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## The application of multivariate analysis of the community structure of macrobenthos to water environment quality monitoring at a river in the Pearl river Delta, China

Feng Li<sup>1,2\*</sup>, Yan-Yan Lang<sup>1</sup>, Xiang-Yun Zeng<sup>1</sup>, Kai-Xuan Shen<sup>1</sup>, Jun-Yong Lin<sup>1</sup>, Dui-Lin Wu<sup>2</sup>, Zhi Tan<sup>2</sup>, Jia-Yu Yang<sup>3</sup>

<sup>1</sup>School of Civil Engineering and Transportation, South China University of Technology, Guangzhou 510630, (CHINA)

<sup>2</sup>Environmental Monitoring Center Station of Dongguan City, Dongguan, 523009, (CHINA)

<sup>3</sup>School of Environmental Science and Engineering, Guangdong University of Technology, Guangzhou, 510006, (CHINA)  
E-mail: hjlifeng@scut.edu.cn

### ABSTRACT

A representative polluted river in Pearl River Delta has been selected. The samples of macrobenthos were collected and analyzed. The biological monitoring data were analyzed by Shannon-Wiener diversity index (SWI), the multivariate analysis (combining two methods of MDS and Cluster analysis), and the analysis results of them were compared. The investigation results show there are rare species in the study area; furthermore the dominants were mainly contributed by the two pollution indicator species, *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi*. Analytical results indicated that the community structure of macrobenthos was highly associated with the condition of water environment in the river. The results of SWI cannot effectively reflect the difference of pollution status of various stations in the polluted river; despite the presence of some problems, multivariate analysis method is more suitable than SWI as far as information mining of biological monitoring in the polluted river is concerned.

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### KEYWORDS

Biological monitoring;  
Macrobenthos;  
Multivariate analysis;  
Non-metric multidimensional  
scaling analysis (MDS);  
Cluster analysis.

### INTRODUCTION

The water environmental monitoring is the prerequisite of environmental protection. Generally physical and chemical methods have been used to monitor water environment quality in the past, but they can indirectly reflect transient condition of water environment only through single index, and health condition of water environment cannot be reflected comprehensively<sup>[1-3]</sup>. The biological monitoring has been introduced to com-

pensate for this defect<sup>[4-6]</sup>. The biological monitoring is one comprehensive technology developed with biological methods to monitor environmental quality, which can both save funding and illustrate problems easily<sup>[7,8]</sup>. Aquatic community monitoring is an important part of biological monitoring<sup>[9,10]</sup>, which plays an important role in evaluation of water environment quality<sup>[11,12]</sup>. With characteristic of long life span, high sensitivity to environmental change, small capacity and scope of activity, macrobenthos living in the water for a long term can

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comprehensively reflect effects to environment and creature imposed by pollutants<sup>[13,14]</sup>. With the change of water quality, the community structure of macrobenthos will change accordingly: in polluted water body, communities of macrobenthos are dominated by pollution tolerant species, whereas communities are composed of various species in clean waters. Hence macrobenthos is regarded as good tool of water quality monitoring<sup>[15,16]</sup>.

The keys and difficulties of biological monitoring are the information mining of biological monitoring data we gain, and analyzing the valuable information among them<sup>[17]</sup>. When water quality is monitored by using community structure of macrobenthos, experimental results should be analyzed deeply to dig the useful information. In the early stage, Shannon-Wiener diversity index (SWI) has been widely used to explain the results of aquatic biological monitoring previously<sup>[18]</sup>. However, SWI suffering the problem in a polluted river, which it cannot identify the water polluted degree because of the species and quantities of aquatic animals are scarce and SWI tends to homogenization<sup>[19]</sup>. Recently, multivariate analysis method has been introduced to remedy these defects. Recently, multivariate analysis method has been applied in some researches<sup>[20,21]</sup>, and certain results have been achieved<sup>[22,23]</sup>, however, the application of multivariate analysis of the community structure of macrobenthos to water environment quality monitoring in polluted river has been rarely reported.

In this study, Xinan River, a representative polluted river in Pearl River Delta, has been selected. The samples of macrobenthos were collected and analyzed. The biological monitoring data were analyzed by SWI, the multivariate analysis (combining two methods of MDS and Cluster analysis), and the analysis results of them were compared, so as to verify the effects of multivariate analysis of macrobenthos to monitoring water quality.

## MATERIALS AND METHODS

### Study area

A typical river, Xinan River in the Pearl River Delta (PRD), China, has been selected as the object. The Pearl River Delta (PRD) is formed by three main streams of the Pearl River, which is China's second largest river

after the Yangtze in terms of annual average flow. The Xinan River is upstream of the Guangzhou section of the Pearl River, connecting the major cities of Guangzhou and Foshan (shown in Figure 1). This research chose most typical segment of the Xinan River (the segment was customarily called Fenggang section of the Xinan River). The section is located in the upper mid region of the Xinan River with three streams (Damian Creek, Dalang Creek, Fenggang Creek) flowing into it. With a rapidly growing economy and a sharp increase in the population, a large amount of industrial and domestic wastewater discharges into the Xinan River and worsens the water quality. Because of complicated and changeable condition of water environment, it's difficult to assess the water quality by using physical and chemical methods of monitoring<sup>[24]</sup>.

### Sampling and analysis

According to the previous investigation concerning pollution status of the river<sup>[24,25]</sup>, 7 representative stations were selected in the river (the specific location of various sampling stations was shown in Figure 1). The collection and processing of macrobenthos were carried out according to Chinese national standards<sup>[26]</sup>. The samples of macrobenthos were collected by using a 17 cm×29 cm Peterson grab sampler. The benthic invertebrate samples were placed on a white porcelain plate to be sorted after being screened through a 40 mesh (380 μm) sieve. The sorted samples were then washed with Milli-Q water and fixed in bottles that were transported to the laboratory for further processing. The fixed samples were examined by microscopy and enumerated and identified to the species level. Shannon-Wiener diversity index were calculated by Excel 2007<sup>[26]</sup>. The two multivariate statistical analyses (Cluster analysis and MDS analysis) were conducted by SPSS 19.0<sup>[27,28]</sup>.

## RESULTS AND DISCUSSION

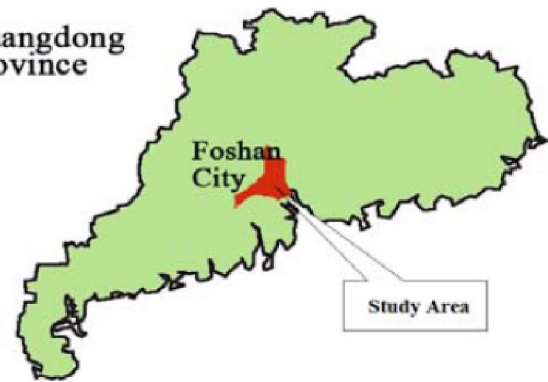
### Features of community structure of macrobenthos in the river

TABLE 1 shows the investigation results of macrobenthos in the river. Generally, rare species have been found in the study area: only 15 species of macrobenthos were found in 7 stations, 7 mollusks accounted for 46.67%, 4 annelid accounted for 26.67%,

1. China



2. Guangdong Province



3. Study Area

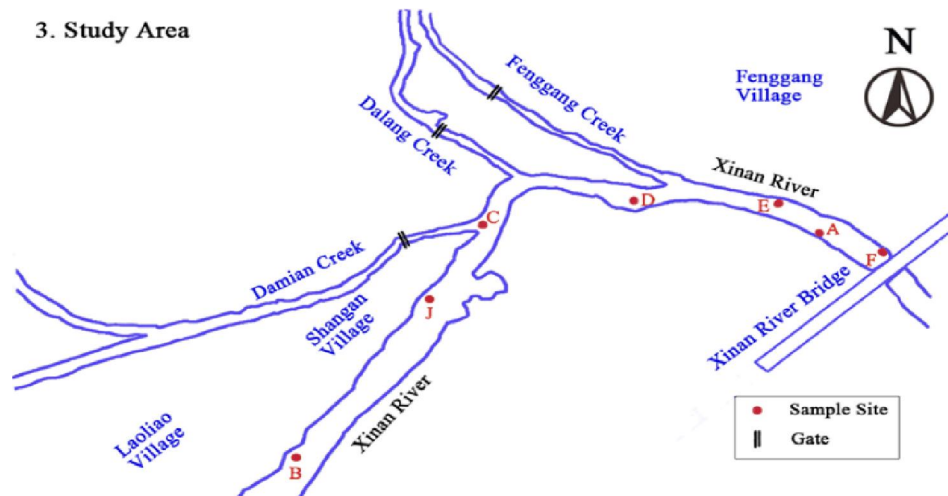


Figure 1: The study area and locations of the sampling stations

TABLE 1 : The density of macrobenthos (ind.m<sup>-2</sup>)

Stations Species	A	B	C	D	E	F	J
<i>Hippeutis cantori</i>	53	122	0	0	30	0	0
<i>Cipangopaludina chinensis</i>	13	0	0	10	10	0	0
<i>Bellamyia aeruginosa</i>	0	41	0	0	0	0	0
<i>Alocinma longicornis</i>	0	10	0	0	0	0	0
<i>Katayama nosophora Robson</i>	0	41	0	10	0	10	0
<i>Radix swinboei</i>	0	0	0	0	10	0	0
<i>Corbicula fluminea</i>	0	10	0	0	0	0	0
<i>Branchiura sowerbyi</i>	0	0	467	152	20	203	193
<i>Limnodrilus hoffmeisteri</i>	13	913	14138	3550	2901	7677	7505
<i>Nais communis</i>	13	0	0	0	0	0	0
<i>Tylorrhynchus heterochaetus</i>	0	0	20	0	0	0	0
<i>Tanypus chinensis</i>	13	0	0	0	0	0	0
<i>Ephaceella</i> sp.	13	0	0	0	0	0	0
<i>Planocera reticulata</i>	13	0	0	0	0	0	0
<i>Dugesia gonocephala</i>	26	0	0	0	0	0	0
Total Density / ( ind.m <sup>-2</sup> )	157	1137	14625	3722	2971	7890	7698
Total Number of Species	8	6	3	4	5	3	2

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aquatic insects 2 accounted for 13.33%, platyhelminth 2 accounted for 13.33% (TABLE 1). Community compositions of benthos varied in different stations. Populations of species were relatively low in the stations C, D, F, J: station C with 3 species, station D with 4 species, station F with 3 species, station J with 2 species. Furthermore, community structure of macrobenthos in the 4 stations was mainly composed of pollution tolerant species. Species at A, B, E station is relatively abundant: station A has 8 species, and the dominant species is *Hippeutis cantori*. B station has 6 species, and E station has 5 species. It can be conclude from TABLE 1 that one of the features of the community structure of macrobenthos is that density of macrobenthos varied widely in different stations: station C with the highest density of 14625 ind·m<sup>-2</sup>, station A with the lowest density of 157 ind·m<sup>-2</sup>, and there is 93 times difference between them.

It can be also been seen in TABLE 1 that the two pollution indicator species, that is *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi*, were widespread. *Limnodrilus hoffmeisteri* appeared at every station, but density varied from each station. Maximum value appeared at the C position; its perched density reached 14138 ind·m<sup>-2</sup>. The lowest perched density of A station was only 13 ind·m<sup>-2</sup>. Dominant species of other 6 stations except station A was *Limnodrilus hoffmeisteri*. The other indicator of pollution, *Branchiura sowerbyi*, was found in most stations (5 stations), and its density ranged from 20 ind·m<sup>-2</sup> (station E) to 467 ind·m<sup>-2</sup> (station C).

From TABLE 1 it can be concluded that there are rare species in the river and dominates were mainly composed of pollution tolerant species in most of the stations, which is in accordance with the characteristics of pollution areas.

### The assessment results of Shannon-Wiener diversity index (SWI)

Species diversity is an important sign of a healthy ecosystem, and Shannon-Wiener diversity index has been regarded as an appropriate measure of species diversity in the field survey<sup>[29,30]</sup>. Hence, it is often used to analysis the community construct of macrobenthos. The SWI is calculated as follows:

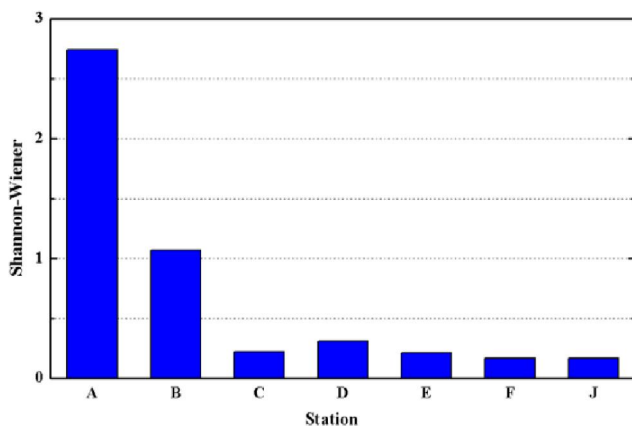
$$H' = -\sum_{i=1}^S P_i \log_2 P_i \quad (1)$$

$H'$ — Shannon-Wiener diversity index;  $P_i = n_i / N$ ;  $n_i$ — number of creature individuals in the sample  $i$ ;  $N$ — total number of individuals in the sample;  $S$ — total number of species in the sample

The calculating results of SWI are shown in Figure 2. The evaluation standard is that the smaller SWI value is, the worse ecological environmental quality is<sup>[29,30]</sup>. According to the evaluation criterion, the pollution degree of the stations from heavy to light was  $J \approx F > E \approx C > D > B > A$ . There are two problems existing in the evaluation results. Firstly, it is difficult to differentiate between stations of J and F, stations C and E. It can be seen from Figure 2: the SWI values of 5 stations are very close, making it impossible to distinguish pollution degree because of equal SWI value of J and F, C and E. Secondly, the environmental conditions may be misjudged in station E. The reason of first problem could be that quantities of macrobenthos species are so much scarce in the study area that most of the data of macrobenthos in TABLE 1 are zero. It can be seen from TABLE 1 that the differences between J and F is very small. There are 3 species in station F, 2 species in station J, and dominant species of two stations are *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi*, furthermore density of two species are similar. The subtle differences lie in that there are little *Radix swinboei* in station F, which cannot be distinguished by SWI. The second question above mentioned was proposed on the basis of the results of *in-site* inspection, combining the analysis to the structure of macrobenthos community, distribution of polluted indicators species as well as the previous study<sup>[24]</sup>.

The characteristics of hydrology and water quality in Xinan River are that they are both complicated and variable. The Xinan River is the upstream of the Guangzhou section of the Pearl River, flowing from Xinan Gate (located in Sanshui District in the city of Foshan) through the town of Guangyao in Nanhai District Foshan to the towns of Lishui, Heshun, and at last into the Guangzhou section of the Pearl River, connecting the major cities of Guangzhou and Foshan. The Fenggang section of Xinan River is greatly influenced by semidiurnal tide, which causes constant changing





**Figure 2 :** The calculating results of the Shannon-Wiener diversified index (SWI) of the sampling stations in the Xinan river

conditions, and it is an intersection of water quality<sup>[24]</sup>. Water quality in the upstream of is relatively good because its inflow water comes from Beijiing River (one of the most important drinking water resources) via Xinan Gate. The water quality began to deteriorate in Fenggang section (the study area in this paper, as shown in Figure 1) because of pollution discharge along the river on both sides<sup>[24]</sup>. Among the 3 tributaries, Damian Creek has maximum watershed and sewage flow. Sewage of Damian Creek is contributed by domestic sewage and industrial wastewater; sewage of Dalang Creek is mainly composed of domestic sewage and mixed with some industrial wastewater; under the influence of the large printing factory, water quality in Fenggang Creek is worst, and its water is black and stinky. The range of its effect has extended to the stations D, E, A, and F in downstream. Black water holds half of watercourse and extended to the bottom of Xinan River Bridge<sup>[24]</sup>. In collusion, the characteristics of water quality in the study area is that water quality of upstream is relatively good while the quality turn to be worse after convergence of tributaries, and the water quality is the worst nearby the sewage outfalls and the intersections of creeks and main watercourse.

Station B is located in upstream and relatively far from sewage outfall (The wastewater outfall of Laoliao Village). Furthermore tributaries have not yet flown into it. Hence water quality is relatively good, and there are relatively rich species in the station, including 5 mollusca. In comparison to station B, station J was heavily polluted owing to its location near the sewage outfall of

Shang'an Village, hence the water quality of station J is worse. Only two pollution indicator species, *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi*, were found in this station. Similar to station J, station C is located in intersection of Damian Creek and main channel with abundant industrial wastewater and living sewage of Damian Creek flowing into it. So water quality of station C is relatively worse and the community of macrobenthos is mainly composed of *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi*. Though the 3 stations of A, E, and F are all located in main channel, the environmental conditions are very difference. In the section of the river, the three creeks flow into Xinan River but the pollutants carried by the creeks have not yet mixed with the water from upstream, resulting the huge spatial differentiation of water quality. It can be seen from TABLE 1 that the structure of macrobenthos community in the 3 stations is considerably different, which reflects the space differences of water quality. The number of species in station A reached 8, the highest of all stations in this study; while the number of species in station F was only 3 with the two polluted indicators, *Limnodrilus hoffmeisteri* and *Branchiura sowerbyi* as the dominant species. The number of species of station E is 5, including the clean water and pollution indicator species.

Judging from biological diversity, the environment quality of station E is better than station C and D because the number of species in station E is larger than station C, and D; furthermore density of polluted indicators in stations E is lower than C and D. Secondly, water quality of station E is better than station C and D on the basis of *in-site* survey. Thirdly, the previous results of water monitoring show that water quality of station C and D is obviously worse than station E<sup>[24]</sup>. So there may be misjudgment in the assessment results of station E by SWI.

To sum up, the community structure of macrobenthos was highly associated with the conditions of water environment in the river. There are some problems including misjudgment and failing to distinguish pollution degree when SWI was used to assess the water environment quality of Xinan River.

#### The assessment results of multivariate analysis

The difficulty of biological monitoring in polluted

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rivers lies in the rare aquatic organisms<sup>[31]</sup>. The situation often occur that half of biological data in matrix data is zero. According to this characteristic, the choice of method to measure the similarity of the biological samples shall not be subject to the situation<sup>[32]</sup>. The MDS analysis based on the Bray-Curtis similarity coefficient, one of multivariate analysis, has been regarded as is a good method to deal with the problem<sup>[33,34]</sup>. When combined with Cluster analysis, another multivariate analysis method, the MDS analysis can better explore biological monitoring data in polluted rivers<sup>[18,20,21]</sup>.

The steps are as follows<sup>[35]</sup>. First of all, based on Bray-Curtis similarity measurement, hierarchical cluster of group average clustering method was used to draw the clustering tree diagram (Figure 3), and MDS was used to analyze. To make the results have more practical significance, MDS analysis results should compare with hierarchical clustering analysis results, and the corresponding cluster group should be sketched in MDS graph (Figure 4).

$$d_{ij}(B) = \frac{\sum_{a=1}^p |x_{ia} - x_{ja}|}{\sum_{a=1}^p (x_{ia} + x_{ja})} \quad (2)$$

$d_{ij}(B)$ —Bray-Curtis non-similarity coefficient of sample I and sample j;  $x_{ia}$ —the number of species individuals in position i;  $x_{ja}$ —the number of species individuals in position j;  $p$ —total number of species

Results of Cluster analysis were shown in Figure 3. The stations could be divided into four groups: group 1 included the stations of F, J, and D; group 2 included the stations of C and E; group 3 included station B; group 4 included station A. The analysis results of MDS were shown in Figure 4. The results were divided into 4 groups according to their position in the diagram: group 1 included the stations of F, J, and C; group 2 included the stations of D and E; group 3 included station B; group 4 included station A. The grouping results are similar to the results of Cluster analysis and the slight difference lies in the classification of station C, and D. Although the MDS results also fail to distinguish station F and J (station F and J nearly overlap in Figure 4), Cluster analysis distinguish subtle difference between them (Figure 3). According to the above mentioned

results of multivariate analysis, the pollution degree of various stations from heavy to light was:  $J > F > C > D > E > B > A$ . The results distinguish pollution degree between station J and F, station C and E; furthermore the misjudgment of station E may be corrected. Thus, multivariate analysis avoided the mistakes by SWI. In addition, the results of multivariate analysis are more intuitive to express by diagram. The reason why multivariate analysis does better than SWI lies in that it is based on sample similarity, which can judge the difference of pollution degree in various stations through similarity determination of community structure, making it capable to reflect the water environment quality within a wide range from clean water to polluted water body where the species are rare. However, there are still some problems of multivariate analysis, such as the integrity and information mining of different methods of multivariate analysis and the expression of the treat-

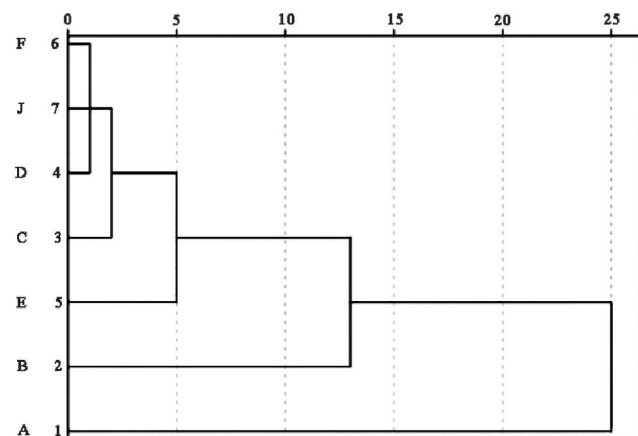


Figure 3 : Cluster dendrogram based on Bray-Curtis similarity measurement

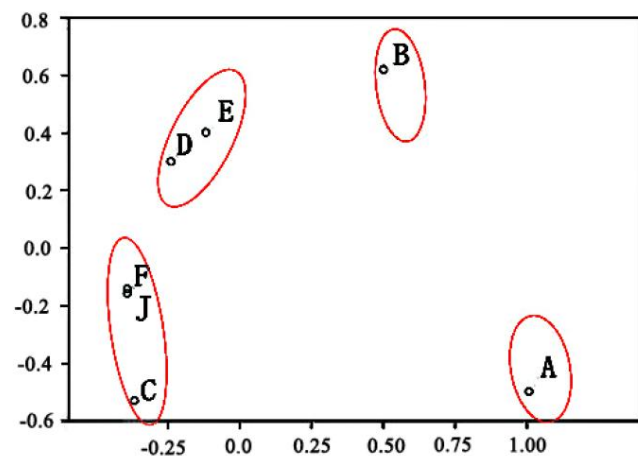


Figure 4 : The analysis results of non-metric multivariate scaling (MDS) of macrobenthos in the Xinan river

ment of abnormal values. Although multivariate analysis method is not perfect, the case in this paper show that the method is superior to the SWI in water environment quality monitoring in the polluted river.

### CONCLUSIONS

Analytical results indicated that the community structure of macrobenthos was highly associated with the water environmental conditions in the polluted river where hydrology and water quality present a huge spatial variation. Hence the community structure of macrobenthos may provide useful information for the water environment quality monitoring of the river. The comparison between SWI and multivariate analysis show the results of SWI cannot effectively reflect the difference of pollution status of the polluted river; despite the presence of some problems, multivariate analysis method is more suitable than SWI as far as information mining of biological monitoring in the polluted river is concerned.

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