Section Division of a Large River Main Stream Based on Bio-monitoring: A Case Study in the Dongjiang River, China

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The benthic diatom community and environmental factors were investigated in Dongjiang River, China. Section division of the main stream was then conducted by using diatoms based on these investigations. The value of bio-monitoring is also discussed. According to the benthic diatom and environmental factor data on 40 sites, the main stream was divided into 4 sections by using several analysis methods (clustering, multidimensional scaling (MDS), and self-organizing map (SOM)). The results of canonical correspondence analysis (CCA) showed the existence of a phosphorus restriction for the diatom community in the mid and downstream sections, while the distribution of diatom was mainly influenced by dissolved oxygen, pH, and geographical factors. A Biological Diatom Index (IBD) was also calculated, and the results showed that this type of section division meets the requirement of ecosystem health management in Dongjiang River.

Key words: Dongjiang River, bio-monitoring, diatom, SOM, river section division, river management.

Section division in river ecosystem management is a very sensitive issue because of the difficulty in ecosystem data analysis. Due to numerous biological and non-biological factors involved at each level of river ecosystem activities, there is a complex relationship between environment and biota[1]. How to obtain valuable information, to sort this from the vast biological and environmental data, and to realize the gathering use of dispersed data is a key problem in a river ecosystem study.

The "Convention on Biological Diversity" stipulates the obligations that the signatories had to accept in the monitoring, protecting, and sustainable utilization of bio-diversity. Bio-diversity had been said to be the ultimate standard of ecosystem health. The prerequisite for biodiversity conservation and improvement was to set up a full set of measurements with high performance, in order to evaluate and monitor biodiversity. The existing experience showed that using habitat clustering based on the habitat type and species occurrence in the environment design, monitoring, and evaluation had been proved to be feasible[2,3,4].

Under existing conditions of bio-diversity, a single species may become a useful indicator, and can be related to some other species[5]. Multivariate statistical analysis is considered very sensitive in the analysis community dynamics. Self-organizing map model (SOM)[6], cluster analysis (CA), principal component analysis (PCA), multidimensional scaling (MDS)[7,8], canonical correspondence analysis (CCA)[9], and other multivariate statistical methods have been widely used.

In 1982, the self-organizing network was

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proposed by Professor Kohonen. This could be effective in extracting and manifesting ecological information, and was widely used in engineering, agronomy and ecology data processing[6,10]. Since the 1980s, SOM has been widely used in the community pattern analysis of benthic macro-invertebrates in disturbed streams[1]. In the SOM result of macro-invertebrates, community mode includes community's parameter (species abundant, diversified index)[11], function group[12], grades[13], etc. Obach used SOM and radial base function (RBF) to predict abundance of aquatic insect population under specific environmental conditions[14]. Meanwhile, some other researchers successfully applied SOM in clustering analysis of benthic animals in different geographic regions of glacial rivers[15]. Nonparametric method was applied in the early 1980s. It was widely used initially in the study of marine bottom communities[16]. CCA, which was proposed by Harold Hotellin, is a nonlinear multivariate direct gradient analysis method, and its essence is the covariance matrix. CCA also combined multiple regression analysis and correspondence analysis. In the calculating process, environmental factors are regressed in every step in order to study the relationship between the species composition, site distribution, and environmental factors[9].

To sum up, ecologists and environmental managers demand the following: (1) a clear understanding of the environmental characteristics of the ecosystem studied, (2) sufficient quantitative data, including the biological taxa in a proper time and space, (3) solid classification and introduction of the biological taxa present, (4) understanding of the ecological consequence that the environmental change may produce. In China, river management is still in the physical-chemical water quality monitoring phase at present, and the management region mainly depends on the administrative regional division. Taking Dongjiang River (a tributary of the Pearl River Basin) as an example, traditional section division should consider its flow across Jiangxi and Guangdong provinces. Currently, benthic diatom-based monitoring had been applied in some sections of the Dongjiang River[17].

In this paper, an investigation was conducted on the aquatic organisms in the main stream of Dongjiang River. Due to the influence of dispersed habitat structure along the bank, on-site survey data (mainly physical, chemical and biological parameters) had always been more complicated, the section division becomes difficult to be interpreted and it is difficult to draw meaningful conclusions. The application of different multivariate statistical methods (e.g., self-organizing map model (SOM), cluster analysis (CA), principal component analysis (PCA), multidimensional scaling (MDS), canonical correspondence analysis (CCA), etc.) was helpful to draw conclusions from the complex data for a better understanding of the river water quality and ecological conditions, which is significant to the river section division[18,19]. On the basis of the investigation of Dongjiang River main stream, we uncover the response of different type organisms to river habitat, and evaluate the main stream biological health according to single taxa index, which will provide a basis for the study of river ecosystem health assessment in the future.

MATERIALS AND METHODS

Dongjiang River is located in the Pearl River Basin in southern China, sampling and bio-monitoring were conducted in the main stream (Fig. 1). Dongjiang River flows from northeast to

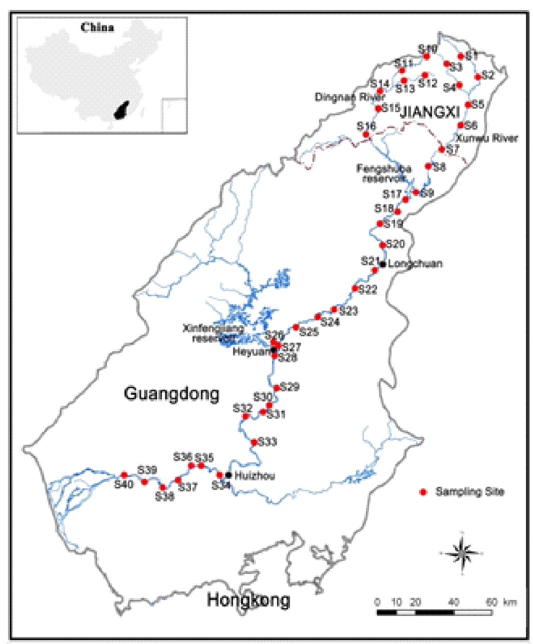

Fig. 1. The distributionof sample sitesin Dongjiang River

southwest, originated from Jiangxi Province, intersected in the Fengshuba reservoir with Dingnan River and Xunwu River. The river basin includes Longchuan, Heyuan, Huizhou, Dongguan and other cities extending to 562 km.

40 sites were set in the main stream of Dongjiang river (shown in Fig. 1). The investigation was conducted in October 2010, April 2011, April 2012 and November 2012. Benthic diatoms and environmental conditions were investigated in the first two survey, At each sampling site, latitude, longitude, altitude (Garmin eTrex H), pH, temperature, conductivity and dissolved oxygen (YSI ProPlus) were measured simultaneously. In the main stream of Dongjiang river, water samples were collected at the 40 sites for further chemical analyses in October 2010, April, 2011, May and November 2012. Suspended solids (filtration), total nitrogen spectrophotometric method), nitrate (spectrophotometric method), ammonium (spectrophotometric method), total phosphorus (spectrophotometric method) were measured. All these measurements followed Chinese reference methods20 (Table 1).

Table 1. Mean value of environment variables and quality status according to the Chinese assessment method (GB3838-2002, 2002)

Environmental index	Oct-10	Apr-11	May-12	Nov-12
Parameters mean (SD)				
T (°C)	25.06	25.40	25.15	18.56
	(2.30)	(2.29)	(1.99)	(2.10)
pH	7.88	7.87	7.33	7.00
	(0.35)	(0.41)	(0.65)	(0.54)
Conductivity (¼S/cm)	101.81	132.78	97.05	108.68
	(53.70)	(47.08)	(35.30)	(56.91)
DissolvedOxygen (mg/L)	5.62	5.01	5.50	5.82
	(2.02)	(2.22)	(2.09)	(2.39)
Total nitrate (mg/L)	3.28	2.15	2.51	2.46
	(1.08)	(0.66)	(0.87)	(0.77)
Nitrate (mg/L)	1.71	0.85	1.62	1.58
	(0.65)	(0.41)	(0.58)	(0.55)
Ammonia (mg/L)	1.25	1.02	0.83	0.70
	(0.72)	(0.62)	(0.42)	(0.57)
Total phosphorus (mg/L)	0.07	0.10	0.05	0.06
	(0.01)	(0.02)	(0.03)	(0.05)
Phosphate (mg/L)	0.05	0.07	0.02	0.04
	(0.03)	(0.01)	(0.02)	(0.03)
Chlorophyll *a* (mg/L)	7.07	N.D	4.82	3.25
	(12.64)		(3.08)	(2.83)
Parameters scope				
Distance (km)	0-460.41			
Altitude (m)	0-309			

N.D., not determined

Diatoms were ultimately considered to be the most appropriate bio-indicator[17]. Diatom samples were collected according to a semi-quantitative field protocol followed Stevenson and Bahls[21] in October, 2010 and April, 2011, as the application of standardized methods was possible for all river types (shallow/deep rivers; good/poor water quality). Water samples were collected in the same time and site. At each sampling site, five cobble-sized stones were chosen as preferred substrata randomly. In exceptional cases where there were no suitable-size stony substrata, we sampled on artificial hard substrata, such as concretes and piersor two dots. The samples were pooled into a composite sample subsequently. Phase contrast microscopy had been used in benthic diatom identification; species identification and silicon shell number counting were conducted under the resolution of 1000. Diatom species was identified according to KLB classification system and standard method provided by American Patrick environmental research center[22-25]. As the miniature diatom was difficult to be identified with optical microscope, the scanning electron microscopy was used.

Before analysis, the data must be transformed (logarithmic transformation: log 10 (x + 1)). In the clustering analysis of environmental data, it is necessary to calculate the Euclidean distance of environmental factors, The sites were grouped by clustering analysis of group average as well.

Multidimensional scaling (MDS) was used to examine relationships between river sites and diatoms. MDS was performed based on Bray–Curtis similarities using log 10 (x + 1) abundance data with species frequency greater than 12.5% at each site, and relative density of species higher than 1% at least one site. Finally, the sites were grouped and MDS distribution map was drawn. Confidence level was determined according to the stress coefficient of MDS. Significant differences between groups of sites were tested by single factor ANOSIM analysis. CCA analysis is mainly used to verify the response relationship between environmental factors and benthic diatoms. The Biological Diatom Index (IBD) was calculated with reference to Wang's method[26].

Self-organizing map network methods were used for zoning utilizing[27]. In this study, the majority of non-parametric multivariate analysis, namely cluster analysis, MDS, PCA, ANOSIM and RELATE test, were conducted using software package PRIMER 5 which was developed by Plymouth laboratory of Britain[28] CCA was completed by ecological data processing software CANOCO for Window 4.5.

RESULTS AND DISCUSSIONS

The environmental factor clustering of 40 sample sites of main stream of the Dongjiang River, was was used to divide the river into three groups (Group A: S1–S16, Group B: S17–S33; Group C: S34–S40) (Fig.2). According to Fig. 1, the sites of group C were located in the downstream section of the Dongjiang River. Group A, which includes

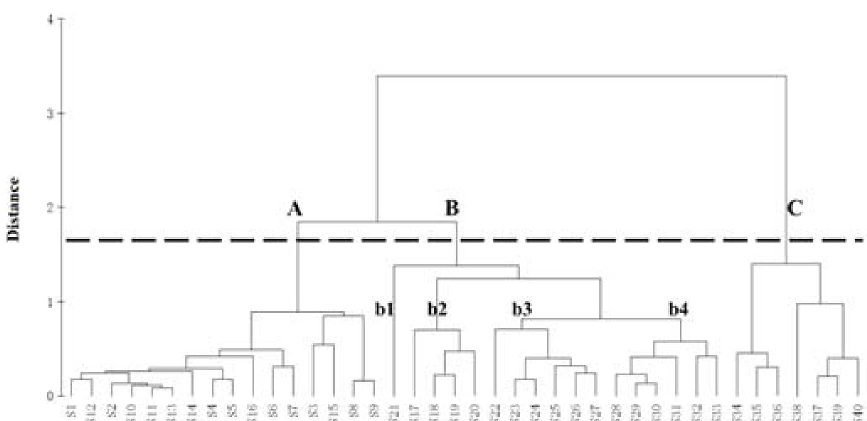

Fig. 2. Cluster analysis of sample sites based on environment variables (Euclidean distance; Primer)

two wadeable rivers (Dingnan River and Xunwu River) and belongs to the headwaters area of the Dongjiang River. The sites of group B cover the main stream of Dongjiang in the upstream and midstream, and are further subdivided into four groups (b1, b2: S17–S20, b3: S22–S27, b4: S28–S33). However, this does not clearly show differentiation degree of group B, using Euclidean distance cluster difference between midstream and upstream was not obvious.

From the river management perspective, Dongjiang River is currently still at the stage of physical-chemical water quality monitoring; therefore, it is essential to divide the main stream of the Dongjiang River into sections according to the environmental factor of water quality. However, this involves the problem of whether clustering analysis of environmental factors can reflect the true state of the ecosystem of a large river. From the results of this study (Fig. 2) and the geographical distribution analysis, the environmental factor cluster analysis of Dongjiang River, which divide the main stream into three major sections showed that these sections can be summarized as the headstream (Group A), upstream and midstream (Group B), downstream (Group C) sections. Whether the segmentation is accurate or not needs to be confirmed by organism distribution.

Cluster analysis results for benthic diatoms at different sites according to the Bray-Curtis similarity analysis are shown in Fig 3-a. At the 77% level of similarity, the sites could be divided into five groups, among which, group B and C included only one site. Group A, B, and C included the sites of S1–S16, which belong to the headwaters range, and group E included sites S34–S40, belonging to the main stream of the downstream section of Dongjiang River. Group D

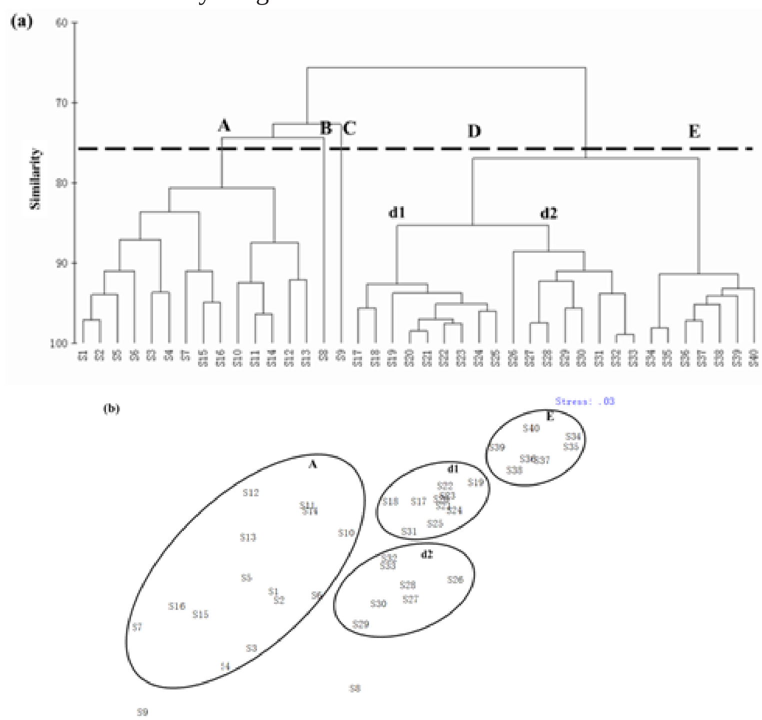

Fig. 3. Cluster and MDS analysis of benthic diatomsamong sitesin the Dongjiang River. (a):Cluster analysis result, (b):MDS analysis result

was subdivided into two groups—d1 (S17–S25) and d2 (S26-S33)—at the 85% similarity levels. These two sites are attributed to the upstream and midstream segments of Dongjiang River respectively. ANOSIM analysis of all diatom showed significant differences among the three groups A, D, and E ($p < 0.01$), and a significant difference between groups d1 and d2 ($p < 0.05$). MDS analysis of the cluster result showed stress coefficient = 0.03 (Fig. 3-b).

The algae community showed complicated response to environmental impact, and they are also important ecosystem indicators. The algae have an extremely important effect on elementary productivity and ecological integrality of the ecosystem. Therefore, they are used as a principal index for water quality monitoring in the aquatic ecosystem[29]. Research on the use of biological or environmental cluster analysis had been proved to be effective[30,31]. In this study, benthic diatoms and environmental factors were considered in the section division of the Dongjiang River. The results (Fig. 3) showed that benthic diatoms with biological similarity in Dongjiang

River can be divided into two sections, namely, the wadeable river of the headstream section and the non-wadeable river segment. Meanwhile, the non-wadeable river was further divided into upstream, midstream, and downstream. Clustering results based on diatoms division are close to the real situation, which is concerned with the advantages of biological partition. This is because the biota can reflect not only environmental factors, but also the real situation where habitat conditions are objectively reflected[31]. The RELATE analysis of benthic diatom community structure and environmental factors indicated that in Dongjiang River, benthic diatom distribution and environmental factors were significantly correlated ($R^2 = 0.527$, $p = 0.001$).

Table 2.1st and 2nd principal components of the PCA results (2009–2012).

Parameter/PC	1st PC	2nd PC
Distance	0.93	0.28
Altitude	0.85	0.27
T	-0.26	-0.16
DO	-0.02	0.72
Con	-0.15	-0.24
pH	0.13	0.73
Chla	-0.12	0.01
TP	0.87	-0.21
TN	0.77	0.30
NO_3-N	-0.28	-0.14
PO_4-P	0.80	-0.05
NH_3-N	0.82	-0.12
Explained variance	4.55	2.05
Proportion of total	0.405	0.253

The impact of environmental factors on the distribution of benthic diatoms was verified by principal component analysis. The main environmental factors using 2009–2010 data (table 2), are higher than 0.70, selected only in main composition factors which carry on the environmental variable CCA analyzes in the next step. The first principal component ordination axis explains 40.5% of the total amount of the data. Distance, elevation, total nitrogen (TN), total phosphorus (TP), orthophosphate (PO_4-P) and ammonia nitrogen (NH_4-N) were significant environmental factor loadings ($p < 0.05$). The second axis explained 25.3% of the total variation, and dissolved oxygen (DO), and pH were significant principal environmental factor loadings ($p < 0.05$). The total accumulative explanation of the two axes was 65.8%.

The maximum length of the ecological gradient is 3.503 (>3) in the first two axes in DCA analyzes, indicating that the benthic diatom community and ecological gradient had a non-linear uni-modal response relationship in the main stream of the Dongjiang River. Therefore, CCA was selected to study the relationship between environmental factors and diatom distribution. CCA analysis showed (Table 3) that the characteristic value of two axes was 0.380 and 0.233 respectively and that the coefficient correlation between species and environmental gradient was 0.870 and 0.767 respectively. This indicates that the ordination diagram provides a good reflection of the relationship between diatom distribution and environmental factors. Meanwhile, the first and second CCA axes explained 58.4% of the diatom community variation. The CCA result of the site relationships between benthic diatoms and environmental factors showed (Fig. 4) that group A and B contained the sites S1–S25. Group A was significantly influenced by distance, altitude, and pH, while group B's site distribution was correlated with TN. Groups C (S26–S33) and D (S34–S40), belonged to midstream and downstream sections, and benthic diatom distribution was positively correlated with TP and PO_4-P at these sites.

Table 3. Eigenvalues for CCA axis and species-environment correlation

Axes	1	2	3	4
Eigenvalues	0.380	0.233	0.160	0.133
Species-environment correlations	0.870	0.767	0.821	0.651
Cumulative percentage variance of species data	13.4	21.6	27.2	31.9
Cumulative percentage variance species-environment relation	36.2	58.4	73.7	86.4

CCA analysis was used to analyze the response relationships between environmental

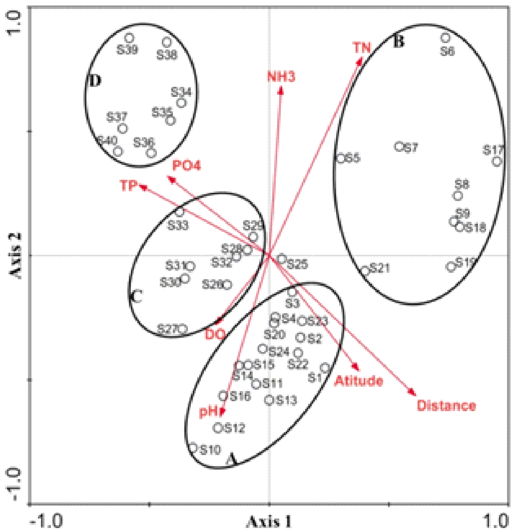

Fig. 4. CCAbiplot of benthic diatoms site and environmental variables in Dongjiang River (2010-2011)

factors and benthic diatom communities in different sections. The results showed that the diatom communities in different sections of Dongjiang River was commonly affected by water quality and geographical factors, which are similar to the finding of other studies[32-34]. Unrestricted Monte Carlo permutation tests and randomization indicated that the first CCA axis reflected the change gradient of phosphorus. In the downstream section, phosphorus was the main factor influencing diatom community structure. This result is consistent with the results of many studies[34,35]. Nitrogen in this section was relatively high, causing the imbalance between nitrogen and phosphorus, which was an important reason for the algae phosphorus limits[36]. The second CCA axis was a gradient axis of geographical factors and dissolved oxygen and pH in the river. Many studies have proved that the diatom respond sensitively to the acidity and alkalinity of rivers, and might indicate pH variation of rivers[37]. Leira et al. studied the structural influence factor of the diatom in rivers, and confirmed that the nutrient component of water was an obvious influencing factor[33]. The larger the watershed scale is , the more differences in water quality will be caused by geographical factors. This will influence the distribution of diatom communities[38]. The results of this study (Fig. 4) showed that, the diatom distribution in the headstream and upstream sections were mainly influenced by functional factors such as the geographical factor, dissolved oxygen, pH, etc. This indicated that the environment in these sections suffered only slightly from human disturbance, and that the distribution of benthic diatoms still existed under natural conditions. However, in the midstream and downstream sections, due to increasing human activities, high nitrogen concentrations directly affect the diatom distribution.

SOM was used in cluster analysis based on the relative abundance of benthic diatoms in these sites. The result (Fig. 5) showed that all the

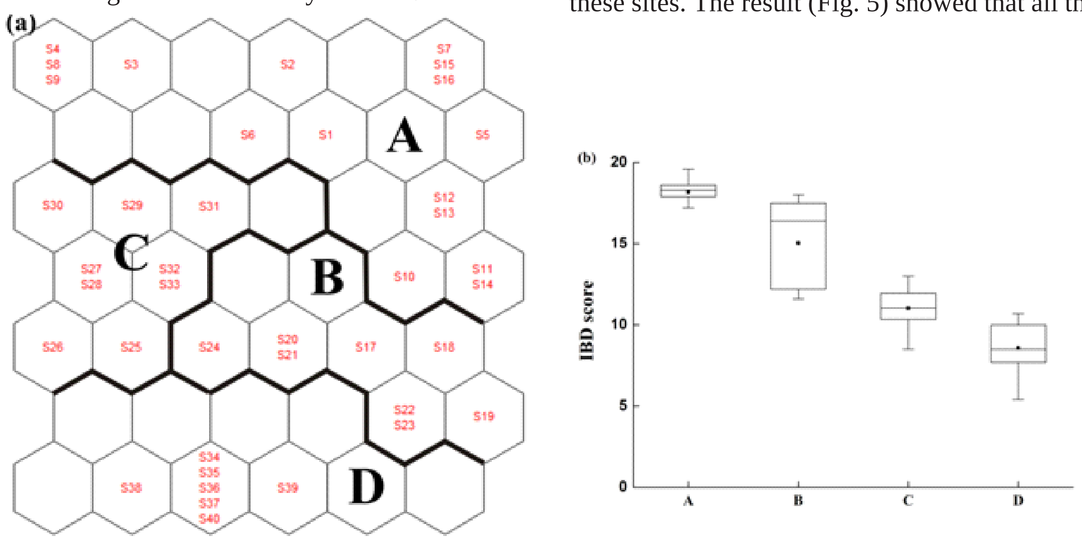

Fig. 5. The map trained by SOM for pattering benthic diatoms reported from DongjiangRiver: (a) sample sites; (b) box-plot of IBD scores in different clusters defined in the SOM

sites were divided into 4 groups. Group A included S1–S16, which belongs to the headstream section. Group B included site S17–S24, belonging to upstream section. Groups C and D included the sites of S25–S33 and S34–S40 respectively, which belonged to the section of midstream and downstream. From the classification results, the IBD value was the highest in the headstream, and there was a significant difference between these sections ($p < 0.05$).

SOM is widely used for benthos cluster analysis in different areas of the river[15]. In this study, diatom distribution based SOM analysis was conducted. The results combined with the site geographic distribution clearly showed that benthic diatoms community was divided into four regions in Dongjiang River (Fig. 5). The Xunwu River and Dingnan River belonged to wadeable section of the headwaters. The upstream section was from the Fengshuba reservoir to Longchuan; the midstream segment was from Longchuan to Heyuan; the downstream section was from Huizhou to Dongguan. The benthic fauna community was clustered by simulating the temporal-spatial scale change according to community development trend. Some researchers have analyzed the long-term data of benthic diatoms to reflect the impact of natural or artificial activities on large-scale river environments[39]. The results of clustering, further confirmed the accuracy of the aforementioned section division. Meanwhile, the results of IBD confirmed that large differences exist among the different sections in the Dongjiang River, which provided a new monitoring mode and section division method.

Statistical analysis was used to analyze the relationship between biological and environmental factors in the main stream of Dongjiang River. Section division was conducted using environmental factors and benthic diatom. The results were as follows: (1) environmental factor analysis showed that high nutrient concentration was the most important factor in the downstream section, and four sections were divided by clustering analysis; (2) clustering analysis using diatom showed similar results to the environmental factors analysis. CCA results showed phosphorus limitation in the diatom community in mid-downstream section, which indicates an obvious imbalance in the N:P ratio in this section. This was due to the high N concentration. In the upstream and headstream sections, the diatom distributions were mostly influenced by geography factors, DO, and pH, which was consistent with the river environmental section division results; (3) a similar result was shown in the SOM clustering analysis using benthic diatoms, and the IBD score of benthic diatoms is consistent with the mainstream section division results.

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