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Spatiotemporal variations of benthic macrofaunal community in the Xiamen Amphioxus Nature Reserve, eastern South China Sea

Yiyong Rao[1], Lizhe Cai[1, 2*], Wenjun Li[1], Xinwei Chen[1], Deyuan Yang[1]
[1] College of the Environment and Ecology, Xiamen University, Xiamen 361102, China
[2] Key Laboratory of the Coastal and Wetland Ecosystems (Xiamen University), Ministry of Education, Xiamen 361102, China

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Abstract
In order to realize the spatiotemporal variations of benthic macrofaunal communities at the "Amphioxus Sand" habitat, six surveys including four seasons and three consecutive summers (i.e., 2014, 2015 and 2016) were conducted in two core sites, i.e., Huangcuo (HC) and Nanxian-Shibaxian (NX), in the Xiamen Amphioxus Nature Reserve in China. A total of 155 species of macrofauna were recorded, therein, polychaetes were dominant in terms of species number and density. Significant spatiotemporal variations of macrofaunal communities were observed. The density of polychaetes and the biomass of molluscs in the HC site were higher than those in the NX site. Macrofauna were more diverse and abundant in the cold seasons (winter and spring) than that in the warm seasons (summer and autumn). The annual variations of macrofaunal communities may be attributed to the changes in sediment texture among the three years of the survey. The variations in macrofaunal communities were mainly related to the proportion of polychaetes within the community. In addition, the density of amphioxus (include *Branchiostoma japonicum* and *B. belcheri*) was negatively correlated to that of polychaetes, bivalves, and crustaceans. Amphioxus was less likely to be found in the sediments with higher silt and clay content. Five biotic indices including Margaref's richness index (d), Peilou's evenness index (J'), Shannon-Wiener diversity index (H'), AMBI and M-AMBI were calculated in the present study. AMBI seems suitable in assessing benthic health at the "Amphioxus Sand" habitat, and a potential risk of ecological health in Xiamen Amphioxus Nature Reserve should be aware.

Key words: spatiotemporal variation, Amphioxus Sand, benthic macrofaunal community, *Branchiostoma*, biotic index

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1 Introduction

Benthic fauna are widely used as indicators of water and sediment quality due to the variable degrees of sensitivity and tolerance of individual species or assemblages (Borja and Dauer, 2008; Dauer et al., 2000; Lancellotti and Stotz, 2004). Macrobenthic assemblages are known to vary at multiple spatial and temporal scales, which may range from days to years and from centimeters to dozens of kilometers (Barnes, 2016; Bessa et al., 2014; Díaz-Tapia et al., 2013; França et al., 2009; Mann and Lazier, 2006; Ramey and Snelgrove, 2003; Varfolomeeva and Naumov, 2013). The variation in composition of benthic communities can be attributed as a response to environmental perturbations, such as seabed dredging, dumping and reclamation (Veiga et al., 2016; Veiga et al., 2017; Olsson et al., 2013; Seitz et al., 2018; Schückel et al., 2015).

The incorporation of multiple spatial and temporal scales into sampling designs is essential for a better understanding of human-induced impacts (Rosenberg et al., 2009). Ecological research has demonstrated that natural disturbances processes play a crucial role in seafloor ecosystems. Only when the space and time scales of human disturbances are greater than those the natural ecosystems are adapted to, changes in community structure are inevitable (Levin, 1992; Thrush et al., 2005). Therefore, elucidating major natural environmental factors that shape spatiotemporal patterns of macrobenthic communities would help to tease apart the effects of natural and anthropogenic disturbances on the variations observed (Glockzin and Zettler, 2008; Dutertre et al., 2013).

Amphioxus prefers coarse sand and shelly sediments, which are then defined as "Amphioxus Sand" (Zinn, 1968; Antoniadou et al., 2004; Chen et al., 2013). The surface of "Amphioxus Sand" is often covered by or enriched with organogenic detritus consisting of coralline algae, broken shells of molluscs, or fragments of bryozoans (Higgins and Kristensen, 1986). The community structures of "Amphioxus Sand" have been studied in some areas, such as the Varna Bay in the Black Sea (Konsulova, 1992) and the Thermaikos Bay in the eastern Mediterranean (Antoniadou et al., 2004). Along the sandy shoals of the Louisiana continental shelf, mole crab *Albunea paretii* and amphioxus *Branchiostoma floridae* typified the community and contributed most of the macrobenthic biomass (Dubois et al., 2009). Three amphioxus species, i.e., *Branchiostoma belcheri*, *B. japonicum* and *B. malayan-*

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*Corresponding author, E-mail: cailizhe@xmu.edu.cn

um, were found coexist at the Tai Long Wan and Pak Lap Wan in subtropical Hong Kong, and polychaetes were numerically dominant in macrofaunal communities herein (Chen et al., 2013).

Aquatic pollution in coastal and marine ecosystems has become a global concern (Shahidul and Tanaka, 2004). There was a world-famous amphioxus fishery which lasted for hundreds of years along the subtropical Xiamen coast, China (Light, 1923). Unfortunately, with the development of marine economy and the intensification of pollution, amphioxus has decreased significantly in the past 50 years (Zeng et al., 1996; Fang, et al., 2002; Weng et al., 2010; Rao et al., 2015). Therefore, a nature reserve was established for amphioxus in the Xiamen, i.e., Xiamen Amphioxus Nature Reserve. Nevertheless, the conservation achievements in terms of both area and quality are declining in some regions in China (Ma et al., 2019). The previous studies in the Xiamen Amphioxus Nature Reserve only focused on the population dynamics of amphioxus (Fang et al., 2002; Weng et al., 2012), and little is known about the structural variability of benthic macrofaunal communities at both spatial and temporal scales here. In order to enhance the understanding of the spatial and temporal variations of macrofaunal communities in the Xiamen Amphioxus Nature Reserve, six sampling surveys including four seasons and three consecutive summers were conducted in summer (August) 2014, autumn (October) 2014, winter (January) 2015, spring (May) 2015, summer (August) 2015, and summer (July) 2016.

2 Materials and methods

2.1 *Study area*

Xiamen is located on the southeast coast of China and the west side of the southern Taiwan Strait. It has a subtropical maritime monsoon climate with semidiurnal tide, the mean annual tidal range is 3.98 m and the mean annual temperature is 21°C. The rainy season is from March to September, and the dry season is from October to February. The mean annual rainfall is 1 200 mm. The northeast wind sustains from September to March and the southeast wind from April to August. The typhoon and tropical storm primarily occur between July and September. The mean annual wind speed is 3.4 m/s. The mean annual salinity is 28.5. The seasonal variation of currents is significant. The direction of the current is from the north to the south of the Taiwan Strait between April and December, and from the south to the north during the remaining year. The current has a reciprocating flow with current speed of less than 40 cm/s.

The Xiamen Amphioxus Nature Reserve was established in 1991 and covers an area of ca. 58 km^2 and consists of four sea areas, they are Huangcuo, Nanxian-Shibaxian, Eyuyu and Xiaodengdao. Two core sites, i.e., Huangcuo (HC) and the Nanxian-Shibaxian (NX), in the Xiamen Amphioxus Nature Reserve were investigated for benthic macrofauna in this study.

2.2 *Sampling and sample processing*

Nine sampling stations were set at the HC and NX sites, respectively (Fig. 1). All sampling stations were located in the subtidal zone. Sampling surveys were conducted in summer (August) 2014, autumn (October) 2014, winter (January) 2015, spring (May) 2015, summer (August) 2015, and summer (July) 2016 at low tide, the water depth ranging from 1 to 2 m. A rabble-like sampling gear (0.1 m^2 area, 10 cm depth) was applied to collect sediment samples because the traditional sampling gear (i.e., van Veen grab) does not work well in this shallow water and sandy sediment environment (Rao et al., 2015). Three sediment replicates were collected for macrofauna at each sampling station. The sediments were washed through a 0.5 mm mesh screen and the residues were fixed in 5% saline formalin for further identification. The fauna was counted and identified to the lowest possible taxon under a dissecting microscope, and was weighed using an electronic balance (0.1 mg) after blotting. In addition, one extra sediment sample was collected from each station for granulometric analysis in August 2014 and in July 2016. The grain size was determined using a SFY-D granulometer, which was capable of measuring grain size from 20 to 4 000 μm.

2.3 *Statistical analysis*

Taxonomic data from the three replicates were pooled to obtain a faunal composition at each sampling station for subsequent analyses. Three classical diversity indices, i.e., Margalef's richness index (d), Pielou's evenness index (J'), and Shannon-Wiener diversity index (H', using $\log_2$ as base) and two common indices used for ecological status assessment, i.e., AMBI and multivariate-AMBI (M-AMBI), were calculated for each sampling station. The three diversity indices were calculated in PRIMER v7 (with PERMANOVA+) (Clarke et al., 2014). The two ecological status indices were calculated in AMBI v5.0 software (http://ambi.azti.es/) until all species or taxa were assigned into one of the five ecological groups (EGs) according to the up to date species list (V. May 2019) (Borja et al., 2000). In SPSS 22.0, non-parametric Kruskal-Wallis test or Mann-Whitney U test were performed to test whether the mean values of the sediment grain size variables and community parameters exhibited significant differences between the two sites, among/between four seasons and among/between the three consecutive summers. The relationships between variables were examined by Spearman's rank correlation coefficient. Before multivariate statistical analyses, abundance data were square-root transformed. Community similarity between each pair of stations was determined using the Bray-Curtis similarity measure based on the transformed abundance data. Non-metric multidimensional scaling (NMDS) ordination based on the Bray-Curtis similarity was performed to explore the spatiotemporal variation of macrofaunal community. Permutational MANOVA (PERMANOVA) was applied to test the significance of the spatiotemporal variation of macrofaunal community. Similarity percentages (SIMPER) analysis was implemented to examine the contribution of each species/taxon to the similarity of macrofaunal community. The above multivariate statistical analyses were run in PRIMER v7 (with PERMANOVA+).

3 Results

3.1 *Sediment grain size*

The mean content of gravel, sand, silt, and clay were 41.1%, 58.3%, 0.5% and 0.0% at the HC site in summer 2014, respectively. The mean content of gravel, sand, silt and clay was 12.7%, 83.8%, 3.2% and 0.3% at the NX site in summer 2014, respectively. The mean content of gravel at the HC site was higher than that at the NX site, but the mean contents of sand, silt and clay at the HC site were lower than that at the NX site in summer 2014. The mean content of gravel, sand, silt, and clay was 6.5%, 32.6%, 57.4% and 3.4%, respectively, at the HC site in summer 2016. The mean content of gravel, sand, silt, and clay was 1.4%, 7.3%, 88.3%, and 3.0% at the NX site in summer 2016, respectively. The mean contents of gravel, sand and clay at the HC site were higher than those at the NX site, but the mean content of silt at the HC site were lower than that at the NX site in summer 2016 (Table 1). Mann-Whitney U test revealed that the content of gravel between the two sites was significantly different (p=0.019). The four sediment

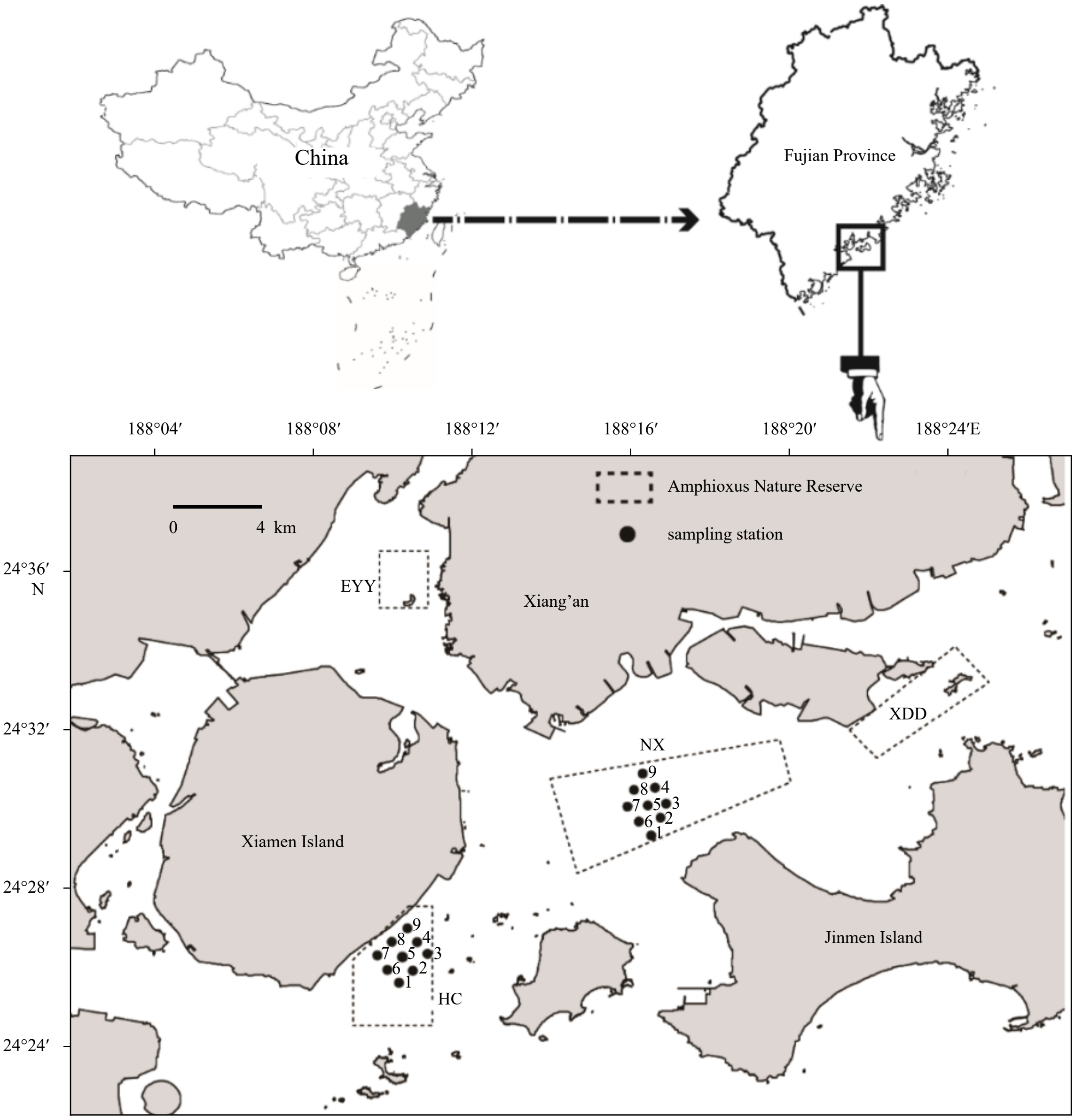

Fig. 1. The sampling stations of macrofauna at Huangcuo (HC) and Nanxian-Shibaxian (NX) subtidal sites in the Xiamen Amphioxus Nature Reserve. EYY represents Eyuyu and XDD Xiaodengdao.

grain size components (i.e., gravel, sand, silt, clay) between summer 2014 and summer 2016 were all significantly different (p<0.001).

3.2 *Community composition and structure*

A total of 155 species of macrofauna were recorded at the HC and NX sites in the Xiamen Amphioxus Nature Reserve during the six surveys. The most diverse taxon was polychaetes (65), followed by bivalves (38), crustaceans (27), gastropods (10) and echinoderms (4). Chordates (4) and the other four taxonomic groups, i.e., cnidarians (3), sipunculans (2), nemerteans (1) and pycnogonid (1) were categorized into "others". According to Zhang et al. (2006), two amphioxus species, i.e., *Branchiostoma japonicum* and *B. belcheri* coexist in Xiamen waters, however, the morphological identification of the two species especially in the juvenile stages is impractical, instead, we use genus *Branchiostoma*. *Branchiostoma* was found at 96 of 106 stations among the six surveys. Figure 2 summarizes the spatial, seasonal and annual variation of macrofaunal species number, density and biomass at the HC and NX sites in the six surveys. The density and biomass of *Branchiostoma* were listed separately from other macrofauna herein. Therefore, if not specifically stated, the density and biomass of macrofauna do not include *Branchiostoma*. Across four seasons and the three consecutive summers (i.e., 2014, 2015 and 2016), the mean species number of macrofauna in the Xiamen Amphioxus Nature Reserve was 43 per site and was constituted mainly by polychaetes (25 per site), and the average contribution rate of polychaetes to per site was 55.6%; the mean density of macrofauna (include *Branchiostoma*) was 137.0 ind./m^2 and was contributed mainly by polychaetes (63.4 ind./m^2, 46.3%) and *Branchiostoma* (36.2 ind./m^2, 26.4%); the mean biomass of macrofauna (include *Branchiostoma*) was 11.15 g/m^2 and was contributed mainly by bivalves (6.65 g/m^2, 59.7%).

In terms of spatial variation, the species number of macrofauna between the two sites was not significantly different (Mann-Whitney U test, p=0.191), while the density and biomass

Table 1. Sediment grain size composition at the sampling stations of the HC and NX sites in the Xiamen Amphioxus Nature Reserve in summer 2014 and summer 2016

Station	Summer 2014				Summer 2016			
	Gravel/%	Sand/%	Silt/%	Clay/%	Gravel/%	Sand/%	Silt/%	Clay/%
HC1	33.6	64.2	2.2	0.0	0.0	0.8	98.3	0.9
HC2	33.3	65.0	1.8	0.0	0.0	1.4	96.6	2.0
HC3	28.5	71.5	0.0	0.0	13.1	61.3	23.9	1.7
HC4	48.8	51.2	0.0	0.0	11.4	59.3	28.2	1.1
HC5	50.1	49.0	0.0	0.0	14.1	72.3	9.6	4.0
HC6	61.4	38.6	0.0	0.0	13.7	70.3	11.4	4.7
HC7	38.7	61.4	0.0	0.0	5.7	26.2	58.6	9.5
HC8	44.8	54.6	0.6	0.0	0.2	1.2	92.3	6.3
HC9	30.9	69.1	0.0	0.0	0.4	0.8	98.2	0.6
Mean	41.1	58.3	0.5	0.0	6.5	32.6	57.4	3.4
NX1	24.5	75.5	0.0	0.0	0.1	1.2	89.8	9.0
NX2	12.1	87.9	0.0	0.0	2.5	10.3	86.3	0.9
NX3	15.5	84.5	0.0	0.0	0.0	1.0	94.5	4.4
NX4	3.9	96.1	0.0	0.0	0.1	0.9	94.0	5.0
NX5	1.5	84.0	13.8	0.7	0.3	2.4	95.0	2.3
NX6	3.4	85.1	10.0	1.4	0.7	4.1	93.9	1.3
NX7	21.5	76.9	1.5	0.1	4.6	25.3	69.9	0.2
NX8	19.3	80.7	0.0	0.0	2.0	9.0	85.9	3.2
NX9	–	–	–	–	2.3	11.8	85.0	0.9
Mean	12.7	83.8	3.2	0.3	1.4	7.3	88.3	3.0

Note: – means no samples.

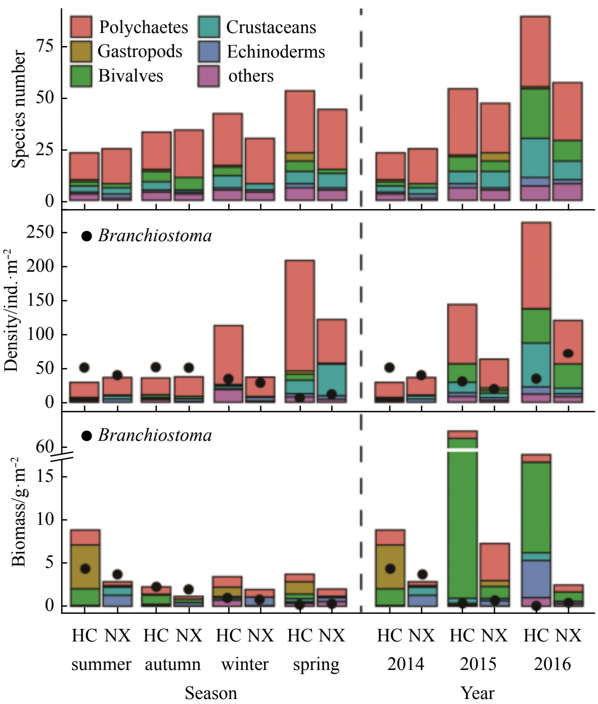

Fig. 2. Taxonomic, density and biomass composition of macrofauna communities at the HC and NX sites in four seasons (left part; summer 2014, autumn 2014, winter 2015 and spring 2015) and three consecutive summers (right part; 2014, 2015 and 2016). The density and biomass of *Branchiostoma* were listed separately from other macrofauna.

of macrofauna between the two sites were significantly different (Mann-Whitney U test, $p<0.05$). Specifically, the mean density of macrofauna at the HC site (132.3 ind./m^2) was higher than that at the NX site (69.4 ind./m^2), especially in winter 2015, spring 2015, summer 2015 and summer 2016, associated with a rapid increase of polychaetes at the HC site. The mean biomass of macrofauna at the HC site (16.70 g/m^2) was higher than that at the NX site (2.94 g/m^2), therein, the biomass of gastropods (1.28 g/m^2) and bivalves (12.79 g/m^2) at the HC site was higher than that at the NX site (gastropods 0.11 g/m^2; bivalves 0.51 g/m^2) (Fig. 2). Notably, bivalve *Ruditapes philippinarum* was predominant in the biomass of macrofauna at HC site in summer 2015 and 2016. In addition, no significant difference between the two sites for the density and biomass of *Branchiostoma* was observed (Mann-Whitney U test, $p=0.311$, $p=0.081$, respectively).

In terms of seasonal variation, the species number and density of macrofauna were significantly different among the four seasons (Kruskal-Wallis test, $p<0.001$), while no significant difference was observed for the biomass (Kruskal-Wallis test, $p=0.671$). The density and biomass of *Branchiostoma* differed significantly among the four seasons (Kruskal-Wallis test, $p<0.001$). The mean species number and density of macrofauna at both sites were higher in the cold seasons (winter 2015 and spring 2015; 40 per site, 119.9 ind./m^2) than those in the warm seasons (summer 2014 and autumn 2014; 27 per site, 34.7 ind./m^2). In contrast, the mean density and biomass of *Branchiostoma* were higher in the warm seasons (48.3 ind./m^2, 3.06 g/m^2) that those in the cold seasons (20.3 ind./m^2, 0.55 g/m^2) (Fig. 2).

In terms of annual variation, the species number and density of macrofauna were significantly different among the three consecutive summers (Kruskal-Wallis test, $p<0.001$), while no significant difference was observed for the biomass (Kruskal-Wallis test, $p=0.632$). The density and biomass of *Branchiostoma* differed significantly among the three consecutive summers (Kruskal-Wallis test, $p<0.05$). The mean species number and density of macrofauna at both sites increased from summer 2014 (24 per site, 32.8 ind./m^2) to summer 2016 (73 per site, 110.5 ind./m^2). The mean density of *Branchiostoma* at both sites decreased firstly from summer 2014 (45.4 ind./m^2) to summer 2015 (25.1 ind./m^2) and then recovered in summer 2016 (55.5 ind./m^2), whereas the mean biomass of *Branchiostoma* at both sites decreased after summer 2014 (4.01 g/m^2) and stayed in low biomass in summer 2015 (0.51 g/m^2) and summer 2016 (0.91 g/m^2) (Fig. 2).

It is noteworthy that the density of *Branchiostoma* was significantly correlated to that of polychaetes, bivalves and crustaceans, with a correlation coefficient of –0.31, –0.23 and –0.40, respectively (p<0.05). The biomass of *Branchiostoma* was significantly correlated to that of bivalves, crustaceans and others, with a correlation coefficient of –0.35, –0.48 and –0.29, respectively (p<0.05).

NMDS ordination plots revealed that both the seasonal and annual variations in macrofaunal communities were clear (Fig. 3). The stations of the HC site in winter 2015 were separated from the rest stations, and the stations of the two sites in spring 2015 were separated from the rest stations (Fig. 3a). The stations of the two sites in different years were separated from each other (Fig. 3b). PERMANOVA main tests confirmed the significant effects of the seasonal and annual factors in the variation of macrofaunal communities (pseudo-F=6.738, p=0.001; pseudo-F=5.708, p=0.001). Further, PERMANOVA pair-wise tests showed that the differences of macrofaunal communities between four seasons and between the three consecutive summers were all significant at the HC site, whereas the significant differences of macrofaunal communities at the NX site were only observed for the two seasonal groups (i.e., summer vs. spring and autumn vs. spring) and for all annual groups (Table 2). In addition, the difference of macrofaunal communities between the two sites was significant (t=2.168, p=0.001).

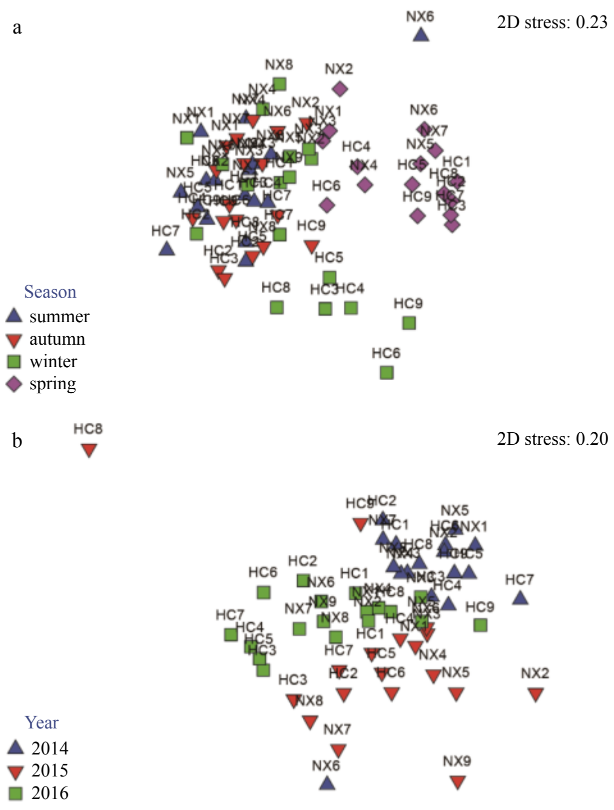

Fig. 3. NMDS ordination plots of benthic macrofaunal communities at the HC and NX sites in four seasons (summer 2014, autumn 2014, winter 2015 and spring 2015; a) and three consecutive summers (2014, 2015 and 2016; b).

The SIMPER analysis identified 21 taxa as most contributing to in-group similarity (Table 3). There were 19 taxa, including polychaetes 13, crustaceans 3, echinoderms 1 and others 2, contributing to 70% in-group similarity at the HC site during the six surveys. Comparatively, there were 12 taxa, including polychaetes 9, echinoderms 1 and others 2, contributing to 70% in-group similarity at the NX site during the six surveys. Polychaetes *Eunice indica*, *Lepidonotus tenuisetosus*, *Marphysa sanguinea*, *Ophelina grandis*, *Paraprionospio pinnata*, *Travisia japonica*, and crustaceans *Corophium* sp. *Typhlocarcinus villosus* and *Byblis* sp. only contributed to the in-group similarity at the HC site, whereas polychaetes *Aglaophamus dibranchis* and *Aonides oxycephala* only contributed to the in-group similarity at the NX site. *Branchiostoma* was one of the top two contributing taxa to the in-group similarity at the HC and NX sites in the six surveys except at the HC site in spring 2015. However, the contribution rate of *Branchiostoma* at the two sites decreased from summer 2014 to spring 2015, and that at the HC site also decreased from 2014 to 2016 but that at the NX site firstly sharply decreased and then slightly increased from 2014 to 2016. Comparatively, the contributions of polychaetes (e.g., *Synelmis albini*, *Prionospio queenslandica* and *Typosyllis* sp.) increased in the cold seasons and in summer 2015 and summer 2016.

3.3 *Biotic indices*

Figure 4 summarizes the spatial, seasonal and annual variation of the five biotic indices at the HC and NX sites in the six surveys. The mean values of Margaref's richness index (d), Peilou's evenness index (J'), Shannon-Wiener diversity index (H'), AMBI and M-AMBI were 2.40, 0.75, 2.64, 1.01 and 0.61, respectively. Except for the AMBI index (Mann-Whitney U, p=0.036), the remaining indices did not differ significantly between the two sites. The mean value of the AMBI index (1.14) at the HC site were higher than that at the NX site (0.87). All five biotic indices showed significant differences among four seasons and among the three consecutive summers. A clear increasing tendency was observed for all five biotic indices at the two sites from summer 2014 to spring 2015. Apart from J' and AMBI index, which the highest mean values occurred in summer 2015, the mean values of the remaining indices at the two sites increased from summer 2014 to summer 2016. The results of the AMBI index indicated that the ecological status of most stations at the two sites in summer 2014 and autumn 2014 was undisturbed, while that in spring 2015 was slightly disturbed. More stations at the HC site were

Table 2. The results of PERMANOVA pair-wise tests between seasonal and annual factors based on the Bray-Curtis similarity of macrofaunal communities at the HC and NX sites

Groups	HC		NX	
	t	p	t	p
Summer, autumn	1.408	0.031*	0.996	0.457
Summer, winter	2.001	0.002**	1.228	0.099
Summer, spring	3.451	<0.001***	2.177	<0.001***
Autumn, winter	1.852	0.002**	1.150	0.181
Autumn, spring	3.025	<0.001***	2.276	<0.001***
Winter, spring	2.460	<0.001***	1.983	<0.001***
2014, 2015	2.300	<0.001***	1.747	0.002**
2014, 2016	2.495	<0.001***	2.171	<0.001***
2015, 2016	1.498	0.008**	1.940	<0.001***

Note: * Significant at the 0.05 level; ** significant at the 0.01 level; *** significant at the 0.001 level.

Table 3. The contributions of taxa to the similarity of macrofaunal communities at HC and in the six surveys based on SIMPER analysis (cut-off applied at 70%)

Species	2014				2015						2016	
	Summer		Autumn		Winter		Spring		Summer		Summer	
	HC	NX	HC	NX	HC	NX	HC	NX	HC	NX	HC	NX
Polychaetes												
Aglaophamus dibranchis								7.9				5.9
Amaeana trilobata							4.6	8.2				
Aonides oxycephala												6.4
Eunice indica					9.6						5.4	
Glycera chirori					7.8				4.9		8.9	7.7
Lepidonotus tenuisetosus			**17.5**				4.1					
Lumbrineris latreilli		**9.0**			8.2		9.7				5.1	
Marphysa sanguinea									3.8			
Ophelina grandis											3.3	
Paraprionospio pinnata									7.5			
Prionospio queenslandica							**18.0**	8.3		9.5		
Synelmis albini							6.5		16.7	**24.5**	3.4	8.3
Tharyx multifilis							8.5	11.7				
Travisia japonica	**11.5**											
Typosyllis sp.						10.1	6.0	**12.1**			3.8	**12.1**
Crustaceans												
Corophium sp.							**11.3**					
Typhlocarcinus villosus									5.0			
Byblis sp.											**9.7**	
Echinoderms												
Amphioplus laevis				**12.2**		**16.0**		9.4	3.2	10.2	5.5	
Others												
Branchiostoma	**65.4**	**68.9**	**54.0**	**58.1**	**26.1**	**50.2**		**12.4**	**23.3**	**21.7**	**19.5**	**30.7**
Cerebratulina sp.					**18.4**		3.3		7.4	7.9	5.8	
Average similarity	47.9	36.9	40.1	41.6	29.9	41.6	43.4	39.1	29.9	29.5	30.1	46.6

Note: Top two contributing taxa in bold.

slightly disturbed compared to the NX site in winter 2015. Most stations at the two sites in the three consecutive summers were undisturbed, however, nearly half of the stations at the HC site in summer 2015 were slightly disturbed. On the contrary, the results of the M-AMBI index suggested that the ecological quality of most stations at the two sites in summer 2014 was moderate, whereas in the remaining surveys was good or high. In addition, the density of *Branchiostoma* was negatively correlated with d (r=–0.36, p<0.001), J' (r=–0.65, p<0.001), H' (r=–0.54, p<0.001), AMBI (r=–0.74, p<0.001), and M-AMBI (r=–0.26, p=0.014), respectively.

3.4 *Relationship between macrofaunal community and sediment grain size*

The sediment grain size components had significant correlations with the axes of NMDS ordination plot produced by the Bray-Curtis similarity matrix of macrofaunal communities at the two sites in summer 2014 and summer 2016. The stations at the two sites in summer 2014 were separated from the stations at the two sites in summer 2016 associated with different sediment grain size composition. In addition, *Branchiostoma* was less likely to be found at the stations with higher silt and clay content (Fig. 5).

4 Discussion

4.1 *Macrofaunal composition in "Amphioxus Sand" habitats*

Our results showed that polychaetes, molluscs, crustaceans and echinoderms were the dominant taxonomic groups contributed to the species composition at the two core sites in the Xiamen Amphioxus Nature Reserve, which is consistent with the findings from other "Amphioxus Sand" habitats (Antoniadou et al., 2004; Chen et al., 2013; Higgins and Kristensen, 1986). Compared with the marcrofaunal communities in "Amphioxus Sand" habitats (i.e., Tai Long Wan and Pak Lap Wan) in Hong Kong waters (Chen et al., 2013), we found that the mean density of macrofauna (include *Branchiostoma*) in present study (167.1 ind./m^2 at the HC site, 106.0 ind./m^2 at the NX site) were much less than that in the Pak Lap Wan (766.6 ind./m^2) and the Tai Long Wan (896.6 ind./m^2), and the mean values of the Shannon-Wiener diversity index (H') and Peilou's evenness index (J') were similar. Besides, Spionida *Prionospio* and Amphipoda *Corophium* were numerically dominant genera in "Amphioxus Sand" community in Xiamen and Hong Kong waters. The species richness of polychaetes (65 species) in the present study was higher than the results from Konsulova (1992) in the Varna Bay, Black Sea (28 species), Antoniadou et al. (2004) in the Thermaikos Bay, Mediterranean, and Chen et al. (2013) in the Tai Long Wan (38 species) and Pak Lap Wan (43 species) in Hong Kong waters (40 species). Compared with previous study at the HC site, the species number of polychaetes in the present study was more than that the result from Zeng et al. (1996), which only four polychaetes species were recorded in a quantitative survey. Higher species richness of polychaetes in "Amphioxus Sand" community probably implied an increase of organic pollution (Antoniadou et al., 2004;

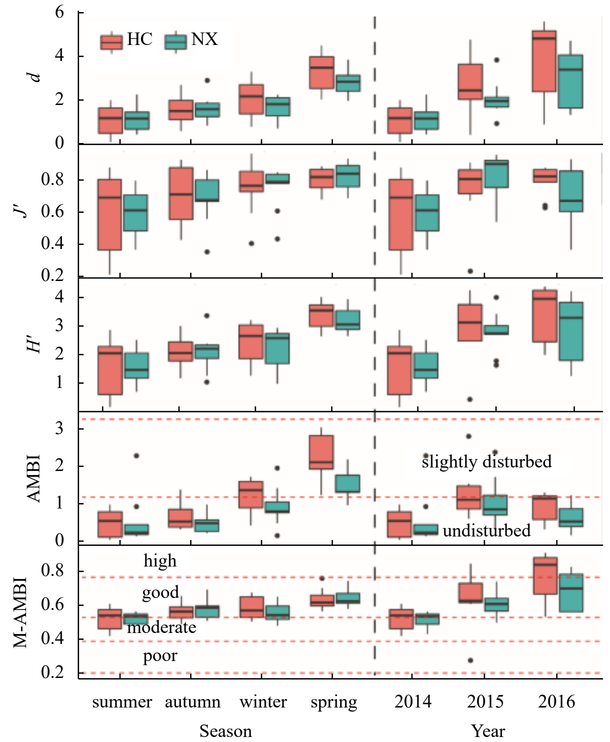

Fig. 4. Boxplots of Margaref's richness index (*d*), Peilou's evenness index (*J*′), Shannon-Wiener diversity index (*H*′), AMBI and M-AMBI at the HC and NX sites in four seasons (summer 2014, autumn 2014, winter 2015 and spring 2015; left part) and three consecutive summers (2014, 2015 and 2016; right part). Red dotted lines indicate the boundaries of the ecological status for AMBI and M-AMBI.

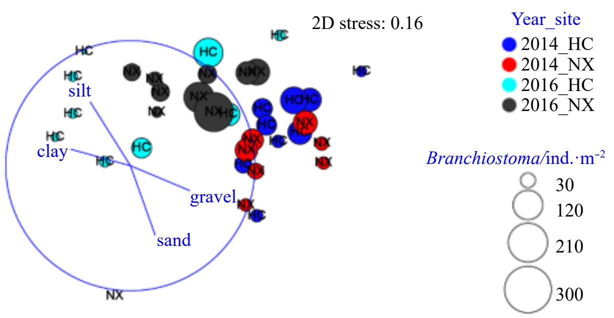

Fig. 5. NMDS ordination plot of macrofaunal communities at the HC and NX sites in summer 2014 and 2016. The bubble size indicates the density of *Branchiostoma* in each station. The length and direction of each vector indicate the strength and significance of the relationship between sediment grain size components and axes based on environmental fitting. The blue open circle is the scale of the correlation coefficient.

Chen et al., 2013; Dean, 2008).

Branchiostoma belcheri and *B. japonicum* were both recorded in the Xiamen Amphioxus Nature Reserve (Zhang et al., 2006) and in Hong Kong waters (Chen et al., 2013). However, *Branchiostoma japonicum* was the only amphioxus species in the adjacent Jinmen (Kinmen) waters (Lin et al., 2015). This suggested a fine-scale habitat differentiation at the overlapped region for these two species. The density of amphioxus (36.2 ind./m^2) in the present study was much less than other "Amphioxus Sand" habitats, e.g., 380.0 ind./m^2 in the Baía de Guanabara in Brazil (Silva et al., 2008), 158.6 ind./m^2 in the Antofagasta Bay in Chile (Vergara et al., 2012), 244.8 ind./m^2 in the Racou Beach in France (de Moura Barboza et al., 2013), 252.2 ind./m^2 in the Tai Long Wan and 383.8 ind./m^2 in the Pak Lap Wan in Hong Kong (Chen et al., 2013). Besides, the density of amphioxus at the HC site in the present study (2014–2015, 35.9 ind./m^2) was much less than that in 1987–1988 (Wang et al., 1989; 150.7 ind./m^2), 1989 (Zhou et al., 1990; 226.8 ind./m^2), 1994–1995 (Zeng et al., 1996; 142.0 ind./m^2), 2001–2002 (Fang et al., 2002; 68.7 ind./m^2), but close to that in 2009–2010 (Huang et al., 2013; 39.0 ind./m^2). Although the Xiamen Amphioxus Nature Reserve was established in 1991, mariculture and other human activities were not under effective control. As we know, beach recreations are extensive at the HC site, and illegal dredged-material disposal was observed in the NX site during our sampling survey in autumn 2015. According to the reports from Weng et al. (2010, 2012), cause of the effects of coastal engineering, amphioxus had disappeared in the other two core areas (i.e., Eyuyu and Xiaodengdao) in the Xiamen Amphioxus Nature Reserve.

4.2 *Spatiotemporal variation of macrofaunal community*

The spatial variation of macrofaunal community between the HC site and the NX site was significant. Although the density and biomass of amphioxus at the two sites was not significantly different, the density and biomass of macrofauna at the HC site was higher than those at the NX site. Polychaetes *T. fauveli*, *P. queenslandica*, *E. indica* and *G. chirori* at the HC site were more abundant than those at the NX site, and bivalves *R. philippinarum* was found at ten stations at the HC site but only one station at the NX site. The spatial variation of macrofaunal community in "Amphioxus Sand" habitats was also observed in the Thermaikos Gulf (Antoniadou et al., 2004) and Hong Kong waters (Chen et al., 2013), which was suggested to be attributed on their granulometric differences. Consistent with our results, *E. indica* was abundant in the Pak Lap Wan but less in the Tai Long Wan where the sediment texture was finer (Chen et al., 2013).

The seasonal variations of macrofaunal community and amphioxus in our study were clear. The seasonal variations were also observed in the Thermaikos Gulf (Antoniadou et al., 2004) but not in Hong Kong waters (Chen et al., 2013). The loss of seasonality in community structure is considered as an indication of deterioration of benthic temperate communities (Karalis et al., 2003). However, for subtropical communities, more stable climate and environmental conditions, might minimize the seasonal variations (Chen et al., 2013). Bertrán et al. (2016) suggested that the seasonal variation of macrobenthos usually associated with the changes of sediment characteristics (i.e., texture and organic material). Our results showed that the difference of community structure between the warm seasons (summer and autumn) and the cold seasons (winter and spring) was apparent. This may be related to the seasonal variation of currents in the Taiwan Strait (Lin et al., 2015), which stronger in the warm seasons and weaker in the cold seasons (Jan et al., 2002). Because of amphioxus specific environmental requirements, sediment suspension under sheet flow would create "clean" environments for amphioxus but "impoverished" environments for other macrofauna, especially for some polychaetes.

We also observed the significant annual variations for macrofaunal community and amphioxus. Similarly, annual discrimination of "Amphioxus Sand" community was revealed in the Thermaikos Gulf and was attributed to the change of the proportions of sediment compounds among the two years of survey (Antoniadou et al., 2004). Indeed, the macrofaunal communities in summer 2016 were separated from those in summer 2014, it might reason to the sediments were finer in summer 2016 than those in summer 2016 (Table 1, Fig. 5). The sediment contains a higher proportion of silt that diversifies its macrofauna (Antoniadou et al., 2004), for example, some polychaete and crustacean species, which prefer to fine sediments (Shin et al., 2004), were abundant in summer 2016 (Fig. 2). We conjectured that the changes in the sediment grain size composition might be related to human activities, such as clam farming and dredged-material disposal. It has been reported that clam (i.e., *R. philippinarum*) farming emerged at the HC site from 2015. The clam farming would cause a significant increase in percentage fines and percentage organic content of the sediment and affect the fauna within the sediment (Johansen et al., 2018; Spencer et al., 1997). Amphioxus prefers sandy or coarse sandy substrata (Lin et al., 2015; Zhou et al., 1990). However, muddy areas expansion has caused a severe decline of amphioxus populations on a global scale (Antoniadou et al., 2004). Interestingly, the density of amphioxus declined from summer 2014 to summer 2015 but recovered in summer 2016. However, the amphioxus collected in summer 2016 stayed in low biomass, i.e., more juveniles appeared during this period. Generally, amphioxus (*B. japonicum* and *B. belcheri*) breed in late spring and summer (Hu, 2013). Therefore, breed season should be considered in survey designs and long-term observations for population dynamics are needed in future work.

4.3 *Ecological status assessment*

The Shannon-Wiener diversity index (H') is usually used to assess the ecological status of benthic environments, especially in the estuarine and coastal waters of China (Cai et al., 2001). Recently, AMBI and M-AMBI indices are worldwide used (Borja and Dauer, 2008) but have rarely been used in the “Amphioxus Sand” habitats. Our results showed that all five biotic indices applied in the present study were negatively related to the density of amphioxus, but the results of ecological status assessment from AMBI and M-AMBI were conflicting. One evaluated as undisturbed by AMBI but as moderate by M-AMBI, vice versa. AMBI and M-AMBI have been demonstrated to effectively assess disturbances caused by bottom aquaculture (Wang et al., 2017). The amphioxus population should be a critical indicator of environmental quality (Rota et al., 2009). Therefore, we suggest that AMBI is a robuster tool to assess the ecological status of “Amphioxus Sand” habitats. Accordingly, the HC site was more affected by anthropogenic pressures (such as clam farming) and a potential risk of ecological health in the Xiamen Amphioxus Nature Reserve should be aware.

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