58	33.3	0.34	Poor
28*	50	33.3	16.7	0	0	1	Undisturbed	6	2.41	53.8	0.49	Moderate
29	0	0	100	0	0	3	Slightly disturbed	1	0	0	0.15	Bad
30	0	0	100	0	0	3	Slightly disturbed	2	1	50	0.24	Poor
32	0	50	50	0	0	2.25	Slightly disturbed	3	1.58	33.3	0.34	Poor
33	0	28.6	57.1	14.3	0	2.79	Slightly disturbed	6	2.48	36.4	0.44	Moderate
35	0	50	50	0	0	2.25	Slightly disturbed	2	1	0	0.28	Poor
36	0	0	50	50	0	3.75	Moderately disturbed	2	1	0	0.21	Poor
37	0	25	50	25	0	3	Slightly disturbed	4	2	0	0.36	Poor
38	0	100	0	0	0	1.5	Slightly disturbed	1	0	0	0.21	Poor
41	0	0	100	0	0	3	Slightly disturbed	2	1	50	0.24	Poor
42	0	50	50	0	0	2.25	Slightly disturbed	4	2	50	0.39	Moderate
JM1	28.6	71.4	0	0	0	1.07	Undisturbed	3	1.38	0	0.38	Poor
JM2	93.1	0	0	6.9	0	0.31	Undisturbed	4	0.68	0	0.37	Poor
JM3*	66.7	4.2	16.7	0	12.5	1.31	Slightly disturbed	11	2.15	68.4	0.56	Good
JM4	100	0	0	0	0	0	Undisturbed	2	0.65	16.7	0.35	Poor
JM5*	16.7	16.7	66.7	0	0	2.25	Slightly disturbed	5	2.1	53.8	0.42	Moderate
ZJ0201*	0	0	100	0	0	3	Slightly disturbed	3	1.37	60	0.29	Poor

*Stations with unassigned taxa abundance over 50%. They were not included in further analysis.

high quality data from 2009 (Fig.2). The precaution method was chosen to provide the reference conditions because it was closer to the virtual condition. This choice was based on our comparison of the M-AMBI results using these three different reference conditions and our knowledge of the coastal area.

3.4 M-AMBI

In 2005, only one station (4.55%) had good ecological status (ES) and two (9.1%) stations had moderate ES. Most stations (18 stations, or 81.8%) had poor ES, and one (4.55%) station had bad ES (Table 2).

In 2009, the benthic ecological health was better than in 2005 based on the M-AMBI, with one station (5.3%) classified as having high ES, three stations (15.8%) with good ES, five stations (26.3%) with moderate ES, nine stations (47.4%) with poor ES, and one station with bad ES (Table 3).

Compared with 2009, benthic ecological health appeared to worsen in 2010. No stations had good-to-high ES, only four stations (21.1%) had moderate ES,

Table 3 Results of AMBI and biodiversity of macrobenthos assessment from the coast adjacent to the Changjiang River estuary in 2009

Stations	I (%)	II (%)	III (%)	IV (%)	V (%)	Mean AMBI	Disturbance classification	Richness	Diversity	Not assigned (%)	M-AMBI	Status
6	26.3	26.3	10.5	36.8	0	2.37	Slightly disturbed	9	2.78	0	0.55	Good
7	16.8	62.1	6.3	14.7	0	1.78	Slightly disturbed	21	3.07	1	0.78	High
12	0	0	0	0	100	6	Heavily disturbed	1	0	0	0.015	Bad
15	65	10	10	15	0	1.13	Undisturbed	9	2.77	0	0.59	Good
17	100	0	0	0	0	0	Undisturbed	2	1	0	0.36	Poor
18	100	0	0	0	0	0	Undisturbed	1	0	0	0.25	Poor
19	20	40	20	20	0	2.1	Slightly disturbed	10	2.96	9.1	0.59	Good
20	77.8	11.1	11.1	0	0	0.5	Undisturbed	3	0.99	0	0.36	Poor
22	0	100	0	0	0	1.5	Slightly disturbed	4	1.79	16.7	0.41	Moderate
24	33.3	50	0	0	16.7	1.75	Slightly disturbed	4	1.92	0	0.41	Moderate
28	4.7	1.9	5.7	87.7	0	4.15	Moderately disturbed	10	0.95	0	0.32	Poor
29	0	66.7	33.3	0	0	2	Slightly disturbed	3	1.58	0	0.35	Poor
30	0	100	0	0	0	1.5	Slightly disturbed	3	1.38	0	0.35	Poor
34	0	83.3	16.7	0	0	1.75	Slightly disturbed	2	0.65	0	0.26	Poor
35	0	90	0	10	0	1.8	Slightly disturbed	5	2.12	0	0.44	Moderate
36	100	0	0	0	0	0	Undisturbed	1	0	0	0.25	Poor
37	50	0	16.7	33.3	0	2	Slightly disturbed	6	2.58	0	0.50	Moderate
39	100	0	0	0	0	0	Undisturbed	1	0	0	0.25	Poor
SH3114	4.9	9.8	2.4	82.9	0	4.0	Moderately disturbed	15	2.06	0	0.51	Moderate

Table 4 Results of AMBI and biodiversity of macrobenthos assessment from the coast adjacent to the Changjiang River estuary in 2010

Stations	I (%)	II (%)	III (%)	IV (%)	V (%)	Mean AMBI	Disturbance classification	Richness	Diversity	Not assigned (%)	M-AMBI	Status
6	0	33.3	0	66.7	0	3.5	Moderately disturbed	3	1.5	25	0.29	Poor
7	0	0	0	0	0	7	Extremely disturbed	0	0	0	-0.04	Bad
15	0	0	100	0	0	3	Slightly disturbed	1	0	0	0.14	Bad
16	0	6.9	27.6	65.5	0	3.9	Moderately disturbed	3	1.18	0	0.24	Poor
17	0	100	0	0	0	1.5	Slightly disturbed	1	0	0	0.20	Poor
19	16.7	16.7	50	16.7	0	2.5	Slightly disturbed	5	2.13	14.3	0.42	Moderate
20	100	0	0	0	0	0	Undisturbed	1	0	0	0.26	Poor
21	0	77.8	22.2	0	0	1.89	Slightly disturbed	6	2.37	10	0.49	Moderate
22	0	100	0	0	0	1.5	Slightly disturbed	2	0.72	0	0.28	Poor
24	25	25	50	0	0	1.9	Slightly disturbed	3	1.5	0	0.35	Poor
28	9.1	0	9.1	81.8	0	4.0	Moderately disturbed	3	0.87	0	0.2	Poor
29	0	0	100	0	0	3	Slightly disturbed	2	1	0	0.25	Poor
33	0	40	10	50	0	3.2	Slightly disturbed	4	1.69	0	0.33	Poor
35	0	83.3	16.7	0	0	1.8	Slightly disturbed	3	1.25	0	0.34	Poor
36	0	0	100	0	0	3	Slightly disturbed	1	0	0	0.14	Bad
37	0	50	50	0	0	2.3	Slightly disturbed	4	1.91	25	0.40	Moderate
39	0	0	100	0	0	3	Slightly disturbed	2	1	0	0.25	Poor
SH3114	0	0	9.1	90.9	0	4.4	Moderately disturbed	4	1.28	0	0.25	Poor
SH31Jm	50	33.3	16.7	0	0	1	Slightly disturbed	6	2.13	50	0.49	Moderate

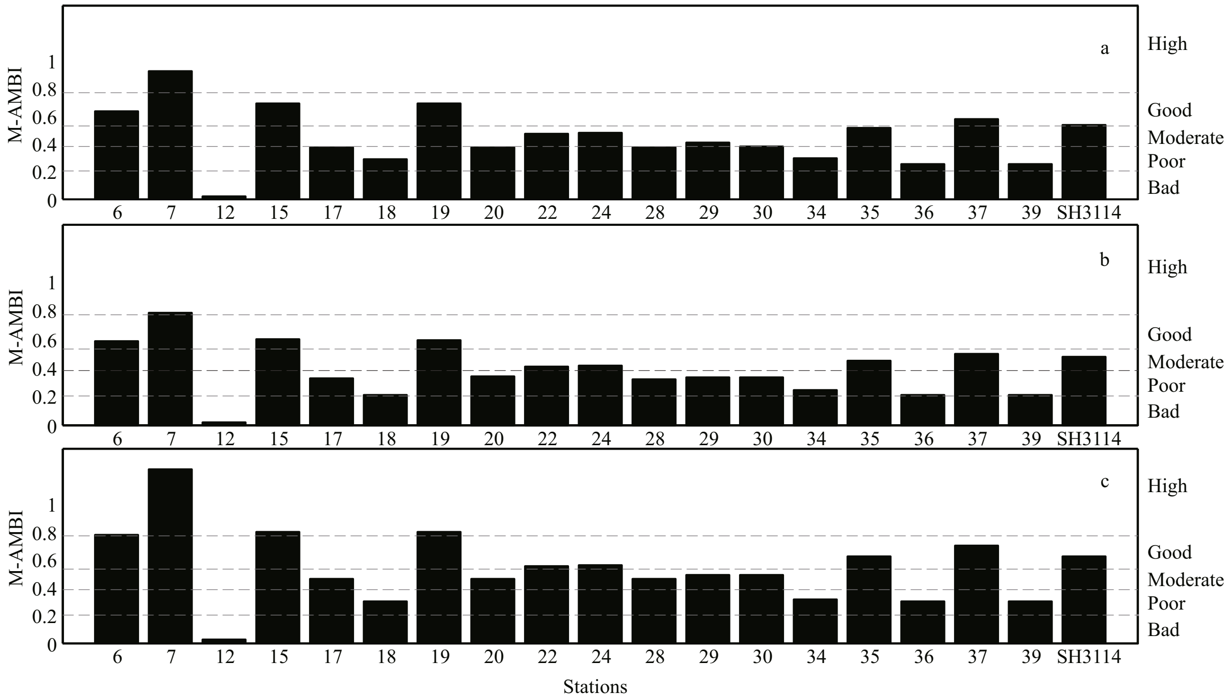

Fig.2 M-AMBI results using different reference conditions (high status) in 2009

a. M-AMBI default (lowest AMBI value and highest diversity *H* and richness *S* from the area), AMBI=0.50, *H*=3.07, *S*=21; b. *H* and *S* values increased by 15%, AMBI=0, *H*=3.5, *S*=28; c. Box-plot analysis by Paganelli et al. (2011), AMBI=0.59, *H*=2.78, *S*=9. Bad status values were: AMBI=6, *H* and *S*=0.

12 stations (63.2%) had poor ES, and another three stations (15.8%) had bad ES (Table 4).

The M-AMBI values of all of the sampling stations among the years 2005, 2009 and 2010 were significantly different (ANOVA, F=4.08, P<0.05). For the 10 sampling stations common to all 3 years, M-AMBI values among the 3 years were also significantly different (ANOVA, F=7.64, P<0.05).

3.5 Analysis of the relationship between AMBI and M-AMBI with environmental parameters

Because the sediment parameters were collected only in the 2005 and 2009 surveys, here we analyse the relationship between the benthic biotic indices and sediment parameters in 2005 and 2009. In 2005, AMBI had a significantly positive correlation with sediment parameters of Cu, Zn, OC%, OM%, and TN. In contrast, M-AMBI was not related to any sediment parameters (Pearson correlate analysis, significance level 0.05). In 2009, AMBI had significantly negative correlation with As, while M-AMBI had no significant correlation with sediment parameters (Pearson correlation analysis, significant level 0.05).

Based on AMBI, M-AMBI and sediment contaminants in 2005, the PCA plot showed a separation zone of sampling stations: MCR and HB, and OMCR and ZA (Fig.5a). The PCA plot of the sediment contaminants in 2009 also separated the sampling sites into two geographic zones: OMCR and the other three zones, namely, MCR, HB and ZA (Fig.5b). In 2009, the eigenvalues of PCA axes 1 and 2 were 5.51 and 2.19, which captured 59.3% of cumulative variation. In 2005, the eigenvalues of PCA axes 1 and 2 were 6.98 and 1.72, respectively, which captured 66.9% of the cumulative variation.

The eutrophication of the bottom water in the sampling area shows a distinct pattern: eutrophication is more serious close to the coast and less severe farther from land (Fig.4a). Although both the AMBI and M-AMBI plots did not exactly match the eutrophication index, they still show a similar tendency on the whole. Furthermore, the M-AMBI plot matched the eutrophication index plot better than did the AMBI plot (Fig.4b and c).

4 DISCUSSION

4.1 Species assignation

To obtain more accurate results, species assignment is of vital importance for the implementation of AMBI and M-AMBI. In this study, most of the species assignations were based on the newest species list of the AMBI software program (http://ambi.azti.es). The AMBI was, however, put forward initially for European macrobenthic fauna. Because there are differences in species composition between European macrobenthic fauna and Chinese fauna, some species local to the coast adjacent to the Changjiang River estuary are not included in the species list. The approaches to assigning those species were primarily according to Borja et al. (2008b), specifically the consultation of references, using the assignment of other species in the same genus and expert opinion (primarily that of Borja). However, because of the limited ecological study of the macrobenthos, particularly the sensitivity of species and their tolerance of the impacts induced by human activities in China, there were still some local species that could not be assigned to any ecological group by consulting references. According to the guidelines for the use of AMBI (Borja and Muxika, 2005), when the percentage of taxa that are not assigned is high (>20%), the results should be evaluated with care because there may be subsequent problems in the interpretation. If it is greater than 50%, the AMBI should not be used. In our results, in 2005, the percentage of unassigned taxa was greater than 20% at nine stations and greater than 50% at nine stations. In 2009, no station's percentage was greater than 20%, whereas in 2010, three stations had percentages were greater than 20%. The high proportion of unassigned taxa in 2005 was caused by the fact that several polychaete species with relatively high abundances could not be identified. According to the guidelines, the values of AMBI and M-AMBI for some stations in 2005 should be evaluated with care. Further research into the ecology of local species is required to clarify their arrangement in ecological groups.

4.2 Reference conditions

The reference condition for a water body type mentioned by the WFD is a description of the biological elements, which corresponds totally, or nearly totally, to undisturbed (pristine) conditions. In other words, this means with no, or with only a very minor, impact from human activities (Muxika et al., 2007). Setting the reference condition is crucial for calculating M-AMBI (Muxika et al., 2007). Four options could be adopted for deriving reference conditions: comparison with an existing pristine/undisturbed (or minimally disturbed) site; (ii) use of historical data and information; (iii) use of models and (iv) expert judgment (see details in Borja et al., 2004a, 2012; Muxika et al., 2007; Forchino et al., 2011). The common challenges encountered in practice are the absence of an appropriate pristine/undisturbed site and the lack of historical data in the literature (Borja et al., 2004a; Forchino et al., 2011; Zheng et al., 2013). Different approaches have been applied by scientists to overcome these difficulties related to reference conditions in different environments and geographical areas. Examples include the default M-AMBI (using the lowest AMBI value and highest *H* and *S* from the area); set minor disturbed sampling stations; literature; and data-driven, knowledge-driven and real reference stations (Forchino et al., 2011; Borja et al., 2012). Borja et al. (2008b) set the reference condition by selecting the highest *H* and *S* values observed in their study and increasing those values by 10%–15%, which was a data-driven approach combined with a subjective data correction (Paganelli et al., 2011). In some cases, the highest *H* and *S* values and lowest AMBI value recorded in an area could perform better as reference values for high status than other types of reference conditions from the literature or from real reference stations (Forchino et al., 2011). This is because the strategy involved was a purely data-driven procedure, which excludes subjective decisions and thus avoids the tricky problem of defining reference conditions (Paganelli et al., 2011). Based on data over a 10-year period, Paganelli et al. (2011) adopted box-plot analysis to calculate the 10^{th} percentile of the AMBI distribution and the 90^{th} percentile of the *H* and *S* distribution, which were taken as references for high AMBI conditions. In an almost pristine area, to avoid the risk of using extreme values for the three indices that could be very difficult to find in a unique sample, Paganelli's method should be better for calculating the AMBI and M-AMBI. In contrast, in a highly degraded area, the method of increasing the best recorded values by 10%–15% or another higher percentage could be a better choice. Which percentage to adopt depends on the level and extent of degradation of the area. In China, because of the high pressure from human activities along the coastal zone, it is difficult to find an almost pristine area. Therefore, the appropriate method is to increase the best value by 15% or by a higher percentage.

The coast adjacent to the Changjiang River estuary has historically been affected by human activities, especially over the last 60 years (Edmond et al., 1985; Meng et al., 2004; Chen et al., 2011b). Related

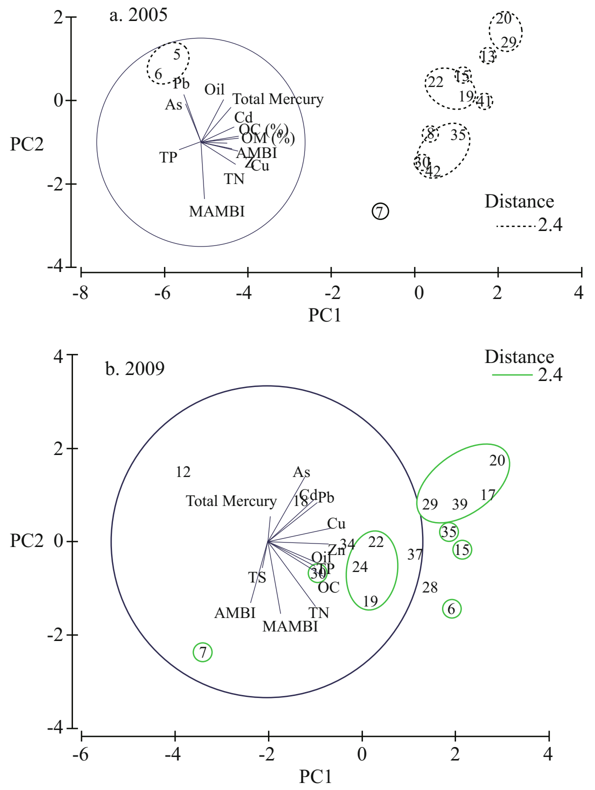

Fig.3 Principal components analysis (PCA) plot for 10 physical and chemical environmental variables on the coast adjacent to the Changjiang River estuary in 2005 (a) and 2009 (b)

Sediment environmental variables data were log (X+1) transformed, then normalized. a. The eigenvalues of PCA axes 1 and 2 are 6.98 and 1.72, which captured 66.9 % of cumulative variation; b. The eigenvalues of PCA axes 1 and 2 are 5.43 and 1.64, which captured 64.2% of variation.

detailed information in the literature is scarce, and this is the first assessment of the ecological status of this estuaryusing M-AMBI. Because reference conditions can change naturally with ecoregion, water body type and habitat (Borja et al., 2009c), it is particularly difficult to define reference conditions for the coast adjacent to the Changjiang River estuary.

The results were similar overall when we used the M-AMBI software default and the highest observed values with the 15% increase as the reference conditions. They were also quite different from the results of the box-plot analysis, a difference that resulted from the lower quantity of data available for the 3 years in the present study compared with data for over 10 years in the study by Paganelli et al. (2011). Because of the stress from human activities in the study region, such as the serious deterioration due to the pollution and eutrophication discussed above, we chose the highest values of H and S of the 3 years. We then increased these values by 15% for use as the reference conditions in the coast adjacent to the Changjiang River estuary, the values being AMBI=0, H=3.5, and S=28. Considering that the natural conditions and benthic community composition in this coastal area were quite different from those in European waters, additional studies for more accurate reference conditions should be undertaken.

4.3 The adaptability of these two indices in Chinese coastal waters

According to the report in the China Oceanic Information Network, eutrophication in Hangzhou Bay seriously escalated from 2005 to 2010 (see: http://www.coi.gov.cn/gongbao/nrhuanjing/nr2010/201107/t20110729_18777_2.html). The most severe environmental problems of the coast adjacent to the Changjiang River estuary were eutrophication and pollution. Our extensive exploration of the estuaryconducted in 2005 also showed that water quality only met the inferior Class VI (Chinese Sea Water Quality Standard GB 3097-1997). Pollution and eutrophication problems were also severe, with an average eutrophication index of 7.2. The worst eutrophication was recorded for Hangzhou Bay, with an index of 34.2 (Fig.4). DIN and DIP were the main two parameters that seriously exceeded the standard.

Because of the complex interactions between water flow, tidal mixing, wind and retention times, eutrophication had a minimal effect on the benthic community condition in the absence of low dissolved oxygen events. However, the benthic community condition was negatively correlated to sediment contaminants levels (Dauer et al., 2000). In the present study, the AMBI and M-AMBI plots could reflect the spatial distribution pattern of sediment contaminants (Fig.3) and eutrophication in the coast adjacent to the Changjiang River estuary (Fig.4) but did not match their plots exactly. Integrated with the analysis of other environmental parameters, we believed that these two indices could be used to assess the ecological status of stations in this coast. The interpretations of ecological status indicated by AMBI and M-AMBI in the coast were significantly different. This degree of difference could be divided into three conditions based on the detailed interpretations of ES by AMBI and M-AMBI: same or almost the same (41.7%), different (38.3%) and opposite interpretations (20%) (Tables 2, 3, and 4). To fully understand the human-induced pressures on the study area, we calculated the two indices for all of the sampling stations, including stations with

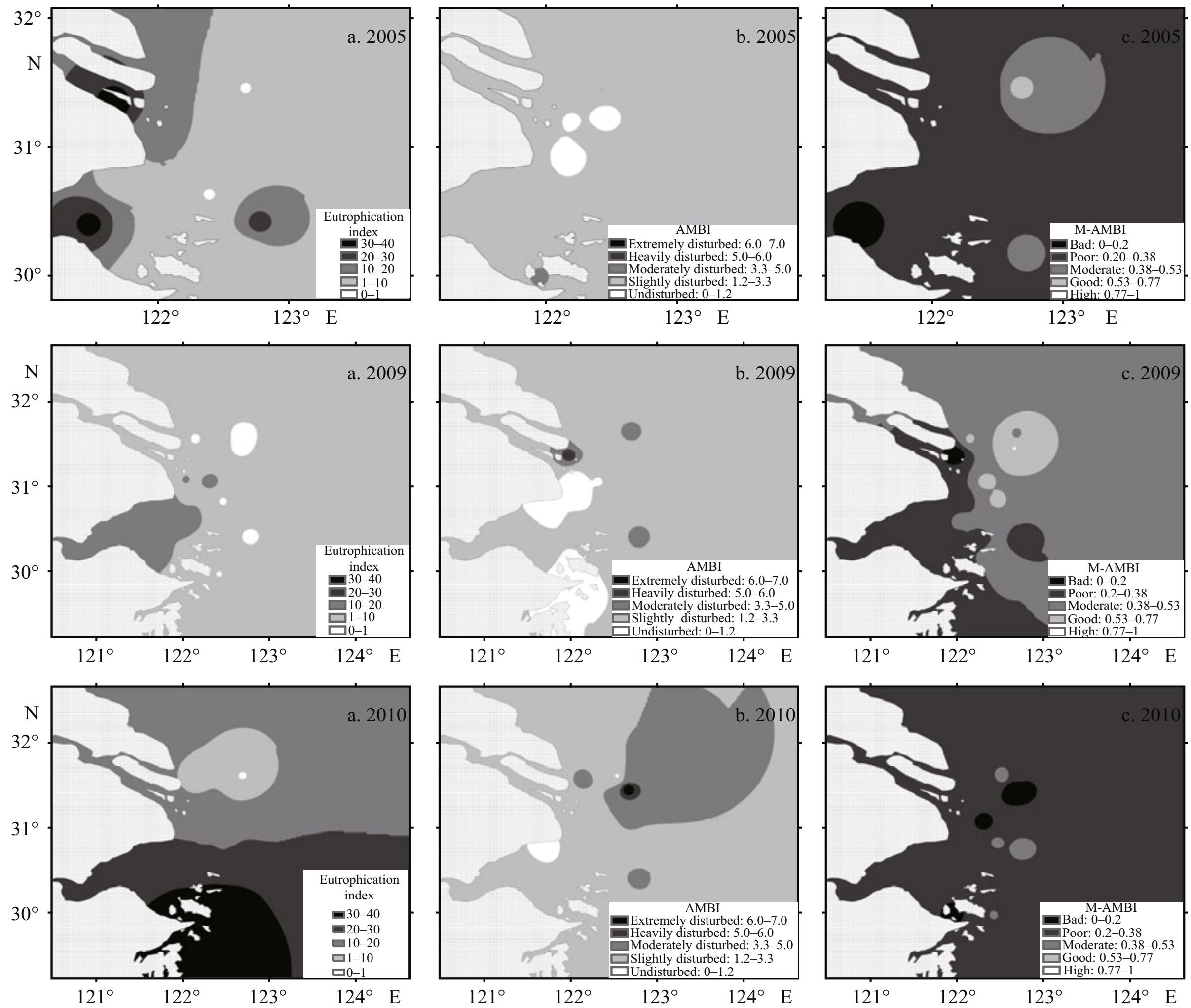

Fig.4 Eutrophication index (a), AMBI (b) and M-AMBI (c) plot of the coast adjacent to the Changjiang River estuary in 2005, 2009, and 2010

fewer than three species. For those stations, the AMBI could not be very informative because of the unreliably high or low value calculated by chance depending on which ecological group to which the observed species were assigned. However, this could be revised using the M-AMBI, in which H and S would be low, and the global value therefore most likely also low, indicating lower quality. Most of the stations with different and opposite interpretations by AMBI and M-AMBI belong to this condition. Those stations might be assigned a high AMBI value (based on species assigned to ecological group I) or a low one (based on species assigned to ecological group III or another group), but because of the low H and S values, the M-AMBI values were still low. However, in stations with more than three species and less than 20% abundance of unassigned species, the interpretations of ecological status indicated by AMBI are comparable with those indicated by M-AMBI, with the same or similar interpretations 65.4% of the time and different and opposing interpretations for only 34.6%. The analysis of the two indices integrated with abiotic parameters showed that the M-AMBI was more suitable for benthic ecological status assessment in the coast adjacent to the Changjiang River estuary.

5 CONCLUSION

The benthic ecological health of the coast adjacent to the Changjiang River estuary was assessed using AMBI and M-AMBI indices in this work. The following conclusions were attained:

(1) The AMBI indicated that the ecological status of the coast adjacent to the Changjiang River estuary in 2005, 2009 and 2010 was only slightly degraded, whereas the M-AMBI indicated that the ecological

status was seriously degraded by pollution and eutrophication.

(2) It was demonstrated that M-AMBI is more suitable than AMBI to assess the ecological status of coastal water in China because the latter integrates Shannon's diversity index and richness. Several species of polychaete with high abundance in certain stations could not be assigned to proper ecological groups in 2005, producing results inadequate for assessment interpretation.

(3) The reference conditions for the biotope of sandy sediment in the coast adjacent to the Changjiang River estuary were AMBI=0, H=3.5 and S=28. In this work we increased the best observed value by 15%. The species assignations in this paper can serve as a reference for Chinese species in similar works.

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Supplementary material A: Chemical and physical parameters analyzed in the sediment of stations sampled in 2005 and 2009 (unit: mg/kg, OC and OM in %)

Stations	Years	Oil	As	Cu	Zn	Cd	Pb	Hg	OC	TS	OM	TN	TP
1	2005	3	7.82	12.5	48.3	0.093	12	0.025	0.46	-	0.856	298	14.2
2	2005	3	6.9	15	60.2	0.127	34.8	0.028	0.274	-	0.51	103	14
3	2005	<2	7.33	13.7	55.9	0.071	14.5	0.033	0.436	-	0.812	110	4.82
4	2005	3	6.34	14	54.3	0.062	21.3	0.025	0.351	-	0.654	235	16.1
5	2005	<2	12.1	5.3	45.7	0.051	33.4	0.011	0.091	-	0.169	26.4	16.7
6	2005	<2	13.1	5.1	40.1	<0.040	23.2	0.02	0.018	<0.3	0.034	36.5	27.8
	2009	11	12.6	25.4	92.4	0.215	26.3	0.032	0.592	7.1	-	473	20.97
7	2005	3	7.57	18.4	73	0.124	20.6	0.023	0.297	-	0.553	110	16.3
	2009	2	6.66	10.2	63.9	0.122	18.7	0.021	0.285	11	-	204	13.45
8	2005	2	10.4	25.8	83.6	0.118	18.2	0.055	0.315	46	0.587	131	9.93
12	2005	<2	8.11	17.8	60.4	0.227	18	0.038	0.095	<0.3	0.177	36.6	18.2
	2009	1	8.56	11.5	72.6	0.195	18.3	0.049	0.114	<0.3	-	43.5	18.5
13	2005	11	8.02	19.1	62.7	0.199	17.6	0.097	0.453	0.8	0.843	94	6.79
15	2005	3	11.5	27.7	75.3	0.164	17.8	0.089	0.503	17.9	0.937	92.2	4.07
	2009	5	12.7	37.7	115	0.264	29.2	0.018	0.653	1.2	-	195	18.42
17	2005	6	9.95	27.4	74.9	0.158	22.7	0.08	0.363	12	0.676	118	9.8
	2009	5	12.4	41.2	124	0.338	30	0.031	0.566	128	-	240	19.16
18	2005	3	12.9	32.8	89.8	0.164	25.5	0.055	0.384	12	0.715	104	5.54
	2009	<1	14.2	25.4	79.9	0.138	18.5	0.02	0.385	19.4	-	156	18.19
19	2005	5	9.43	21.6	63.9	0.126	21.4	0.086	0.525	1.1	0.978	190	14
	2009	4	10.6	28.4	88.9	0.16	22.4	0.022	0.492	<0.3	-	244	18.51
20	2005	7	11.9	32.2	90.4	0.169	28.2	0.085	0.624	0.5	1.16	128	13.2
	2009	3	15.6	50.2	122	0.356	31.8	0.032	0.483	<0.3	-	308	19.84
22	2005	4	10.4	22.6	65.3	0.105	24.1	0.109	0.415	<0.3	0.773	180	13.5
	2009	5	12.2	31.5	105	0.174	23.9	0.023	0.286	<0.3	-	232	17.87
24	2005	<2	11.8	23	71.3	0.11	26.1	0.184	0.497	0.8	0.925	156	13.9
	2009	7	12.4	26.8	87.8	0.15	21.3	0.02	0.467	<0.3	-	194	17.74
28	2005	4	12.8	29.5	85.6	0.117	15.9	0.058	0.72	6	1.34	218	13.6
	2009	6	15	38.9	114	0.124	22.5	0.022	0.743	<0.3	-	332	18.13
29	2005	6	10.5	31	89.9	0.158	20.4	0.084	0.578	6.9	1.08	181	14.5
	2009	6	12.4	34.5	107	0.218	27.4	0.05	0.582	0.5	-	159	18.32
30	2005	<2	8.42	17.5	61.6	0.093	12.3	0.042	0.469	4.1	0.873	146	12.4
	2009	3	10.3	27	96.3	0.103	18	0.024	0.443	<0.3	-	169	18.13

Key: Organic carbon (OC), total sulfur (TS), organic matter (OM), total nitrogen (TN), total phosphorus (TP). <number, meaning the value is below the minimum detectability.

To be continued

Supplementary material A: Continued

Stations	Years	Oil	As	Cu	Zn	Cd	Pb	Hg	OC	TS	OM	TN	TP
32	2005	6	7.78	19.5	62.5	0.108	12.2	0.045	-	0.3	-	230	16.9
	2009	<1	11.4	22.6	94.8	0.157	20.9	0.037	0.35	<0.3	-	134	16.68
33	2005	2	13.2	29.8	84.5	0.104	18.8	0.045	-	2.3	-	150	13.1
	2009	6	13.1	33.7	113	0.202	25.6	0.031	0.815	22.9	-	306	17.68
34	2005	6	10	25	79.2	0.124	18	0.054	0.488	<0.3	0.909	172	14.5
	2009	5	8.5	29.5	87	0.178	24.4	0.019	0.465	0.8	-	134	17.35
35	2005	2	9.95	26.3	76	0.13	16.1	0.056	0.587	4.8	1.09	172	12.4
	2009	4	12.2	35.3	145	0.218	30.1	0.031	0.512	<0.3	-	237	18.03
36	2005	6	-	22.7	74.3	0.085	13.3	0.044	0.372	<0.3	0.693	-	-
	2009	2	15.4	11.3	51.3	0.133	24.9	0.022	0.087	<0.3	-	21.8	9.35
37	2005	6	10.6	26	70	0.109	16.3	0.063	0.513	<0.3	0.955	282	5.1
	2009	6	11.7	31.3	116	0.2	26.9	0.03	0.475	<0.3	-	219	17.48
38	2005	<2	-	22	71	0.106	24.6	0.058	0.346	13.8	0.644	-	-
	2009	10	12.8	34.5	106	0.199	27.9	0.044	0.477	<0.3	-	398	18.22
39	2005	4	9.93	28.7	83.2	0.118	14	0.063	0.257	36.7	0.479	179	14.9
	2009	11	12.6	36.2	120	0.224	24.4	0.027	0.56	1.7	-	298	17.94
41	2005	7	10.9	29.7	84.6	0.106	13.4	0.058	0.553	<0.3	1.03	187	15.7
	2009	3	14.6	46.3	123	0.193	34	0.019	0.68	12.4	-	271	18.77
42	2005	2	12	29.5	76.9	0.118	11.6	0.051	0.38	5.6	0.708	164	13.7
43	2005	6	9.82	24.4	77.6	0.11	25.6	0.064	0.487	5.7	0.908	138	8.82
JM1	2005	4	12.9	32.8	92.1	0.216	26.9	0.042	0.55	-	1.02	129	10.4
JM2	2005	5	9.02	22.2	69.8	0.132	25.9	0.036	0.536	-	0.998	156	13.3
JM3	2005	4	8.14	16.9	62.6	0.11	23.5	0.05	0.508	-	0.946	167	21.2
JM4	2005	7	12.6	34.2	96.2	0.173	25.3	0.033	0.578	-	1.08	134	11.4
SH3114	2009	2	5.79	10.7	70	0.106	18.4	0.029	0.309	32.2	-	204	14.39

Supplementary material B: Species list and their EG assignation in the adjacent coast to the Changjiang River estuary (Species assigned by Ángel Borja, AZTI)

Group	Family	Species	Ecological group (EG)	Group	Family	Species	Ecological group (EG)
Annelida	Cossuridae	*Cossurella dimorpha*	IV	Polychaeta	Capitellidae	*Mediomastus* sp.	III
Cnidaria	Campanulariidae	*Obelia geniculata*	II	Polychaeta	Capitellidae	*Notomastus latericeus*	III
Cnidaria	Virgulariidae	*Virgularia* sp.	I	Polychaeta	Cirratulidae	*Chaetozone setosa*	IV
Nemertea	Cephalothricidae	*Cephalothrix* sp.	III	Polychaeta	Cirratulidae	*Cirratulus filiformis*	IV
Nemertinea			III	Polychaeta	Eunicidae	*Eunice* sp.	II
Sipuncula			I	Polychaeta	Glyceridae	*Glycera chirori*	II
Sipuncula	Sipunculidae	*Sipunculus nudus*	I	Polychaeta	Glyceridae	*Glycera unicornis*	II
Polychaeta	Acrocirridae	*Acrocirrus* sp.	IV	Polychaeta	Goniadidae	*Glycinde gurjanovoae*	II
Polychaeta	Arenicolidae	*Arenicola cristata*	Probably III	Polychaeta	Goniadidae	*Goniada maculata*	II
Polychaeta	Capitellidae	*Capitella capitata*	V	Polychaeta	Lumbrineridae	*Ninoe palmata*	II
Polychaeta	Capitellidae	*Dasybranchus caducus*	III	Polychaeta	Lumbrineridae	*Lumbrineris latreilli*	II
Polychaeta	Capitellidae	*Heteromastus* sp.	IV	Polychaeta	Lumbrineridae	*Lumbrineris* sp.	II

To be continued

Supplementary material B: Continued

Group	Family	Species	Ecological group (EG)	Group	Family	Species	Ecological group (EG)
Polychaeta	Lumbrineridae	*Loboneris pterignatha*	Not assigned	Polychaeta	Terebellidae		I
Polychaeta	Magelonidae	*Magelona cincta*	I	Polychaeta	Terebellidae	*Thelepus* sp.	II
Polychaeta	Magelonidae	*Magelona* sp.	I	Polychaeta	Trichobranchidae	*Terebellides stroemii*	II
Polychaeta	Chalinidae	*Asychis* sp.	II	Polychaeta		Polychaeta spieces A	Not assigned
Polychaeta	Maldanidae	*Euclymene annandalei*	I	Polychaeta		Polychaeta spieces B	Not assigned
Polychaeta	Maldanidae	*Euclymene* sp.	I	Polychaeta		Polychaeta spieces C	Not assigned
Polychaeta	Maldanidae	*Praxillella* sp.	III	Polychaeta		Polychaeta spieces D	Not assigned
Polychaeta	Nephtyidae	*Aglaophamus dibranchis*	I	Polychaeta		Polychaeta spieces E	Not assigned
Polychaeta	Nephtyidae	*Aglaophamus sinensis*	II	Polychaeta		Polychaeta spieces F	Not assigned
Polychaeta	Nephtyidae	*Nephtys oligobranchia*	II	Mollusca	Aplysiidae	*Aplysia* sp.	I
Polychaeta	Nereididae	*Neanthes vaalii*	III	Mollusca	Pholadidae	*Barnea* sp.	Probably I
Polychaeta	Nereididae	*Nectoneanthes* sp.	III	Mollusca	Cancellariidae	*Sydaphera spengleriana*	Not assigned
Polychaeta	Nereididae		III	Mollusca	Nassariidae	*Nassarius variciferus*	II
Polychaeta	Nereididae	*Perinereis* sp.	III	Mollusca	Naticidae	*Neverita didyma*	I
Polychaeta	Onuphidae	*Diopatra amboinensis*	II	Mollusca	Nuculidae	*Nucula faba*	I
Polychaeta	Opheliidae	*Travisia pupa*	I	Mollusca	Pharidae	*Cultellus attenuatus*	I
Polychaeta	Opheliidae	*Travisia* sp.	I	Mollusca	Retusidae	*Retusa boenensis*	II
Polychaeta	Orbiniidae	*Haploscoloplos elongatus*	IV	Mollusca	Tellinidae	*Moerella iridescens*	I
Polychaeta	Orbiniidae	*Orbinia* sp.	I	Mollusca	Triclidae	*Eocylichna braunsi*	II
Polychaeta	Orbiniidae	*Scoloplos* sp.	I	Mollusca	Triclidae	*Eocylichna cylindrella*	II
Polychaeta	Oweniidae	*Owenia* sp.	I	Mollusca	Ungulinidae	*Cycladicama* sp.	Not assigned
Polychaeta	Paralacydoniidae	*Paralacydonia paradoxa*	II	Crustacea	Alpheidae	*Alpheus japonicus*	II
Polychaeta	Paraonidae	*Aricidea fragilis*	I	Crustacea	Gammaridae	*Gammarus* sp.	I
Polychaeta	Pectinariidae	*Pectinaria* sp.	I	Crustacea	Hexapodidae	*Hexapus granuliferus*	Not assigned
Polychaeta	Poecilochaetidae	*Poecilochaetus serpens*	I	Crustacea	Varunidae	*Asthenognathus inaequipes*	Not assigned
Polychaeta	Poecilochaetidae	*Poecilochaetus* sp.	I	Crustacea	Macrophthalmidae	*Tritodynamia intermedia*	Not assigned
Polychaeta	Polynoidae	*Lepidonotus* sp.	II	Crustacea	Porcellanidae	*Raphidopus ciliatus*	I
Polychaeta	Protodrilidae	*Protodrilus* sp.	I	Crustacea	Sergestidae	*Acetes chinensis*	I
Polychaeta	Sabellariidae	*Lygdamis giardi*	I	Crustacea	Squillidae	*Oratosquilla oratoria*	I
Polychaeta	Sabellariidae	*Sabellaria* sp.	I	Crustacea	Varunidae	*Eriocheir leptognathus*	II
Polychaeta	Serpulidae	*Serpula* sp.	I	Crustacea	Varunidae	*Hemigrapsus penicillatus*	II
Polychaeta	Sigalionidae	*Sigalion* sp.	II	Echinodermata	Ampharetidae	*Ampharete* sp.	I
Polychaeta	Spionidae	*Paraprionospio coora*	IV	Echinodermata	Amphiuridae	*Amphioplus depressus*	II
Polychaeta	Spionidae	*Prionospio pacifica*	IV	Echinodermata	Amphiuridae	*Amphioplus* sp.1	II
Polychaeta	Spionidae	*Prionospio pinnata*	IV	Echinodermata	Amphiuridae	*Amphioplus* sp.2	II
Polychaeta	Spionidae	*Prionospio queenslandica*	IV	Echinodermata	Amphiuridae	*Amphiura vadicola*	II
Polychaeta	Spionidae	*Spio* sp.	III	Echinodermata	Synaptidae	*Labidoplax dubia*	I
Polychaeta	Sternaspidae	*Sternaspis scutata*	III	Echinodermata	Synaptidae	*Protankyra asymmetrica*	II
Polychaeta	Terebellidae	*Amaeana trilobata*	I	Echinodermata	Synaptidae	*Protankyra bidentata*	II
Polychaeta	Terebellidae	*Loimia medusa*	III				