

Distribution of Benthic Macroinvertebrates in Relation to Environmental Variables across the Yangtze River Estuary, China

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ABSTRACT

CHAO, M.; SHI, Y.; QUAN, W.; SHEN, X.; AN, C.; YUAN, Q., and HUANG, H., 2012. Distribution of benthic macroinvertebrates in relation to environmental variables across the Yangtze River Estuary, China *Journal of Coastal Research*, 00(0), 000–000. West Palm Beach (Florida), ISSN 0749-0208.

This article reports on a study of the spatial and seasonal distribution of benthic macroinvertebrates and reveals the correlation between benthic macroinvertebrate community and environmental variables in the Yangtze River Estuary, China. A total of 75 species from seven phyla were identified during sample collection in May, August, and November of 2010 and February of 2011; among which, Annelida and Mollusca were found to be the predominant phyla. No significant difference in the community parameters (species, abundance, biomass, Shannon-Winner index, distinctness) of benthic macroinvertebrates was observed among survey trips. In contrast, an increasing tendency of abundance and biomass from river channels to the east waters was observed, and the differences between different geographic zones or community clusters were confirmed with a Kruskal-Wallis test. Compared with the data of the 1980s in the Yangtze River Estuary, the species richness, biomass, and abundance has decreased greatly. Distinct groups were classified based on clusters and nonmetric multidimensional scaling (nMDS) analysis. *Corbicula fluminea*, *Potamocorbula amurensis*, *Nassarius variciferus*, and *Notomastus latericeus* were the dominant species in abundance in clusters I, II, IIIa, and IIIb, respectively. The most abundant species during all four survey trips were *Glycera chirori*, *Notomastus latericeus*, *Heterospio sinica*, and *Cossura longocirrata*, all of which belong to the polychaetes. Our study demonstrated that the distribution of benthic macroinvertebrates in the Yangtze River Estuary was closely related to environmental variables. Salinity, bottom water temperature, sand content (C_S), silt content (C_T), and pH were explanatory variables for the spatial and seasonal distribution across the Yangtze River Estuary. Salinity was the factor that most determined the spatial distribution of the species, temperature determined the species occurrence on the entire estuarine scale, and the high content of silt favored the abundance of polychaetes.

ADDITIONAL INDEX WORDS: *Multivariate analysis, species distribution, community composition, environmental variables.*

INTRODUCTION

The Yangtze River (Changjiang) Estuary is the fifth largest river system in the world in water discharge (Yang *et al.*, 2011). Its mean annual water discharge into the East China Sea has occurred at a stable rate of 9.0×10^{11} m³/y (Chen, Yu, and Gupta, 2001) during the past 60 years. However, by 2000, the mean annual suspended sediment loading from the Yangtze River was 34% lower than the 440 mt/y recorded in the 1950s (Yang, Zhao, and Belkin, 2002). Yang *et al.* (2006) found that the Changjiang had entered a third phase of sediment reduction, with annual sediment loads at Datong (upstream

of the estuary) being less than 200 mt/y. A decrease in sediment loading has the potential to cause severe channel erosion in estuarine areas unless current watershed management policies are adjusted (Yang *et al.*, 2006). Therefore, a scientific assessment of the integrated impacts of large hydraulic facilities on the ecosocial systems along estuarine zones was needed.

Benthic macroinvertebrates play a crucial role in estuarine ecosystems. They are important food sources for higher trophic consumers (Seitz *et al.*, 2009; Zhu *et al.*, 2007), provide nutrient cycling through their burrowing and feeding activities (Arnott and Vanni, 1996), and are a potential indicator of environmental deterioration (Wildsmith *et al.*, 2011; Zorita *et al.*, 2008) and the state of ecosystem health (Dauvin *et al.*, 2007; Hale and Heltsh, 2008). Therefore, the composition and spatiotemporal distribution of macroinvertebrates, as well as their relation to

DOI: 10.2112/JCOASTRES-D-11-00194.1 received 27 October 2011; accepted in revision 15 January 2012.

Published Pre-print online 15 May 2012.

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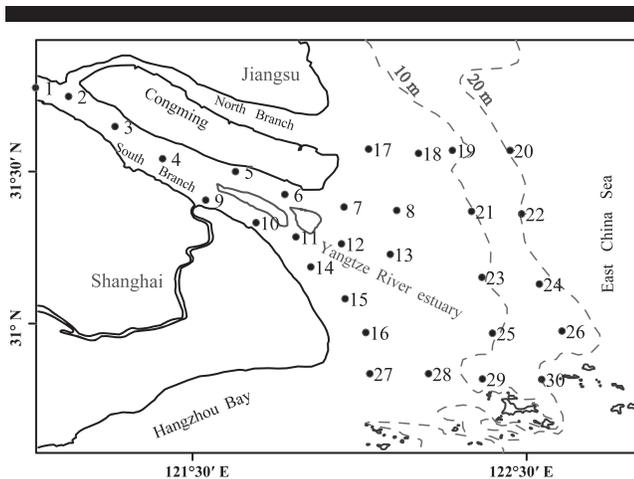


Figure 1. Map of the Yangtze River Estuary with the locations of the benthic macroinvertebrate sampling stations.

environmental variables or predators, have largely been studied (Fujii, 2007; Hampel, Elliott, and Cattrijsse, 2009; Wildsmith *et al.*, 2009). Numerous previous studies on the macroinvertebrate ecology of estuaries analyzed community characteristics and their correlation with environmental variables through multivariate analysis methods, such as cluster analysis, nonmetric multidimensional scaling (nMDS), and canonical correspondence analysis (CCA) (Gaudêncio and Cabral, 2007; Gogina, Glockzin, and Zettler, 2010; Mutlu, Cinar, and Ergev, 2010; Tomiyama *et al.*, 2008).

In the 1980s, the ecology of benthic macroinvertebrates in the Yangtze River Estuary was studied as a joint venture between China and the United States. The following subjects were studied: the sediment dynamics of the East China Sea in 1980–81 (Boesch *et al.*, 1986), a survey of the effects of pollution on macrobenthic organisms along the southern coast of the Yangtze River Estuary in 1982–83 (Dai, 1991), and environmental assessment surveys for the Three Gorges Dam (TGD), which predicted effects on the estuary in 1985–86 and 1988 (Liu, 1986, 1992; Sun and Dong, 1985, 1986; Sun *et al.*, 1992). These studies were valuable resources for measuring the species richness, abundance, and biomass in the Yangtze River Estuary. However, since the 1980s, the community characteristics of macroinvertebrates in the subtidal waters of the Yangtze River Estuary have not been well studied. Most studies on estuarine macroinvertebrates have been small scale

(Luo *et al.*, 2011) or lacked data on long-term scales (Li *et al.*, 2007; Meng *et al.*, 2007; Wang *et al.*, 2009; Zhang *et al.*, 2007). A few studies reported multiple survey data (Liu *et al.*, 2008), but those data were insufficient and did not reveal correlations between the species and their environment.

In this article, we report on surveys conducted across the Yangtze River Estuary to sample for benthic organisms. We sought to investigate the spatial and seasonal distribution of benthic macroinvertebrates in this estuary, to classify extant communities, and to detect how macroinvertebrate communities change because of variations in environmental variables *via* multivariate analysis.

MATERIALS AND METHODS

Study Site

The Yangtze River Estuary is a mesotidal estuary with a tidal range of about 2.7 m. The river below Xuliujing (121° E) is divided into the South Branch and the North Branch by the Congming Island. Since the 1950s, nearly all riverine discharge has flowed *via* the South Branch system (Chen *et al.*, 1985). A special high suspended particle materials (SPMs) content zone stood out in the river mouth-bar area because of the interaction between the freshwater and the tidal flows, where the turbidity maximum (TM) existed year-round. According to the research of Shen and Pan (1999), the range of the TM could extend to the 10-m isobath. The eastern waters of the estuary were controlled by salt water. The average runoff to the East China Sea was 9.0×10^{11} m³/y; of which, the greatest percentage occurred during the flood season from May to October.

Thirty sampling stations were set up across the estuary from 121° E to the 121°40' E to assess the aquatic ecosystem health of the Yangtze River Estuary (Figure 1). Four subzones were created according to variations in their characteristics of salinity, surface sediment characteristics, and depth to compare the differences of macroinvertebrate communities across the estuary (Table 1). Because the South Branch was the main discharge path of the Yangtze River for freshwater and loading of suspended sediment (Chen *et al.*, 1985), eight stations were set up in the South Branch and encoded as the Z1 subzone. Those stations were normally controlled by the freshwater, and the sediment was characterized by the sand and silty sand (Chen, Yan and Li, 2009; Liu *et al.*, 2007, 2010). Eight stations locating at the TM areas were encoded as Z2 subzone. They were controlled by brackish water, and the predominant sediment type found there was silt and sometimes

Table 1. Site characterization of survey areas.

Subzone Code	Station Included	Location Range	Mean Depth	Site Description
Z1	No. 1 to 6, No. 9 to 11	121° E–121°50' E of the South Branch	13.4 m	River channel; freshwater controlled; sediment type: sand and silty sand
Z2	No. 7, 8, No. 12 to 16 and No. 27	121°50' E–121°15' E	8.9 m	Maximum turbidity; brackish water controlled; sediment type: silt and sometimes clayey silt or sand
Z3	No. 17 to 19, No. 21, 23, 25, 28, and 29	along 10 m isobath from north to south of the estuary	11.7 m	Estuarine front; sediment type: silt and sandy silt
Z4	No. 20, 22, 24, 26 and 30	along 20 m isobath from north to south of the estuary	21.1 m	Eastern estuary; sea water controlled; sediment type: silt and clayey silt

clayey silt or sand. The sediment type of the TM varied occasionally because of the complicated water cycles and interactions between the Yangtze River and the tides of the East China Sea (Chen, Yan, and Li, 2009; Liu *et al.*, 2007, 2010). The Z3 subzone was located at the eastern boundary of the TM and contained an estuarine front with strong interactions between the river and the sea (Bi *et al.*, 2009). Five stations along 10-m isobath were included in the Z3 subzone, and stations 17, 18, and 28 were also included in this group because of the salinity and water-depth variations commonly found there were similar to the conditions found at the estuarine front. Five stations were located in the eastern estuary along 20-m isobath and were included in Z4 subzone, which was controlled by sea water, and the predominant sediment type was silt or clayey silt.

Sample Collection and Laboratory Analysis

Samples were collected during four survey trip in May, August, and November of 2010 and February of 2011. The survey time of each trip included a different season (spring, summer, autumn, winter) according to variations in the atmospheric temperature in the Yangtze River Estuary. Thirty sampling stations were surveyed during each trip. At each station, samples of benthic macroinvertebrates were collected with a 0.025-m² box corer with four successful grabs and then sieved through a 0.5-mm screen (Luo *et al.*, 2011). Samples were preserved in 75% alcohol. Once in the laboratory, organisms were sorted, identified to the lowest possible taxa, and counted. Concurrent to our sampling, the hydrographic characteristics of the near-bottom layer were measured by a conductivity–temperature–depth (CTD) profiler equipped with sensors for temperature, salinity, and pH.

The sediment was analyzed for trace metals (Cu, Zn, Pb, Cd, Hg, and As), grain size, and total organic carbon content (TOC). The detailed pretreatment and measurement procedure for the sediment samples used for trace metal analysis was described by Yang *et al.* (2009). In brief, concentrations of four heavy metal species (Cd, Pb, Cu, and Zn) were measured by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Scientific, Waltham, Massachusetts). Replicate analyses of blanks and reference materials showed good accuracy, and the reference material concentrations found were within 92%–117% of the certified values for all measured elements. Atomic fluorescence spectrometry (AFS) was used to analyze the concentration of As and Hg; As was measured at 193.7 nm and Hg at 253.65 nm. The detection limits were 0.2 µg/g for As, 1 µg/g for Cu, 2 µg/g for Pb and Zn, 0.02 µg/g for Cd, and 0.002 µg/g for Hg. The grain size of the sediment was measured on Particle Size Analyzer (LA950, Horiba, Kyoto, Japan) according to the method described in Liu *et al.* (2010). Sediment components were classified as clay (very fine clay, fine clay, medium clay, coarse clay), silt (very fine silt, fine silt, medium silt, coarse silt), and sand (very fine sand, fine sand, medium sand, coarse sand, very coarse sand, very fine gravel, fine gravel), using the method described by Shepard (1954). C_S , C_T , and C_Y were used to represent the sand, silt, and clay content in the sediment, respectively. Liu *et al.* (2010) distinguished five primary sediment types in the Yangtze River Estuary and the

adjacent region: sand, silty sand, sandy silt, silt, and clayey silt. After acidification (1N HCl) of the air-dried sediment, the TOC in the sediment was measured using a TOC analyzer (Multi N/C 2100, Analytik Jena AG, Jena, Germany) according to the thermal conductivity method (China National Standards GB 17378.5-2007, 2008).

Data Analysis

For each station, species richness (S), abundance, biomass, biodiversity indices, including the Shannon-Wiener index (H' , base = 2), and the taxonomic-distinctness index (von Euler and Svensson, 2001) were calculated.

Both cluster analysis and nMDS were used to classify the benthic macroinvertebrate communities. Before conducting the analysis, abundance data (individuals/m²) were fourth-root transformed. Species collected only from one or two stations were excluded from analysis (Tomiyama *et al.*, 2008). The cluster analysis was performed by the group-average method, based on a Bray-Curtis dissimilarity matrix. The taxa most responsible for similarities and dissimilarities between each cluster of stations were identified using the SIMPER procedure (Labruno *et al.*, 2007).

Kruskal-Wallis tests were used to evaluate differences of environmental variables and macroinvertebrate assemblage parameters among subzones, survey trips, and clusters.

A CCA was used to study the relationships between benthic macroinvertebrate abundance and environmental variables (Ter Braak, 1986). Fourteen environmental variables, including one category variable (sediment type), were chosen as representative variables, and they were the bottom water temperature, salinity, grain size, TOC, C_S , C_T , pH, trace metals (Cu, Zn, Pb, Cd, Hg, and As), and sediment type (sand, silty sand, sandy silt, silt, and clayey silt). The abundance data for each survey trip were fourth-root transformed, and species collected only from one station were excluded before analysis. Significant environmental variables were explored using a backward stepwise selection based on Akaike information criteria (AIC), with subsequent Monte Carlo permutation tests ($n = 1000$ permutations). The weighted averages of site scores were based on the weighted averages of species scores, and the latter was used in constrained ordination, given that weighted-average scores were more robust against random error in environmental variables than linear combination scores (Oksanen, 2008). The results of CCA were presented as ordination diagrams containing continuous explanatory variables plotted as arrows and points for sites (sampling stations) and species, respectively. Using these diagrams, the variation of species, sampling stations, and environmental variables were clearly detectable. cluster analysis, nMDS, and CCA were calculated using the software R 2.13.0 (www.r-project.org) with version 1.17.9 of the vegan package, and SIMPER was conducted with Primer 5.0 software (Clarke and Warwick, 2001).

RESULTS

Environmental Measurements

The mean values of the environmental variables measured during the four survey trips are listed in Table 2. Nonparametric

Table 2. The mean value of the environmental data in the Yangtze River Estuary.

Factors	Subzone				Survey Trip				Cluster			
	Z1	Z2	Z3	Z4	May 2010	August 2010	November 2010	February 2011	I	II	IIIa	IIIb
	Temperature (°C)	20.0	19.5	16.4	16.3	21.8	26.5	17.6	7.0	20.0	19.7	16.4
Salinity	0.47	4.90	26.80	31.23	11.19	13.66	13.50	16.84	0.61	3.90	27.89	27.51
Grain size (µm)	76.5	28.9	31.2	18.0	45.2	46.6	35.3	38.1	71.9	22.6	32.8	17.7
TOC (%)	1.72	1.99	1.99	2.11	1.93	1.93	1.93	1.93	1.65	2.07	1.98	2.21
Cu (mg/kg ¹)	23.28	31.54	27.91	26.66	33.91	23.86	26.80	24.67	23.44	35.08	27.88	27.15
Zn (mg/kg ¹)	66.35	79.94	74.61	75.25	47.75	78.07	86.58	82.90	66.95	86.05	73.59	75.44
Pb (mg/kg ¹)	25.14	25.15	24.17	23.22	21.68	23.39	29.54	23.79	24.95	25.57	24.28	23.51
Cd (mg/kg ¹)	0.195	0.172	0.147	0.111	0.139	0.154	0.161	0.192	0.188	0.181	0.143	0.125
Hg (mg/kg ¹)	0.057	0.049	0.048	0.040	0.038	0.049	0.058	0.053	0.054	0.052	0.046	0.043
As (mg/kg ¹)	7.76	8.73	9.52	8.88	7.34	8.99	9.26	9.15	7.78	9.16	9.54	8.86
C _S (%)	46.1	17.8	18.0	9.8	26.5	26.3	21.3	24.2	43.6	13.5	18.7	10.0
C _T (%)	46.6	67.2	65.7	73.7	60.0	58.5	62.7	66.7	48.4	70.8	65.6	72.4
pH (%)	8.03	7.96	8.02	8.03	8.28	8.14	7.84	7.78	8.03	7.93	8.03	8.02

test results from the Kruskal-Wallis method showed that the environmental factors salinity, grain size, C_S , and C_T differed significantly between the subzones ($df = 3, p < 0.05$). Bottom water temperature, TOC, and trace metals (Cu, Zn, Pb, Cd, Hg, As) did not show significant differences between the subzones ($df = 3, p > 0.05$). Seasonal differences were measured for bottom water temperature and salinity ($df = 3, p < 0.05$). The mean value of temperature was higher in both May (21.8°C) and in August (26.5°C) than that measured in November (17.6°C) and February (7.0°C). The mean value of salinity varied and increased significantly from 0.47 in Z1 to 31.23 in Z4. Salinity was highest during the February trip (16.84). The mean value of the grain size in Z1 (76.5 µm) was larger than that found in the other three subzones, and the predominant sediment type found there was sand, which was different from what was found in the other three subzones.

The TOC value varied from 1.03% to 2.85%. Most trace metal values were far below the Effects Range-Low (ERL) and Effects Range-Median (ERM) guideline values (Long et al., 1995), with the exception of Cu. There were 28 stations out of total 120

stations during four survey trips in which the concentration of Cu was higher than the ERL guideline (34 mg/kg) value.

Distribution of Benthic Macroinvertebrates

Seventy-five species from seven phyla were identified during four survey trips (Table A1), including Cnidaria (3 species), Nemertinea (1 species), Echiura (1 species), Annelida (31 species, all belonging to polychaeta), Mollusca (24 species, with 10 species belonging to Bivalvia and the others belonging to Gastropoda), Arthropoda (11 species), and Echinodermata (4 species). Annelida and Mollusca were the predominant phyla in the Yangtze River Estuary.

Species richness during each trip from May 2010 to February 2011 was 35, 40, 39, and 36, respectively, and species numbers occurring in Z1, Z2, Z3, and Z4 were 10, 14, 37, and 48, respectively, during four trips. No significant difference was observed among survey trips based on Kruskal-Wallis test ($p > 0.05$) (Table 3). However, significant difference was observed for average taxonomic distinctness (Δ^+) between November and

Table 3. Kruskal-Wallis test comparing species richness (S), abundance, Shannon-Winner index (H'), biomass and distinctness (taxonomic distinctness, Δ ; average taxonomic distinctness, Δ^+) among subzones, survey trips and clusters. Degrees of freedom, chi-squares (χ^2), and their significance levels (p) are listed. Significant results are highlighted in bold.

Group Variable	df	S		Abundance		H'		Biomass		Δ		Δ^+	
		χ^2	p	χ^2	p								
Subzone													
Z1-Z2	1	1.00	0.3	1.80	0.2	0.38	0.5	0.26	0.6	1.3	0.3	0.02	0.9
Z2-Z3	1	25.7	0.0	22.5	0.0	20.4	0.0	14.7	0.0	7.5	0.0	0.48	0.8
Z3-Z4	1	17.5	0.0	12.7	0.0	11.0	0.0	0.85	0.4	3.1	0.1	7.7	0.0
Total	3	65.9	0.0	62.9	0.0	62.1	0.0	29.5	0.0	10.4	0.0	3.9	0.3
Cluster													
I-II	1	0.01	0.9	0.10	0.7	0.06	0.8	1.13	0.3	1.0	0.3	3.5	0.1
II-IIIa	1	21.5	0.0	21.3	0.0	17.9	0.0	4.95	0.0	4.8	0.03	5.6	0.02
IIIa-IIIb	1	4.71	0.0	6.53	0.0	1.71	0.2	0.54	0.5	2.6	0.1	5.0	0.03
Total	3	61.5	0.0	66.5	0.0	55.0	0.0	25.9	0.0	9.0	0.03	10.0	0.02
Survey trip													
May-August	1	1.31	0.3	0.85	0.4	0.23	0.6	0.00	0.9	0.4	0.5	1.7	0.2
August-November	1	1.37	0.2	2.20	0.2	0.32	0.5	0.92	0.3	1.5	0.2	0.9	0.4
November-February	1	0.07	0.8	0.01	0.9	1.32	0.3	0.00	0.9	2.5	0.2	3.9	0.04
Total	3	5.9	0.1	3.1	0.4	5.2	0.2	1.3	0.7	2.8	0.4	7.3	0.1

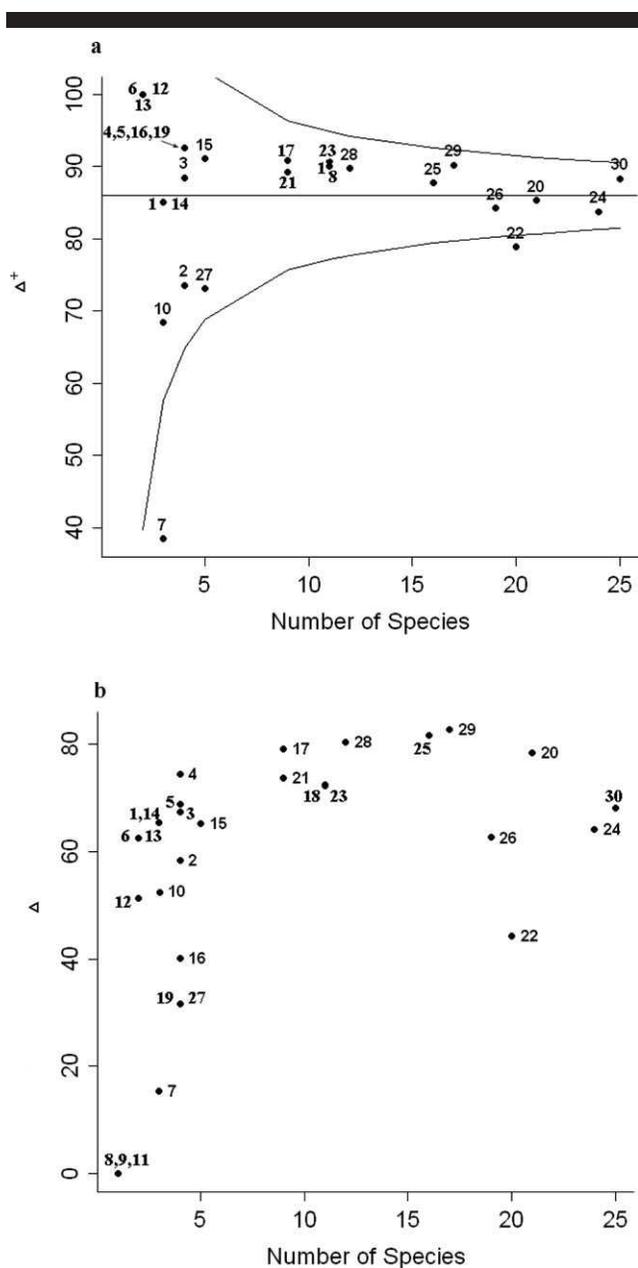


Figure 2. The biodiversity plot of taxonomic distinctness with (a) average taxonomic distinctness (Δ^+) and (b) taxonomic distinctness (Δ) based on benthic macroinvertebrate taxa abundance. Δ^+ was expressed as a funnel plot with 95% probability curves.

February trip. Significant difference was observed among subzones ($p < 0.05$). Results of comparison of bisubzones indicated that the significant difference was due to the differences between Z2 and Z3 and between Z3 and Z4 (table 3). Z4 had the highest species richness, which was also confirmed by the funnel plot of Δ^+ (Figure 2a). There were 25 stations that fell within the 95% confidence interval, and that indicated that the diversity at those stations was within the expected limits of Δ^+ . However, the scores from stations 8, 9, and 11 were not calculated because only 1 species occurred there during all four

survey trips. Two stations fell below the lower 95% confidence interval. Listed in decreasing order of their number of species, these were stations 22 and 7. However, no significant difference was observed for Δ^+ among the subzones. In contrast, significant differences for taxonomic distinctness and Δ (taxonomic distinctness) were observed. The species number and score of stations located in both Z3 and Z4 were higher than that of stations in Z1 and Z2 (Figure 2b).

The mean benthic macroinvertebrate abundance from the four survey trips in the subzones of Z1, Z2, Z3, and Z4 were measured as 11.94, 21.25, 65.94, and 218.5 individuals/m², respectively. Significant difference was observed among subzones ($p < 0.05$) (Table 3). Further comparison between subzones showed that significant difference existed between Z2 and Z3 and between Z3 and Z4 (Table 3). Z1 had the lowest abundance, and only half of the stations in Z1 successfully sampled macroinvertebrate specimens during the four survey trips. The abundance in Z2 was also very low. No specimens were obtained in 37.5% of the stations. The predominant species in each of the four subzones were *Corbicula fluminea*, *Nephtys* sp., *Nassarius variciferus*, and *Notomastus latericeus*, respectively. Mean abundance of each survey trip from May 2010 to February 2011 was 65.33, 69.0, 62.67, and 56.67 individuals/m², and the predominant species were *Glycera chirori*, *Notomastus latericeus*, *Heterospio sinica*, *Cossura longocirrata*, respectively, and all of these species were polychaetes. The most seasonally abundant species was *Heterospio sinica*, with 690 individuals/m² sampled in station 22 during the November trip. Some other relatively dominant species, such as *Heteromastus filiformis*, were only sampled during the February trip. No significant difference was observed between survey trips ($p > 0.05$) (Table 3).

The biomass also increased from Z1 to Z4. The mean biomass of the four subzones was 7.44, 17.28, 24.19, and 38.13 g/m², respectively. Significant difference was observed among subzones ($p < 0.05$) (Table 3). Detailed comparison between subzones indicated that the significant difference was caused by the difference between Z2 and Z3 ($p < 0.05$) (Table 3). The mean biomass of all stations of four survey trips from May 2010 to February 2011 was 16.97, 19.61, 12.90, and 29.13 g/m², respectively. Similar to abundance, no significant difference was observed between survey trips ($p > 0.05$) (Table 3). The highest biomass was 301.42 g/m² and was sampled from station 30 during the February 2011 survey. In May and August 2010, station 16 had the highest biomass, and in November, station 15 had the highest biomass. Both stations 15 and 16 were located in Z2.

The results of Kruskal-Wallis test of H' had the same statistical outputs as that of richness (Table 3). Biodiversity (H') was very poor in the stations found in Z1 and Z2. Most station scores there were marked as 0 based on finding no—or only one—sampled species.

Classification of the Benthic Macroinvertebrate Community

Based on an 80% dissimilarity level, the quantitative cluster analysis showed the existence of three main clusters (Figure 3a). Cluster I included all of the stations in Z1 and

Table 4. Contribution and cumulative contribution of the species most responsible for similarities within clusters and dissimilarities between clusters according to the SIMPER analysis.

Cluster	Species	Contribution (%)	Cumulative Contribution (%)
Within clusters			
Cluster I	<i>Corbicula fluminea</i>	70.33	70.33
	<i>Nephtys</i> sp.	18.72	89.05
	<i>Nephtys californiensis</i>	6.15	95.20
Cluster II	<i>Potamocorbula amurensis</i>	54.75	54.75
	<i>Eriocheir leptognathus</i>	45.25	100
Cluster IIIa	<i>Nassarius variciferus</i>	34.74	34.74
	<i>Amphiura vadicola</i>	13.07	47.81
	<i>Protankyra bidentata</i>	9.46	57.27
	<i>Glycera chirori</i>	6.58	63.85
	<i>Pista</i> sp.	6.11	69.96
Cluster IIIb	<i>Notomastus latericeus</i>	21.28	21.28
	<i>Sternaspis scutata</i>	16.12	37.28
	<i>Glycera chirori</i>	13.07	50.27
	<i>Eocylichna braunsi</i>	9.00	59.27
	<i>Virgularia</i> sp.	6.72	65.99
Between clusters			
I, II	<i>Corbicula fluminea</i>	21.68 (I)	21.68
	<i>Potamocorbula amurensis</i>	19.54 (II)	41.21
	<i>Eriocheir leptognathus</i>	18.08 (II)	59.29
II, IIIa	<i>Nassarius variciferus</i>	10.53 (IIIa)	10.53
	<i>Potamocorbula amurensis</i>	10.27 (II)	20.80
	<i>Eriocheir leptognathus</i>	9.26 (II)	30.07
	<i>Amphiura vadicola</i>	5.61 (IIIa)	35.68
	<i>Protankyra bidentata</i>	4.93 (IIIa)	40.60
II, IIIb	<i>Potamocorbula amurensis</i>	8.62 (II)	8.62
	<i>Notomastus latericeus</i>	7.96 (IIIb)	16.59
	<i>Eriocheir leptognathus</i>	7.26 (II)	23.85
	<i>Sternaspis scutata</i>	6.33 (IIIb)	30.18
	<i>Glycera chirori</i>	6.29 (IIIb)	36.47
IIIa, IIIb	<i>Magelona cincta</i>	5.27 (IIIb)	41.74
	<i>Nassarius variciferus</i>	7.04 (IIIa)	7.04
	<i>Notomastus latericeus</i>	5.42 (IIIb)	12.45
	<i>Magelona cincta</i>	4.15 (IIIb)	16.60
	<i>Eocylichna braunsi</i>	3.94 (IIIb)	20.54
	<i>Amphiura vadicola</i>	3.92 (IIIa)	24.47
	<i>Sternaspis scutata</i>	3.89 (IIIb)	28.36

stations 7 and 14 in Z2. Cluster II included the remaining stations in Z2. Cluster III included all of the stations in Z3 and Z4 and was separated into two subclusters (IIIa and IIIb). The results of the hierarchical clustering analysis were supplemented by nMDS ordination (Figure 3b). The classification results of the stations of cluster I and II approximated the geographic classification of subzone Z1 and Z2. All the stations of Z3 were classified in cluster IIIa, except stations 23 and 25. Those two stations and all the stations of Z4 were included in the cluster IIIb. Kruskal-Wallis test results for the indices of benthic macroinvertebrate community among clusters showed a similar pattern to that among subzones, with the exception of Δ^+ (Table 3). Detailed comparison of Δ^+ between cluster groups showed that the difference existed between clusters II and III and between IIIa and IIIb.

The similarity in clusters I, II, IIIa, and IIIb were 46.45%, 47.27%, 32.15%, and 41.17%, respectively. Further SIMPER

analysis identified the species responsible for the similarity within each cluster. Within cluster I, those species were identified as *Corbicula fluminea*, *Nephtys* sp., and *Nephtys californiensis*. *Potamocorbula amurensis* (Liu and He, 2007) and *Eriocheir leptognathus* contributed the most to the similarity within cluster II. *Nassarius variciferus*, *Amphiura vadicola*, *Protankyra bidentata*, *Glycera chirori*, and *Pista* sp. were the greatest contributors within cluster IIIa. *Notomastus latericeus*, *Sternaspis scutata*, *Glycera chirori*, *Eocylichna braunsi*, and *Virgularia* sp. most contributed to the similarity within cluster IIIb (Table 4). These species cumulatively contributed to more than 60% of the similarities in each cluster. SIMPER also identified species that contributed to dissimilarities between the clusters (Table 4). The dissimilarity ranged from 77.29 (between IIIa and IIIb) to 100 (between II and IIIa). The transition between clusters I and II generally corresponded to the decrease in the abundance of *Corbicula fluminea* and to the increase of *Potamocorbula amurensis* and *Eriocheir leptognathus*. The transition between clusters II and IIIa generally corresponded to the decrease of *Potamocorbula amurensis* and *Eriocheir leptognathus* and to the increase of *Nassarius variciferus*, *Amphiura vadicola*, and *Protankyra bidentata*. The transition between clusters II and IIIb generally corresponded to the decrease of *Potamocorbula amurensis* and *Eriocheir leptognathus* and to the increase of *Notomastus latericeus*, *Sternaspis scutata*, *Glycera chirori*, and *Magelona cincta*. High degrees of dissimilarity existed between clusters IIIa and IIIb. That dissimilarity came from the decrease of *Nassarius variciferus* and *Amphiura vadicola* and the increase of *Notomastus latericeus*, *Magelona cincta*, *Eocylichna braunsi*, and *Sternaspis scutata*.

Relationship between Benthic Macroinvertebrate Community and Environmental Variables

The relationship between the benthic macroinvertebrate community and environmental variables was analyzed by CCA. Five environmental variables, including salinity, bottom water temperature, C_T , C_S , and pH were detected as significant factors by stepwise selection based on AIC ($p < 0.05$) and with subsequent Monte Carlo permutation tests ($n = 1000$ permutations). The five variables explained 19.6% of the benthic macroinvertebrate community found in the Yangtze River Estuary. Axis 1 and axis 2 accounted for 38.8% and 22.0% of total inertia, respectively. The ordination diagrams for the site and species scores from CCA are shown in Figure 4. Salinity, temperature, C_S , and C_T were important explanatory variables for site and species scores in which salinity, C_S , and C_T correlated more with axis 1, and temperature correlated more with axis 2. The ordination results showed a clear distribution pattern of sampling stations and species in line with variations in the environmental variables. Stations in Z1 and Z2 (including clusters I and II) were located in the left side of the CCA biplot (Figure 4a), and that was determined by salinity, C_S , and C_T . All of the predominant species in Z1 and Z2, such as *Corbicula fluminea*, *Nephtys* sp., *Nephtys californiensis*, *Potamocorbula amurensis*, and *Eriocheir leptognathus*, occurred in the left side of the biplot (Figure 4b). Stations of Z3 and Z4 (cluster IIIa and IIIb) were in the right side of the biplot.

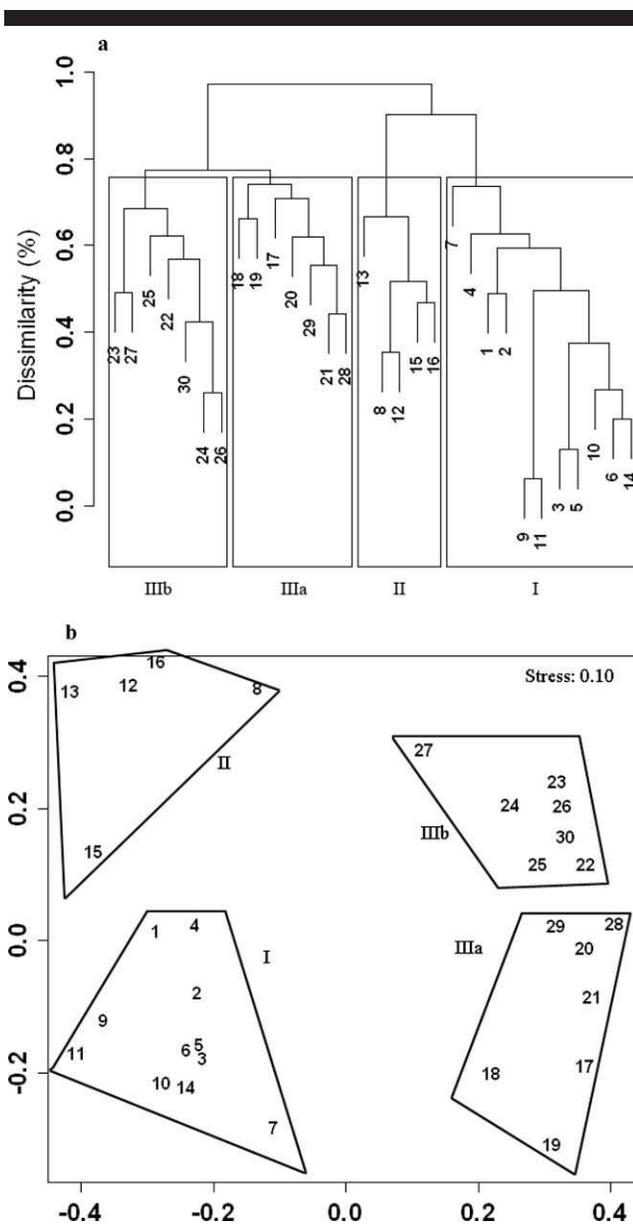


Figure 3. The classification of a benthic macroinvertebrate community in the Yangtze River Estuary by the (a) cluster and (b) nMDS methods. The dendrogram was produced by the group-average linkage method, Bray-Curtis dissimilarities, and the fourth-root transformed abundance data from the four survey trips. nMDS-ordination was produced by a Bray-Curtis dissimilarity index based on fourth-root transformed abundance data from the four trips.

The predominant species from the Z3 and Z4 subzones, such as *Nassarius variciferus*, *Amphiura vadicolica*, *Glycera chirori*, *Sternaspis scutata*, and *Eocylichna braunsi*, occurred in that same area. All these species occurred in each survey trip, with the exception of *Protankyra bidentata*, which was only found here in the February trip, and *Notomastus latericeus* and *Pista* sp., which were both absent during the February trip. For other incidental species, their distribution was also related to seasonal fluctuations in bottom water temperature. For

example, *Dosinia derupta* and *Heteromastus filiformis* were only found during the February trip, and although *Heterospio sinica* was the most abundant species, it was only found in the November trip at station 22. Comparison of both ordination diagrams highlighted the importance of salinity, C_S , C_T , and temperature.

DISCUSSION

Benthic Macroinvertebrate Habitats across the Yangtze River Estuary

The Yangtze River Estuary has developed diverse habitats for benthic macroinvertebrates because of the interaction between freshwater and saltwater. Because the estuary is situated in a subtropical monsoonal region of China, the temperature of the estuary habitats varies between seasons. Salinity patterns also vary between the wet and dry seasons because of variations in flow discharge. Significant seasonal differences in temperature and salinity were observed during our survey trips. The grain size in the main channel was coarser, with the presence of strong hydrodynamics, and was finer at the mouth of flats, with weak hydrodynamics, and in adjacent sea area off the estuarine mouth (Chen, Yan, and Li, 2009). Liu *et al.* (2010) also suggested that fine particle sediments were transported into the mouth bar area and the outer estuary, whereas the coarse sediments were deposited in the upper reaches of the estuary because of selective deposition. According to Yang *et al.* (2011), the bottom sediments 5 to 8 m below the lowest tide were at a high risk of sediment erosion, which suggested a high probability of a change in sediment composition in the next few decades. We observed peak concentrations of Cu in the TM areas. Trace metals such as Cu are more easily adsorbed by fine-grained, suspended sediments (Chen *et al.*, 2004) and are deposited with the suspended sediments because of the effects of salinity in the TM and seaward stations (Shen and Pan, 1999). In comparison with other estuaries around the world, trace metals of the Yangtze River Estuary were present in much lower concentrations (Chen *et al.*, 2004).

Comparison of the Benthic Macroinvertebrate Community between 2010–11 and the 1980s

Annelida and Mollusca were the predominant phyla in our survey results. These two phyla contributed 72.4% to the total macroinvertebrate phyla in the estuary, and this phyla composition pattern was similar to that found in the 1980s. The contribution of Annelida and Mollusca in 1982–83 (Dai, 1991) and 1985–86 survey (Liu *et al.*, 1992) was 86.1% and 80.7%, respectively. In contrast, species richness had decreased greatly. We sampled 35 and 39 species in May and November, which were significantly lower than that reported by Sun *et al.* (1992). Sun sampled 135 and 115 species in April and October, respectively, with a few more stations and wider sampling efforts in the 123°E waters, compared with our 122°40'E location.

Compared with the data reported in the 1980s, the increasing tendency of abundance and biomass didn't change from the river channels to the east waters. However,

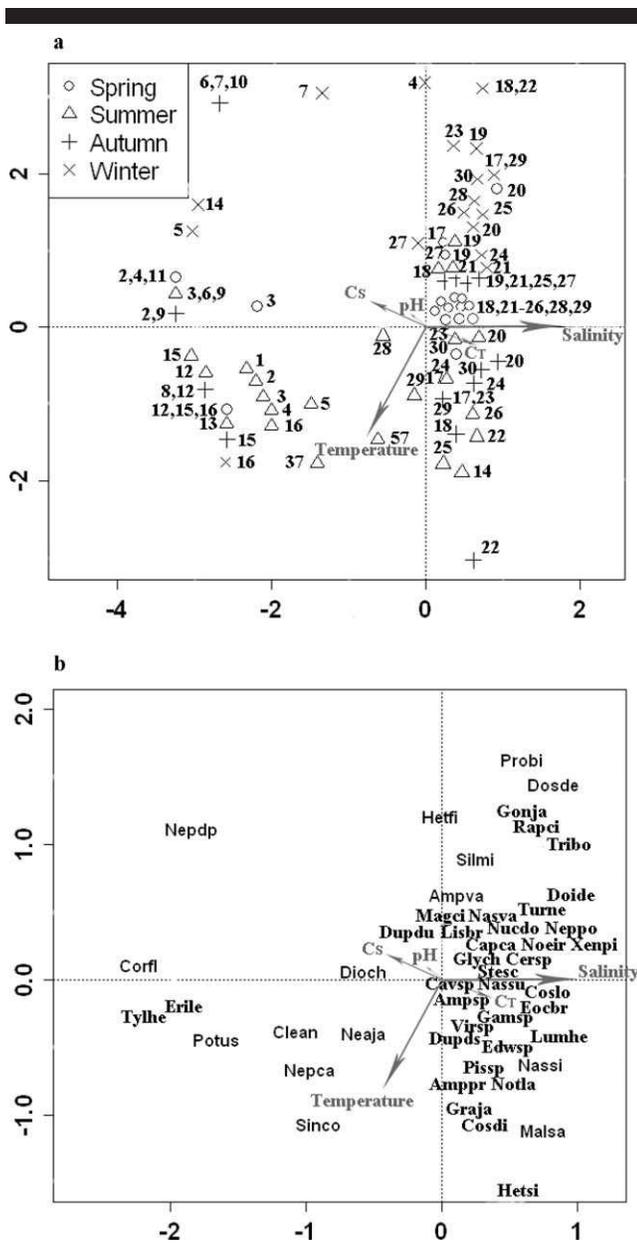


Figure 4. Canonical correspondence analysis (CCA) ordination diagrams for (a) sampling site scores and (b) species scores. Arrows indicate significant environmental variables. The seasons in which we visited each site are indicated by different symbols. The species names are abbreviated, and the full names are given in Appendix.

abundance was significantly reduced in comparison with that reported data of 126.6 individuals/m² in 1982–83 (Dai, 1991) and 258 individuals/m² in 1988 (Sun *et al.*, 1992). Compared with the seasonal data of Dai (1991), the abundance found in our survey in August decreased by 251.8 individuals/m², but the abundance of the other survey trip didn't show significant variation ($p > 0.05$). Biomass was also significantly reduced in comparison with those data of 44.33 g/m² reported in 1982–83 (Dai, 1991), and only the biomass in our February survey didn't

decrease. However, biomass was not significantly different compared with those data reported in 1985–86 (Liu *et al.*, 1992). Compared with the measurements of Liu *et al.* (1992), the biomass of stations close to the G section at 31°N changed significantly, even though the average biomass of the total section was in accordance with our data. At station 16, biomass increased from 0.02 g/m² to 15.82 g/m², whereas the biomass at station 26 decreased from 37.70 g/m² to 7.83 g/m². The biomass variation of these two stations was attributed to biomass-dominated species alteration. In August 1986, only two polychaete species were sampled at station 16. However, our survey found the Mollusca *Potamocorbula amurensis* to dominate the biomass of that area. Compared with those data recorded in the 1980s, the predominant species also varied to a small degree in the northeastern stations (cluster IIIa), such as, the small crab *Xenopthalmus pinnotheroides*, which had been replaced by the Mollusca *Nassarius variciferus*. In the southeastern stations (cluster IIIb), the polychaetes *Notomastus latericeus* and *Sternaspis scutata* remained the predominant species.

Spatial and Seasonal Variation of Benthic Macroinvertebrate Community in Relation to Environmental Parameters

Salinity, bottom water temperature, C_s , C_T , and pH were representative factors of the seasonal and spatial characteristics of the Yangtze River Estuary. Through CCA analysis, our results demonstrated that these factors were significant explanatory variables for species distribution in the Yangtze River Estuary. Salinity and sediment characteristics, such as C_s and C_T , significantly affected the community composition of macroinvertebrates in estuarine areas, which has also been revealed in several other studies (Hampel, Elliott, and Cattrijsse, 2009; Teskeand Wooldridge, 2003; Tomiyama *et al.*, 2008).

Comparison of the Kruskal-Wallis analysis results showed that all variables had significant differences among clusters. In contrast, the difference of variable Δ^+ was not significant among subzones. It can be concluded that classification of the communities by cluster groups was more reasonable than that by subzone groups. In our study, the spatial distribution of predominant species in different clusters was primarily determined by salinity. Freshwater resident species, such as the Mollusca *Corbicula fluminea*, were only sampled in low-salinity waters. The Mollusca *Potamocorbula amurensis* lived in brackish waters and was abundantly sampled from the TM and 10-m isobath waters. A few polychaetes, such as *Nephtys* sp., were limited by their ability to live in brackish and salt waters, but most polychaete and Mollusca species adapted to live in the high-salinity zones, based on the CCA results (Figure 4b). Polychaetes *Notomastus latericeus*, *Sternaspis scutata*, and *Glycera chirori* were abundant in cluster IIIb. In contrast, Mollusca *Nassarius variciferus* and Echinodermata *Amphiura vadicola* were found in abundance in cluster IIIa. Combined, the results from the C_T value of cluster IIIb (72.4%) were higher than they were in cluster IIIa (65.6%), and C_T played a role in the distribution of species in clusters IIIa and IIIb, which were confirmed to have an abundance and a biomass of polychaetes that were largely determined by the sediment characteristics under a condition of similar salinity

(Mutlu, Cinar, and Ergev, 2010; Sun and Dong 1986). According to data from the 1980s (Dai, 1991, Liu *et al.*, 1992; Sun *et al.*, 1992), polychaete communities were well developed in the benthic environments, with stable sedimentary rates and fine particles. Because of the higher productivity found in this area than that in river channels (Gao and Song, 2005), macroinvertebrate organisms could feed on more organic detritus than could those living in the zones with higher sedimentary rates (*e.g.*, the TM).

Because the species scattered along the temperature line in a biplot (Figure 4b) and none of species remained the most dominant species in all seasons, temperature was an important variable affecting the seasonal dynamics of species. A significant difference in Δ^+ was observed between the November and February trips, which indicated that temperature also determined species occurrence in different seasons. However, it seemed that the temperature was not a key variable affecting total abundance and biomass of the benthic macroinvertebrates, given that no significant differences in these variables were recorded between our four trips. The stations locating in the left side of biplot (Figure 4a) were from stations of both Z1 and Z2. The abundance, biomass, and species richness in those stations were low. Liu *et al.* (1992) thought those characteristics were determined by the unstable sedimentary environment, such as the high sedimentary rate and the fluid mud. Besides the above explanatory variables detected in our study, accumulation/erosion and biotic factors should be considered in future studies as potential important variables that affect the dynamics of the benthic community. Based on 50 years of hydrologic and bathymetric data, Yang *et al.* (2011) confirmed that erosion has accelerated in aquatic zones 5 to 8 m deep and noted that this trend had accelerated after the closure of the TGD in 2003. This latter event has made it even more necessary for estuarine managers and biologists to study the response of the benthic macroinvertebrate communities to the stresses of morphological change. Biotic factors, such as predator control, should also be considered in future studies on this subject. Furthermore, although no obvious evidence was found that trace metals affected the macroinvertebrate community in our data, the contaminants, included trace metal, polycyclic aromatic hydrocarbons, and pesticides, as effective variables on the composition of the benthos could not be completely excluded from the group of explanatory variables. Because it had reported that contamination by trace metal in the sediment affects benthic communities by harming sensitive species (Ryu *et al.*, 2011), further study on the response of benthic macroinvertebrates to the effects of contaminants should be conducted through more systematic work, including studying the effects on animal size, vertical distribution (Ryu *et al.*, 2011), the dynamics of sensitive species, and their bioaccumulation and ecotoxicity.

CONCLUSION

The spatial and seasonal variation of benthic macroinvertebrates in relation to environmental variables was investigated in this study. Annelida and Mollusca were the predominant phyla in the Yangtze River Estuary. No significant differences in the community parameters (species, abundance, biomass,

Shannon-Winner index, distinctness) were observed in the benthic macroinvertebrates among survey trips. In contrast, an increasing tendency of abundance and biomass from river channels to east waters was observed, and the differences between different geographic zones or community clusters were confirmed with a Kruskal-Wallis test. Compared with data from the 1980s on the Yangtze River Estuary, the species richness, biomass, and abundance had decreased greatly. Five significant variables, including salinity, bottom water temperature, sand contents (C_S), silt contents (C_T), and pH, were detected as explanatory variables for the spatial and seasonal distribution across the Yangtze River Estuary. Salinity was the factor that determined spatial distribution of the species, temperature determined species occurrence on a whole-estuarine scale, and the high content of silt was favorable to an abundance of polychaetes.

ACKNOWLEDGMENTS

This research was funded by the National Basic Research Program of China (973 Program, no. 2010CB429005) and the Special Research Fund for the National Nonprofit Institutes (East China Sea Fisheries Research Institute) (2007M02). We appreciated all of the crew members of the Hupuyu 47058 and Hucongyu 1381 for their help in sample collection and processing and Chenghong Feng, Yuanye Wang, Xianyin Ping, and Cuihua Wang for their aid in the analysis of our sediment samples.

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APPENDIX

Abundance (Individuals/m²) of Species and Species Code Considered in the Analysis.

Taxa and Species	Species Code	Abundance (individuals/m ²)			
		May 2010	August 2010	November 2010	February 2011
Cnidaria					
<i>Edwardsia</i> sp.	Edwsp	0.33	0.67	0.33	
<i>Cavemularia</i> sp.	Cavsp	0.67			
<i>Virgularia</i> sp.	Virsp	2.33	2.00	3.33	0.67
Nemertinea					
<i>Cerebratulina</i> sp.	Cersp	0.67	0.33	1.33	0.33
Annelida					
<i>Pectinaria</i> sp.	Pecsp			0.33	
<i>Pista</i> sp.	Pissp	1.00	1.67	2.67	
<i>Cirriformia</i> sp.	Cirsp			0.67	
<i>Pherusa cf. bengalensis</i>	Phebe				0.33
<i>Ampharete</i> sp.	Ampsp	0.33		1.67	0.33
<i>Magelona cincta</i>	Magci	2.33	2.67	0.67	4.00
<i>Heterospio sinica</i>	Hetsi		0.33	23.00	
<i>Harmothoe imbricata</i>	harim			0.33	
<i>Glycera chirori</i>	Glych	16.67	2.00	0.33	2.33
<i>Goniada japonica</i>	Gonja			0.33	3.33
<i>Nephtys californiensis</i>	Nepca		3.00		
<i>Nephtys polybranchia</i>	Neppo	0.67	1.00		2.00
<i>Nephtys</i> sp.	Nepdp	0.67		2.00	1.00
<i>Inermonephtys inermis</i>	Inein			0.33	
<i>Tylorrhynchus heterochaetus</i>	Tylhe		1.67		
<i>Neanthes oxyroda</i>	Necox	0.33			
<i>Neanthes japonica</i>	Neaja	0.33	0.33		
<i>Neanthes succinea</i>	Neasu	0.33			
<i>Lumbrineris heteropoda</i>	Lumhe		1.00	0.67	0.33
<i>Diopatra chileensis</i>	Dioch		0.33		0.33
<i>Capitella capitata</i>	Capca	1.00			
<i>Notomastus latericeus</i>	Notla	8.00	21.00	1.67	
<i>Heteromastus filiformis</i>	Hetfi				5.00
<i>Maldane sarsi</i>	Malsa		1.33	1.00	
<i>Orbinia dicrochaeta</i>	Orbdi			0.33	
<i>Orbinia</i> sp.	Orbsp	0.33			
<i>Aricidea</i> sp.	Arisp			0.33	
<i>Cossura longocirrata</i>	Coslo		3.33		8.67
<i>Cossurella dimorpha</i>	Cosdi		0.33	2.00	
<i>Sternaspis scutata</i>	Stesc	5.67	2.67	1.67	5.67
<i>Branchiomma serratibranchis</i>	Brase		0.33		
Echiura					
<i>Listeolobus brevis</i>	Lisbr	0.33		0.67	0.67
Mollusca					
<i>Nucula donaciformis</i>	Nuedo		0.67		0.33
<i>Scapharca suberenata</i>	Scasu			0.33	0.33
<i>Moerella iridescens</i>	Moeir	0.67	0.33	0.33	1.00
<i>Sinonovacula constricta</i>	Sinco		0.67		
<i>Siliqua minima</i>	Silmi	0.33	0.33		0.67
<i>Corbicula fluminea</i>	Corfl	2.67	2.33	2.00	1.33
<i>Dosinia derupta</i>	Dosde				0.67
<i>Dosinia japonica</i>	Dosja	0.33			
<i>Potamocorbula amurensis</i>	Potus	1.33	2.67	1.33	0.33
<i>Trigonothracia jinxiangae</i>	Triji	0.67			
<i>Neverita didyma</i>	Nevdi				0.33
<i>Rapana bezoar</i>	Rapbe	0.33			
<i>Mitrella bella</i>	Pyrbe				0.33
<i>Nassarius variciferus</i>	Nasva	6.00	2.67	3.67	3.67
<i>Nassarius succinctus</i>	Nassu	1.33	1.33	1.00	1.00
<i>Nassarius siquijorensis</i>	Nassi		0.67	0.33	
<i>Trigonaphera bocageana</i>	Tribo	0.33			0.33
<i>Lophiotoma leucoptropis</i>	Lople	0.67			
<i>Turricula javana</i>	Turja			0.33	

Continued.

Taxa and Species	Species Code	Abundance (individuals/m ²)			
		May 2010	August 2010	November 2010	February 2011
<i>Turricula nelliae spurious</i>	Turne			0.33	0.33
<i>Duplicaria duplicata</i>	Dupdu	1.33			
<i>Duplicaria dussumierii</i>	Dupds	0.67	0.33		
<i>Eocylichna braunsi</i>	Eocbr	2.67	1.33	0.67	1.00
<i>Philine kinglipini</i>	Phiki			0.33	
Arthropoda					
<i>Corophium acherusicum</i>	Corac		0.33		
<i>Corophium sinensis</i>	Corsi			1.00	
<i>Grandidierella japonica</i>	Graja		2.33		
<i>Gammarus</i> sp.	Gamp	1.00	0.33	0.33	
<i>Cleantoides annandalei</i>	Clean	0.33	1.67		0.33
<i>Macrobrachium nipponense</i>	Macni				0.33
<i>Leptochela gracilis</i>	Lepgr		0.33		
<i>Raphidopus ciliatus</i>	Rapci			0.33	1.00
<i>Diogenes deflectomanus</i>	Diode				0.67
<i>Eriocheir leptognathus</i>	Erile		2.00	2.00	
<i>Xenophthalmus pinnotheroides</i>	Xenpi		0.33	0.67	1.00
Echinodermata					
<i>Protankyra bidentata</i>	Probi				3.33
<i>Acaudina molpadioides</i>	Acamo		0.67		
<i>Amphioplus praetans</i>	Amppr			2.00	
<i>Amphiura vadicola</i>	Ampva	2.67	1.33		3.00