

Article

# Assessment of Aquatic Ecosystem Health of the Wutong River Based on Benthic Diatoms

Hao Xue <sup>1,2</sup> , Binghui Zheng <sup>1,2,\*</sup> , Fansheng Meng <sup>2,\*</sup> , Yeyao Wang <sup>1,3</sup>, Lingsong Zhang <sup>2</sup> and Peixuan Cheng <sup>2</sup>

<sup>1</sup> College of Water Sciences, Beijing Normal University, Beijing 100875, China; xh715810629@163.com (H.X.); yeyao wang@163.com (Y.W.)

<sup>2</sup> Chinese Research Academy of Environmental Sciences, Beijing 100012, China; zlingsong@163.com (L.Z.); cheng\_peixuan@126.com (P.C.)

<sup>3</sup> China National Environmental Monitoring Centre, Beijing 100012, China

\* Correspondence: zhengbinghui@craes.org.cn (B.Z.); mengfs@craes.org.cn (F.M.); Tel.: +86-10-849-15178 (F.M.)

Received: 23 March 2019; Accepted: 5 April 2019; Published: 8 April 2019



**Abstract:** The community structure of benthic diatoms and water environmental characteristics were extensively investigated to assess the aquatic ecosystem health of the Wutong River (Heilongjiang Province, China). Several diatom indices were calculated, and a benthic diatom index based on biotic integrity (BD-IBI) was developed. Principal component analysis (PCA), Spearman correlation analysis (CA), cluster analysis, redundancy analysis (RDA), and the box plot analysis were used to analyze the benthic diatom communities, assess the river ecosystem health, and compare the applicability of different indexes. The results indicated that *Gomphonema parvulum* and other tolerant species were the dominant species. Meanwhile, most sites were in “poor” or “very poor” condition according to the diatom indexes evaluation, indicating that the river has been disturbed by human activities. The sampling sites of the Wutong River were divided into three groups based on different pollution levels. The derived BD-IBI included four individual metrics of different aspects, showed strong distinguishability for three grouping and robust correlation with environmental variables. Of all the indexes selected, IBI performed the best, followed by the species-level diatom indexes and the genus-level diatom indexes.

**Keywords:** benthic diatom; diatom index; index of biotic integrity; Wutong River

## 1. Introduction

Human disturbance has severely affected the health of river ecosystems, which leads to the significant degradation of biodiversity and ecosystem integrity [1]. As a result, river ecosystem health assessment is attracting more and more attention from scholars globally, and a large number of aquatic ecosystem health assessment methods have been reported [2]. There are now many approaches for assessing the health of freshwater systems that use biological communities, such as fish, macrophytes, macroinvertebrates, and algae [3–5]. Algae is considered to be more efficient than other biological communities as it has a shorter generation time than fish and macroinvertebrates and responds rapidly to environmental changes [6–8].

Benthic diatoms are single-celled microscopic algae that possess an ornamented cell wall composed of silica (SiO<sub>2</sub>). As an important part of aquatic resources and river ecosystems, benthic diatoms are a good indicator of water quality changes [9] and human disturbance activities [10]. Several studies on the performance comparison between a single assemblage (diatoms, or soft algae that include cyanobacteria) and a combination of diatoms and soft algae (“hybrids”) have been published. Kelly et al. [11] reported

that benthic soft algae did not improve the predictability of chemical composition in lakes compared to benthic diatoms. Schneider et al. [12] reported that benthic diatoms reflected environmental changes faster than other benthic algae. Fetscher et al. also considered the diatom index of biotic integrity (IBI) a more appropriate method for routine monitoring applications, because less information on bioindicator development and performance is available for soft algae than for diatoms [13]. Therefore, benthic diatoms were selected as bioindicators of the Wutong River in our research.

According to Stevenson et al. [14], diatom-based autecological index (diatom index) and IBI are two basic methods to assess environmental conditions in rivers and streams using diatoms. Rimet et al. [10] observed that many biotic indexes were developed before 1999 in Europe, Australia, and America. Meantime, a few diatom indexes have been developed in Asia. Wu [15] developed a generic diatom index and tested it successfully in Taiwan, China. Watanabe et al. [16] developed the diatom assemblage index of organic pollution (DAIPo) in Japan. Between 1999 and 2009, numerous studies reported the applications of diatom indexes in neighboring countries or very different regions from the area they were created. Most diatom indexes were successfully validated [17,18], but a few indexes have not achieved the desired effect [19]. Diatom indexes developed in Europe and Japan were applied and verified successfully in the Pearl River Basin, Guangdong, China, but the authors deemed that the diatom indexes needed further adjustment [20]. Benthic diatom-based indexes of biotic integrity (BD-IBI) have been developed and applied in ecosystem health assessment in America, Europe, and Asia [2,21]. Although, BD-IBI has been applied and achieved good results in river ecosystem health monitoring and assessment in China for the past few years, there were no similar reports in the Songhua River basin [19,22]. The Wutong River is a tributary of the Songhua River, in which obvious agricultural pollution and human disturbance occurs. The main objectives of this study were to (1) analyze the benthic diatom communities, (2) assess the river ecosystem health using diatom indexes and BD-IBI, and (3) compare the applicability of different indexes in the Wutong River.

## 2. Materials and Methods

### 2.1. Study Area and Site Locations

The Wutong River (47°10′–47°55′ N, 130°8′–130°48′ E) is a tributary of the Songhua River. The river originates from the Zhewen Mountain in Xiao Hinggan Mountains and drains a total length of 357 km. Most of the area of Wutong River basin belongs to the administrative region of Hegang city, Heilongjiang Province, with a basin area of 4516 km<sup>2</sup>. The Wutong River Basin lies within a north temperate monsoon climate zone, and the temperature and rainfall vary significantly during the year, with the warmest month being July (20–25 °C) and the coldest January (−20 °C). Annual precipitation averages 615.2 mm, 60 to 70% of the annual precipitation occurs from July to August. In this study, a total of 13 sampling sites were selected (Figure 1).

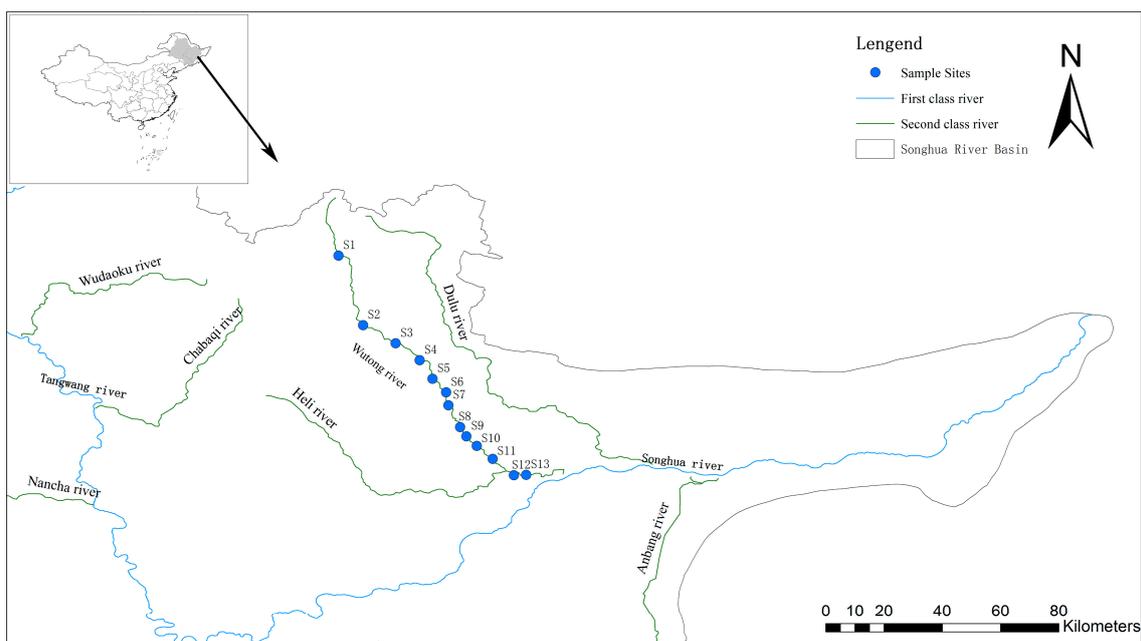


Figure 1. Sampling sites in Wutong River.

## 2.2. Diatom Sampling and Identification

Diatom sampling and water quality analysis were carried out in the Wutong River in July and September 2016. Diatoms were collected from all available habitats. Three representative stones (diameter < 25 cm) were collected from each section. A fixed circular area (diameter = 3 cm) was scrubbed from each of the three rocks, and periphyton was rinsed with distilled water into one wild-mouth plastic bottle as a replicate. A 100 mL sample was then preserved in 4% formaldehyde. Diatoms were mounted with Naphrax™ (Robert Charles laboratories ltd., Bedfordshire, UK) after organic material was removed with acid ( $\text{HNO}_3$  and  $\text{H}_2\text{SO}_4$ ), formaldehyde and acids were washed off with deionized water by centrifugation [8]. Species were identified, and a minimum of 400 valves was counted per slide at  $1000\times$  magnification under microscope (Olympus BX51, Olympus, Tokyo, Japan) [23].

## 2.3. Physical and Chemical Analysis

Water samples were collected from the same sites simultaneously with benthic algae samplings in the river network (Figure 1). Water temperature (Temp), pH, conductivity (Cond), dissolved oxygen (DO), total dissolved solids (TDS), salinity (SAL), and oxidation–reduction potential (ORP) were measured in situ with a multiparameter instrument (YSI Professional Plus, Yellow Springs, OH, USA). Water width, channel width, and current velocity (Velo) were also measured at each site, and the ratio of water width to channel width (Ratio) was calculated. Two liters of water were collected in pre-cleaned plastic containers to measure chemical variables, including total nitrogen (TN), ammonia nitrogen ( $\text{NH}_3\text{-N}$ ), total phosphorus (TP), and phosphate (Phos), using the spectrophotometric method [24]. Samples were stored in the dark at  $4\text{ }^\circ\text{C}$  until the measurement in the laboratory. A qualitative habitat evaluation index (QHEI) developed in China was calculated to evaluate the habitat quality of each site, meanwhile, as an evaluation index in QHEI, the score of the bottom material (Bott) was recorded separately for analysis [25].

## 2.4. Development of the Diatom Index of Biotic Integrity (D-IBI)

### 2.4.1. Selection of Reference Sites

There were no records from earlier times in the Wutong River, and almost all the sampling sites were disturbed by human activities. Therefore, the “least disturbed condition” based on water quality and QHEI was selected as the reference condition in this study [26].

### 2.4.2. Candidate Metrics

Twenty-three metrics were calculated according to the definition, including seven widely used diatom indexes. Most of these metrics have been used as candidate parameters by Xiang et al. [22]. Metrics were classified into 4 categories, i.e., biotic diatom indexes, taxonomic composition, growth form, and diversity. Their details and references are listed in Table 1.

**Table 1.** Attribute and description of candidate metrics of benthic diatom index of biotic integrity (BD-IBI) in Wutong River.

Code	Candidate Metrics	Taxonomic Level	Response to Disturbance	Descriptions and References
<b>Biotic diatom index</b>				
M1	Diatom Bioassessment Index (DBI)	Species	Decline	Yin et al. [27]
M2	Trophic Diatom Index (TDI)	Species	Decline	Kelly and Whitton [28]
M3	Biological Diatom Index (BDI)	Species	Decline	Coste et al. [29]
M4	Pollution Tolerance Index (PTI)	Species	Decline	Muscio C [30]
M5	Generic Index of Diatom (GI)	Genus	Decline	Wu [15]
M6	Diatom Index for Australian Rivers (DIAR)	Genus	Decline	Chessman et al. [31]
M7	Diatom Species Index of Australian Rivers-unweight (DSIAR-uw)	Species	Decline	Chessman et al. [32]
M8	Diatom Species Index of Australian Rivers-weight (DSIAR-w)	Species	Decline	
<b>Taxonomic composition</b>				
M9	Total diatom density	Species	Decline	Total density of benthic diatoms
M10	% <i>Achnanthes</i>	Genus	Decline	Relative abundance of <i>Achnanthes</i>
M11	% <i>Cymbella</i>	Genus	Decline	Relative abundance of <i>Cymbella</i>
M12	% <i>Nitzschia</i>	Genus	Rise	Relative abundance of <i>Nitzschia</i>
M13	% <i>Navicula</i>	Genus	Rise	Relative abundance of <i>Navicula</i>
M14	% <i>Gomphonema parvulum</i>	Species	Rise	Relative abundance of <i>Gomphonema parvulum</i>
<b>Growth form</b>				
M15	Kinetic %	Genus	Rise	Relative abundance of Kinetic genera
M16	Handle %	Genus	Decline	Relative abundance of Handle genera
M17	Sensitive %	Species	Decline	Relative abundance of Sensitive genera
M18	Tolerance %	Species	Rise	Relative abundance of Tolerance genera
<b>Diversity</b>				
M19	Jaccard Index (JI)	Species	Decline	Toporowska et al. [33]
M20	Shannon diversity	Species	Decline	
M21	Pielou index	Species	Decline	
M22	Diatom species richness	Species	Decline	Number of species in the count
M23	Diatom genus richness	Genus	Decline	Number of genera in the count

### 2.4.3. Selection of Metrics

First, metrics with medians of 0 were eliminated from the 23 candidate metrics because they would decrease the separating capacity. Second, metrics with unreasonable trends in response to environmental factors and low separation power (<2) were excluded. For the positive metrics (the better the environment, the higher the score), if the score of the reference group is lower than that of the impaired group, the metrics are considered unreasonable, and vice versa. The separation power was defined as the degree of overlap between boxes (i.e., 25th and 75th quartiles) in the box plot between the impaired and reference sites. If the two boxes did not overlap, the separation power was defined as 3. When the interquartile ranges overlapped but did not reach medians, a value of 2 was assigned.

A value of 1 was given to an attribute when only one median was within the interquartile range of the other box, while a value of 0 was assigned when both medians were within the range of the other box. Finally, Spearman's rank correlation analysis was carried out to test redundancy. Pairs of the metrics with strong correlations ( $r > 0.65$ ,  $p < 0.05$ ) were considered redundant. The redundant metrics were then selected based on their variation [22].

#### 2.4.4. Calculation of IBI

Metrics that passed all the screening procedure were selected for inclusion in the BD-IBI and scored on a 1 to 10 scale, using the following equation and conditions [34].

$$M_s = A + B \times M_r \quad (1)$$

$$\text{If } M_r = M_{\min}, \text{ then } M_s = 1$$

$$\text{If } M_r = M_{\max}, \text{ then } M_s = 10$$

where the standardized metric ( $M_s$ ) was calculated from the raw metric ( $M_r$ ) using a linear function with intercept ( $A$ ) and slope ( $B$ ). For the positive metrics,  $M_{\min}$  was equal to the minimum value of  $M_r$  while  $M_{\max}$  was equal to the maximum value of  $M_r$ . For the negative metrics,  $M_{\min}$  was equal to the maximum value of  $M_r$  while  $M_{\max}$  was equal to the minimum value of  $M_r$ . IBI score was obtained by summing all metric scores.

#### 2.5. Statistical Analysis

In this study, McNaughton dominance index of all species was calculated, species with a dominant degree greater than 0.2 were identified as dominant species, as follows:

$$\text{McNaughton dominance index} = (n_i/N) \times f_i \quad (2)$$

where  $n_i$  = the total number of cells in species  $i$ ,  $N$  = the total number of cells in all species,  $f_i$  = frequency of occurrence of species  $i$ .

A principal component analysis (PCA) on the log transformed environmental variables ( $\log x + 1$ ) was performed to identify the main environmental factors. Variables with a low factor loading (1st axis) were rejected [35]. Correlation analysis (Spearman rank) on the reserved variables was performed, and redundant variables were removed. Relative abundance data of diatom community composition were arcsine square root transformed to reduce skewness. Nonmetric multidimensional scaling (NMDS) was used to reduce dimensions of diatom data. Then, a Ward clustering of the Bray–Curtis matrix was computed, three groups were extracted and the sites according to them were colorized. Redundancy analysis (RDA) was performed on the relative abundance data of diatoms and major environmental factors. Finally, based on the grouping results of Ward clustering, the box plot analysis was implemented to analyze the adaptability of different indexes. PCA and RDA were performed in R with the function "rda" from the "vegan" package, correlation analysis was performed in R with the function "cor", NMDS was performed in R with the elegant function "metaMDS" from the "vegan" package, Ward clustering was performed in R with the function "hclust". All analyses above were performed by using the R platform (version 3.5.2).

### 3. Results and Discussion

#### 3.1. Diatom Community Structure

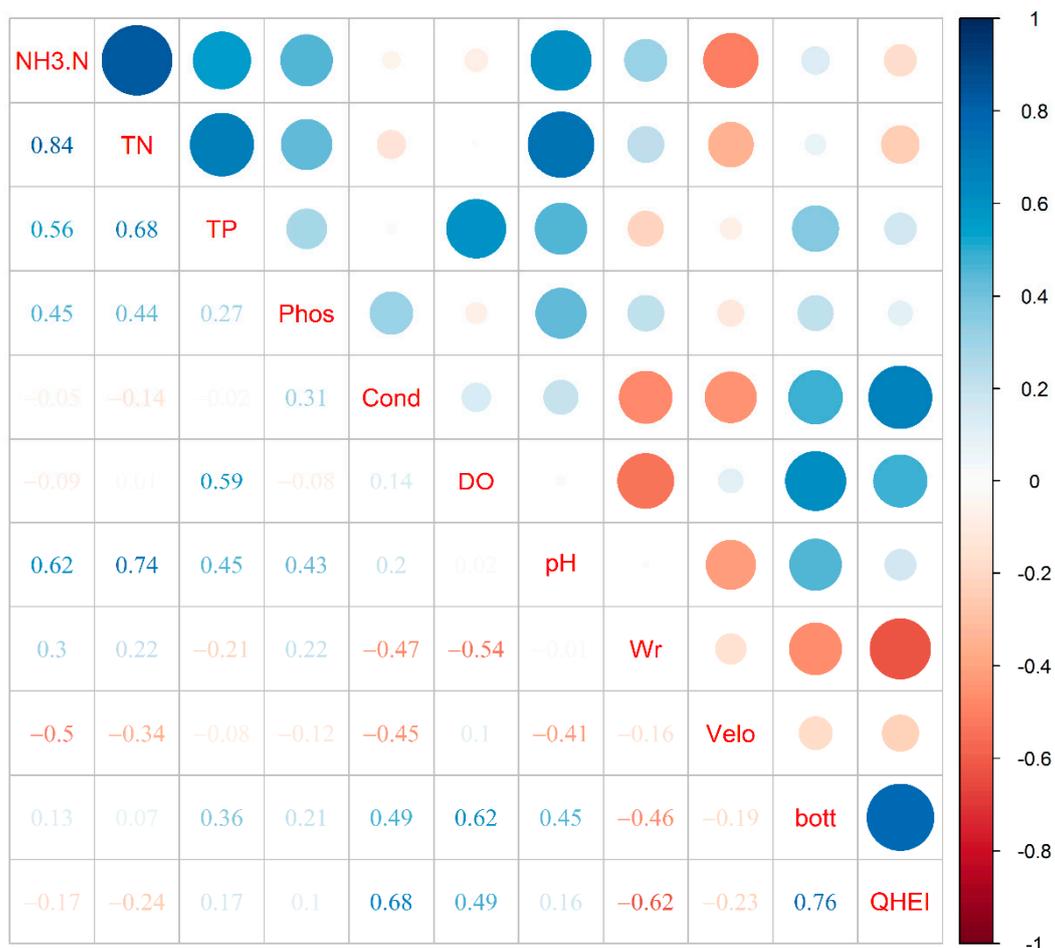
In the Wutong River, a total of 122 diatom species belonging to 29 genera were recorded in 13 diatom samples collected. The species richness varied from 18 to 54 with an average of 29. The absolute dominant species of diatom in Wutong River was *Gomphonema parvulum* (Kützing) Kützing (Table 2), which is considered as organic pollution-tolerant species [28] with a fairly low pollution

tolerance value [30]. Previous researches have shown that *G. parvulum* was often found in highly disturbed, organic enriched water, and it can be used as an indicator species for river eutrophication [36]. In addition, *Gomphonema angustatum* (Kützing) Rabenhorst, *Cymbella naviculiformis* Auerswald, *Cymbella sinuata* Gregory, and *Melosira varians* Agardh are also indicators of mild or moderate pollution [28,30]. In summary, benthic diatoms in the Wutong River were dominated by the pollution-tolerant species, which indicates that the river was at a poor health status and might have organic pollution or other human disturbance.

**Table 2.** Dominant species of benthic diatom in the Wutong River.

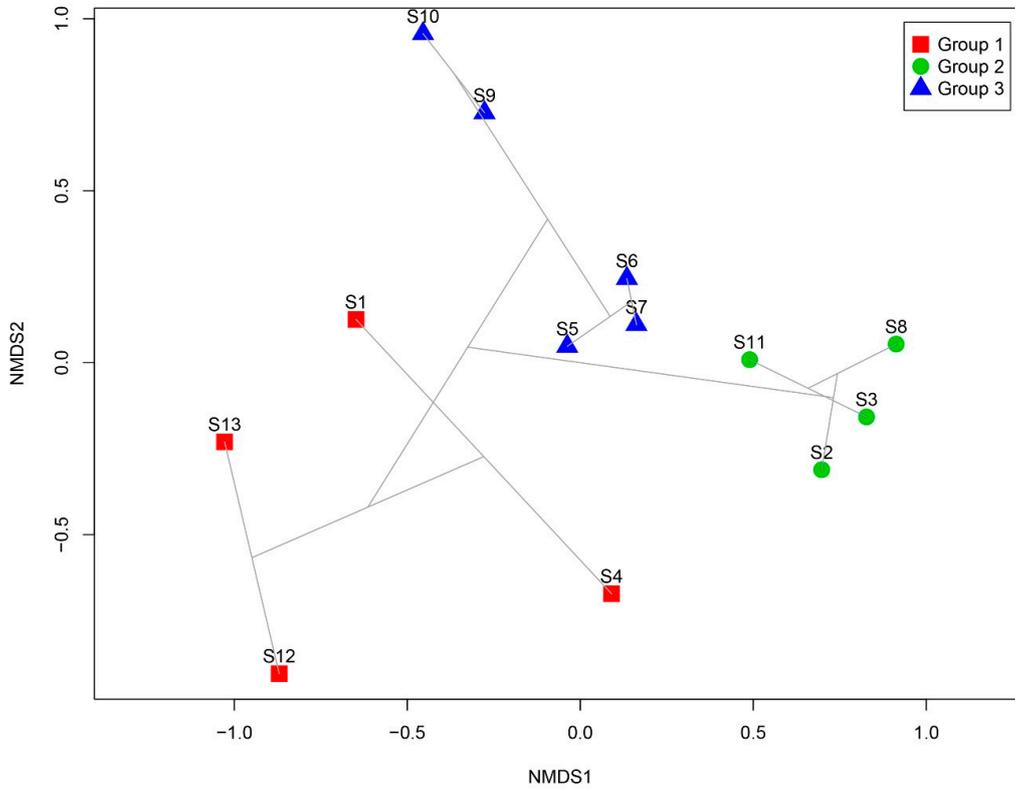
Taxon	Authority	Dominance Index
<i>Gomphonema parvulum</i>	(Kützing) Kützing	0.21
<i>Cymbella naviculiformis</i>	Auerswald	0.09
<i>Cymbella sinuata</i>	Gregory	0.09
<i>Fragilaria capucina</i>	Desmazières	0.04
<i>Gomphonema angustatum</i>	(Kützing) Rabenhorst	0.03
<i>Melosira varians</i>	Agardh	0.03

Eleven major environmental factors were retained based on PCA results. Correlation analysis was performed for the above 11 environmental factors (Figure 2), and seven environmental factors with weak correlation (NH<sub>3</sub>-N, TP, Phos, Cond, DO, pH, and Velo) were selected for RDA with diatom community data.

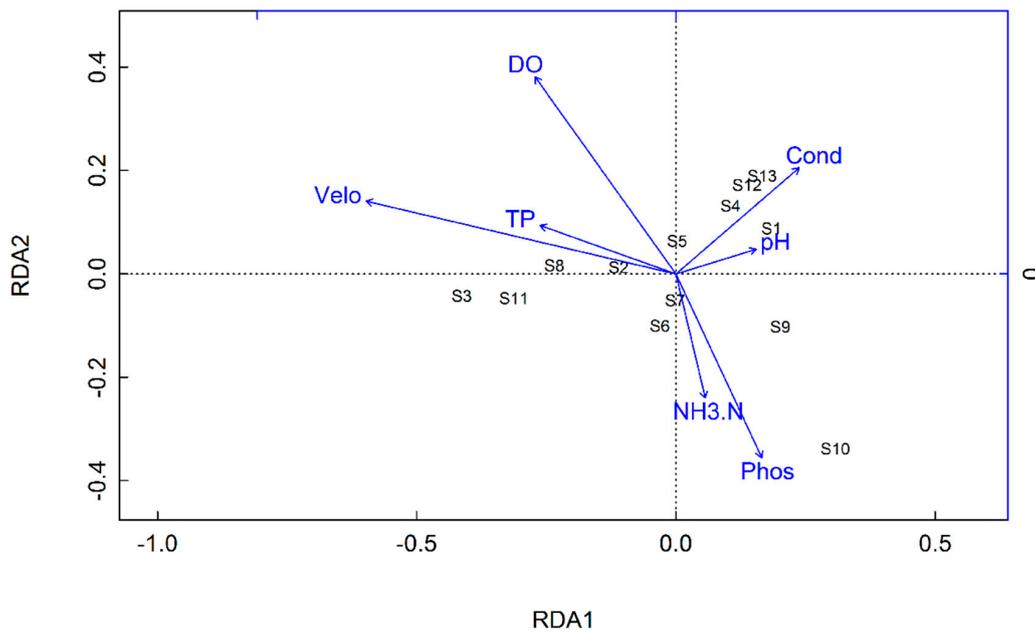


**Figure 2.** Spearman's rank correlation coefficient chart of 11 environmental factors.

All the sites were classified into three groups according to the cluster analysis (Figure 3). RDA results (Figure 4) showed that the main environmental factors of Group 1 (S1, S4, S12, and S13) were Cond and pH, Group 2 (S2, S3, S8, and S11) were Velo and TP, Group 3 (S5, S6, S7, S9, and S10) were NH<sub>3</sub>-N, Phos, and DO.



**Figure 3.** Ward clustering result based on the nonmetric multidimensional scaling (NMDS) ordination plot.



**Figure 4.** Ordination diagram of redundancy analysis (RDA) based on benthic diatom communities and environmental factors.

The communities of diatoms in Group 1 was relatively stable, and the average value of the Shannon diversity index was 3, which was the best ecological condition among the three groups. Diatom communities characterizing the sites in Group 3 included medium to high tolerant taxa, such as *Gomphonema parvulum*, *Cymbella naviculiformis*, and *C. sinuata*. The Shannon diversity index of Group 3 was slightly higher than Group 2, belonging to the mild-moderate pollution group. Sites in Group 3 were slightly eutrophic, with high concentrations of NH<sub>3</sub>-N and Phos and low concentrations of DO. In Group 2, the ecological condition was poor, and the average value of the Shannon diversity index was only 1.77, belonging to the medium-heavy pollution group. The main factors that lead to the unhealthy structure of diatom community in Group 2 were high Velo and TP. In freshwater streams, the effects of water velocity and TP on benthic algae are well known and reported. Jowett and Biggs [37] reported that the abundance of benthic diatoms in high-velocity rivers was obviously lower than that in slow-flowing rivers, which was consistent with the results of this study. The dominant species in Group 2 was *G. parvulum*, of which the average relative abundance in S3, S8, and S11 was close to 60%. *G. parvulum* is a typical eutrophic and low profile diatom [38]. According to Passy's study [39], the low profile guild was favored in high disturbance habitats and dominated at high current velocities, which explained why the dominant species in the Group 2 was *G. parvulum*.

### 3.2. Evaluation of the BD-IBI and Diatom Indexes

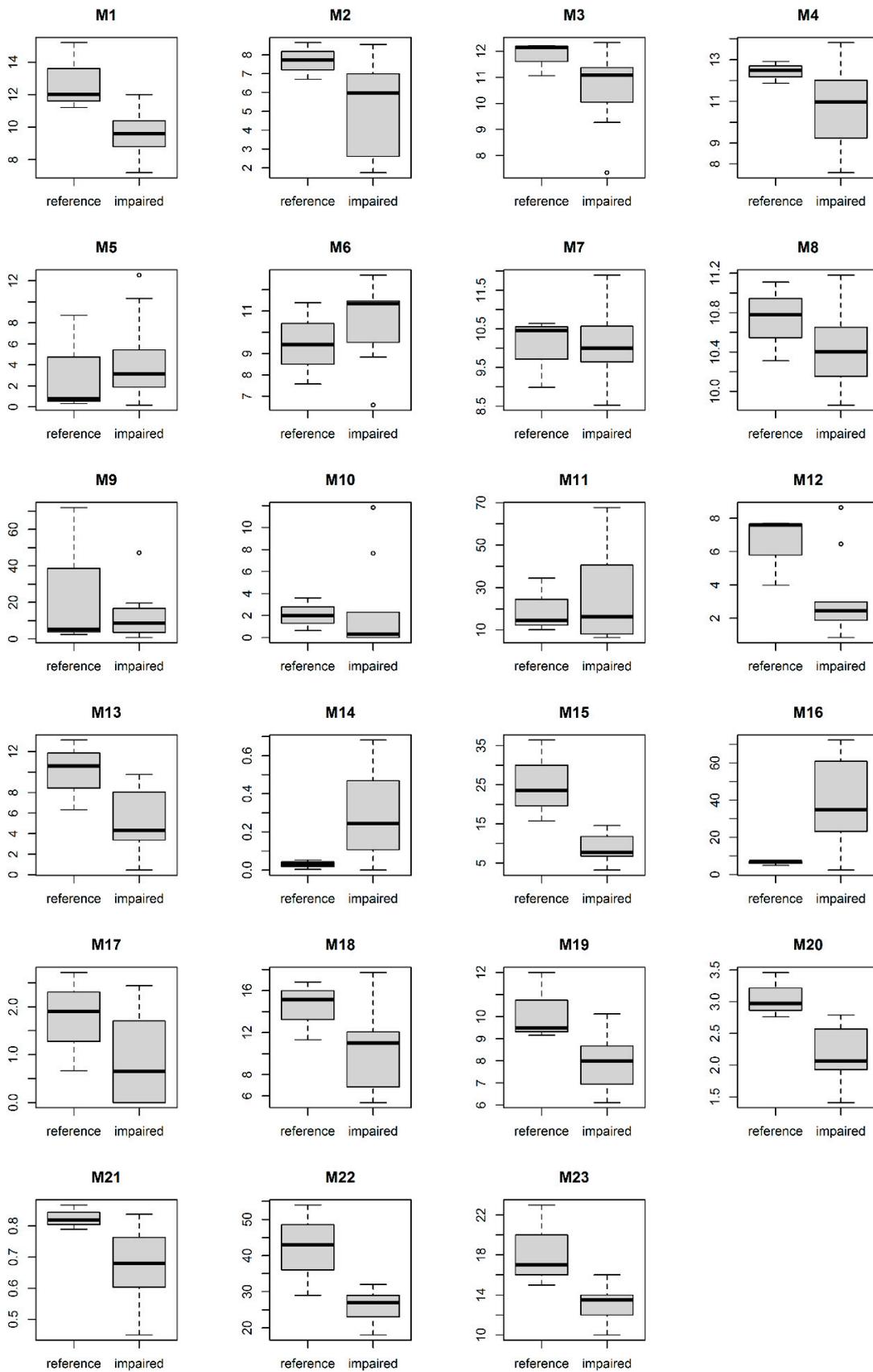
#### 3.2.1. Development and evaluation of the BD-IBI

The maximum score of QHEI is 200. According to the criteria proposed by Zheng et al., QHEI is divided into five grades, Very Good (>150), Good (120–150), Fine (90–120), Poor (60–90), and Very Poor (<60) [25]. A total of five sampling sites (S1, S2, S9, S12, and S13) were rated as good and very good. The results of water quality analysis showed that the TP in S2 and S12 were not up to standard, which may be because there was farmland near the sampling sites. Finally, sites with higher scores for water quality and QHEI were classified as reference sites (S1, S9, and S13), and other sites were classified as impaired sites.

A total of 23 metrics were selected after the first step evaluation (medians > 0), subsequently, box plot analysis was executed for these 23 metrics. Five metrics (M7, M13, M14, M16, and M17) were excluded due to unreasonable trends in response to environmental factors. In addition, five metrics (M6, M8, M10, M11, and M12) were excluded due to low separation power (Figure 5). Therefore, Spearman's rank correlation analysis was performed on the 13 parameters retained (Figure 6), nine metrics were excluded due to strong correlation and large variations. Finally, four metrics (biological diatom index (BDI), diatom species index of Australian rivers (DSIAR), Sensitive%, and Pielou Index) were selected for the BD-IBI development.

The ranges of four selected metrics were normalized on a scale of 1 to 10 (Equation (1)). The IBI value of each site was obtained by summing up the four parameters, which showed an obvious spatial variation. The IBI values ranged from 10 (S8) to 37 (S1) with an average of 24. The average score of the reference group was 32, while the average score of the impaired group was 21, which showed a good ability of differentiation. We used the mean 75th and 25th percentile IBI scores from all sites to set thresholds for good, fair, or poor condition. Therefore, two sites in the reference group were rated as "good" and one was rated as "fair".

The average IBI score of Group 2 (Section 3.1) was the lowest, with only 17 points, and all "poor" sites were in Group 2. Groups 2 scored higher than Group 2, with an average of 23 points, and all sites in this group were classified as "fair". Group 1 was in the best ecological condition, with an average IBI score of 30, and all points were classified as "good" or "fair". The results of IBI evaluation and RDA group analysis showed strong consistency, which indicated that the result of IBI evaluation is reasonable.



**Figure 5.** Box plots of 23 candidate benthic diatom index of biotic integrity (BD-IBI) metrics between reference and impaired sites in the Wutong River.



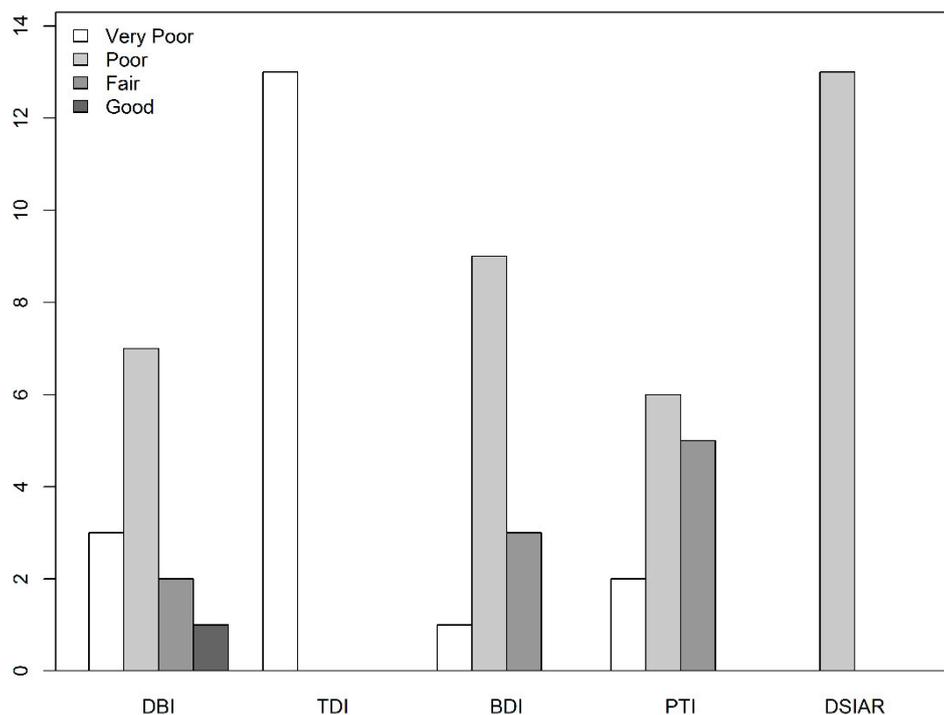


Figure 7. Scores of diatom indexes.

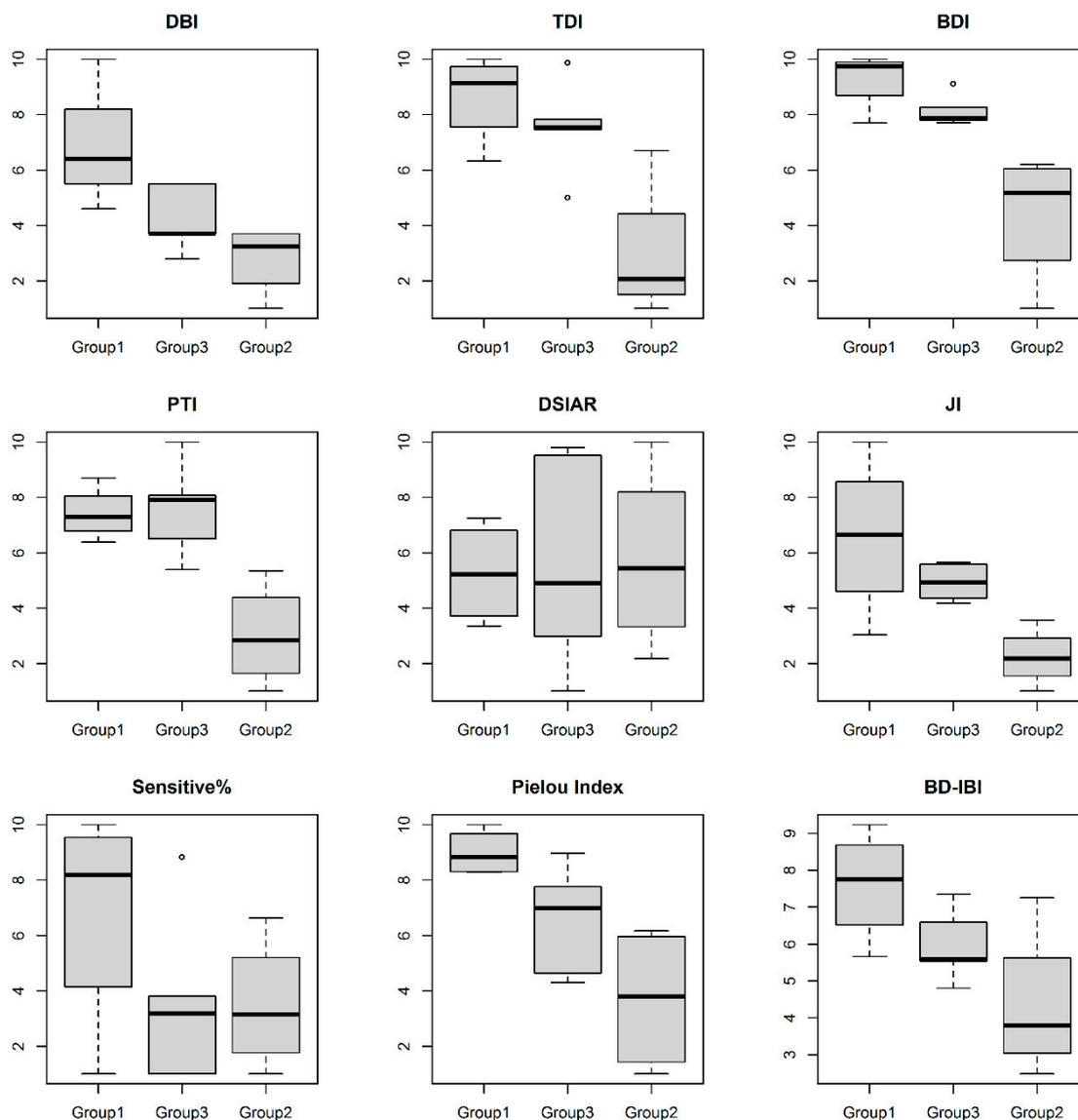
### 3.3. Applicability of Different Indexes

The applicability of seven diatom indexes and BD-IBI in water quality evaluation of the Wutong River were analyzed. In this research, we tentatively hypothesized that grouping by cluster analysis was reasonable and rigorous enough to evaluate the reasonableness of diatom indexes (i.e., diatom indexes, selected metrics, and IBI) as independent evaluation tools. The box plot analysis was adopted to intuitively demonstrate the discriminant abilities of the metrics and IBI (Figure 8). The Spearman correlations among diatom indexes, BD-IBI, physico-chemical variables are displayed in Table 3. Almost all metrics were negatively correlated with TP and Velo, while positively correlated with Cond and pH.

Table 3. The Spearman correlation among metrics (or BD-IBI) and water quality.

	NH <sub>3</sub> -N	TP	Phos	Cond	DO	pH	Velo
DBI	−0.17	−0.27	0.07	0.65*	0.03	−0.01	−0.52
TDI	0.07	−0.02	0.14	0.59*	0.06	0.38	−0.74**
BDI	−0.32	−0.28	−0.03	0.59*	0.21	0.17	−0.42
JI	−0.43	−0.73**	−0.3	0.39	−0.33	−0.15	−0.26
PTI	0.09	−0.29	0.26	0.52	−0.29	0.37	−0.72**
DSIAR	0.04	0.11	−0.14	−0.13	0.15	0.22	−0.11
M18	0.05	−0.16	0.23	0.33	−0.05	0.05	−0.19
M21	−0.38	−0.29	−0.24	0.69**	−0.07	0	−0.4
IBI	−0.25	−0.25	−0.07	0.61*	0.03	0.09	−0.45

Note: \*\* means extremely significant correlation ( $P < 0.01$ ), \* means significant correlation ( $P < 0.05$ ).



**Figure 8.** Box plots of eight diatom indexes and BD-IBI between different groups.

During the establishment of BD-IBI, generic index of diatom (GI) and DIAR were the two diatom indexes excluded because of their poor discriminative ability. Scores of GI and DIAR at the reference group were lower than that at the impaired group (Figure 5), indicating that GI and DIAR were not applicable in the Wutong River.

GI was developed and tested with success in Taiwan, China, based on the diversity of six species of diatoms (*Achnanthes*, *Cocconeis*, *Cymbella*, *Cyclotella*, *Melosira*, and *Nitzschia*) [15]. The low distribution of *Achnanthes*, *Cocconeis*, *Cyclotella*, and *Nitzschia* in the Wutong River may be the main reason for the large deviation of GI evaluation. GI was also used in other river basins of China. Xiang et al. [41] stressed that although GI can evaluate the Taizi River basin well, further studies were needed to ensure whether it can work well in water ecological health evaluation of other rivers due to its own limitations. According to Liu et al. [42], GI is an efficient index which can decrease the time used in species identification, and evaluate the ecological health status of rivers quickly. However, the drawback of GI is the poor accuracy of evaluation results, which causes a discrepancy with the actual situation.

As for DIAR, 55 genera were assigned numbers ranging from 1 to 10 to reflect their inferred sensitivity to common anthropogenic stressors. The higher the score, the more sensitive to human interference. The grade number of *Gomphonema* is 6 in DIAR, while grade numbers of *Gomphonema*

*parvulum*, the absolute dominant species in the Wutong River, were usually low in other indexes. For example, grade number of *G. parvulum* is 1 in PTI. The scores of the damaged group dominated by *G. parvulum* was high in the DIAR evaluation in Wutong River. Therefore, DIAR is not applicable in the Wutong River. In addition, the contribution of different genera to genus-level diatom indexes should be treated differently. For example, *Navicula* and *Nitzschia* have a widely differing range of ecological indicators, while the range of indicators for *Eunotia* and *Achnanthes* is relatively narrow. Feio et al. [43] concluded that the poor execution of genus level diatom indexes maybe due to their low level of taxonomic discrimination. In addition, the authors suggested expanding the diatom list. Although it would increase the difficulty of species identification, the expanded diatom index would be more practical for evaluating water ecosystem health.

Based on the research of DIAR, Chessman et al. [32] subdivided the classification unit into species (DSIAR), which made it more discriminative than DIAR. In this study, the discriminant ability of DSIAR has been improved compared to DIAR (Figure 5). However, DSIAR scores in Group 2 were higher than Group 3 (Figure 8), which indicated that the discriminant ability of DSIAR was still insufficient. The same problem also existed in PTI evaluation; PTI scores in Group 3 were higher than Group 1. In addition, DSIAR showed no significant correlation to environmental factors (Table 3). Therefore, DSIAR and PTI may not be applicable in the Wutong River. The predominant reason is that bioassessment indexes may be influenced by natural environmental gradients and anthropogenic factors. According to Kelly et al. [44], environmental differences can modify species responses to water-quality characteristics. Rimet et al. [45] reported that some diatoms may have different responses to different types of pollution in different regions, leading to different diatom index adaptability in different countries and regions.

TDI performed well in the evaluation of different groups (Figure 8). However, all sites were classified as “very poor” by TDI (Figure 7), which indicated poor variation. Compared with other diatom indexes, TDI showed more rigorous evaluation criteria for it determined more pollution sites, which was consistent with Liu et al. [42], who applied TDI in the ecosystem health assessment of the Wei River basin, China. Tang et al. [19] performed TDI in the Xiangxi River, China, and pointed out that TDI cannot discriminate very clean oligotrophic sites from severely polluted sites. In contrast, TDI has been verified successfully in other regions. For example, TDI can also be used in tropical streams in East Africa [46], Australia [18], and Iran [17]. Therefore, TDI is an effective bioassessment tool, but appropriate adjustments must be made to make TDI applied well in China.

According to the correlation analysis and the box plot analysis, DBI, BDI, Jaccard index (JI), and Pielou index had significant correlations with one or more environmental factors, and presented reasonable gradient between the groups. DBI is a multi-parameter index calculated from the weighted average of five evaluation parameters. JI and Pielou Index are general biological indexes, widely used in ecology [33]. BDI is originally a standardized method developed in France for the surveillance of watercourses quality. A few years later, the species list of BDI was expanded from 209 to 1063 by Coste et al. [29], making it much more suitable for the Water Framework Directive requirements. BDI has been successfully applied to water quality assessment worldwide [23,47,48]. In China, Tan et al. [23] used 14 diatom indexes to assess water quality in a subtropical river, they concluded that BDI has strong correlations with some water quality variables and was more effective than TDI. Similarly, Besse-Lototskaya et al. [49] also stressed that BDI is more robust to uncertainties than TDI. Compared with other parameters, these four indexes are obviously more suitable for the Wutong River.

The IBI is a familiar tool in the environmental assessment, restoration, and conservation of aquatic ecosystems. The original version of the IBI was developed using fish communities [50]. An increasing number of species have since been applied to the establishment of IBI, such as vegetation, macroinvertebrate, plankton, and benthic diatoms [2,51]. Multimetric indexes, such as IBI, has been increasingly used in assessing the ecological status of rivers as well as lakes and wetlands because they are, presumably, much more rational and comprehensive in characterizing ecological conditions in the aquatic ecosystems [21]. In this study, the derived DB-IBI included four individual metrics of different

aspects and effectively separated groups with different levels of interference (Figure 8). Meantime it also showed a strong correlation with environmental variables, such as conductivity and velocity. Overall, the applicability of IBI in the Wutong River was significantly higher than that of 23 alternative metrics. Our conclusion was consistent with previous studies [52], which suggested that multimetric indexes, such as IBI, are often more robust than their component metrics.

#### 3.4. Implications for Watershed Management

With the development of the economy, a large number of river ecosystems in China have been disturbed by human activities. Therefore, the demand for fast, convenient, and efficient water ecological monitoring and evaluation tools has become obvious. Many scholars believe that diatom-based monitoring tools are particularly applicable to river management in developing countries [6], and the results of our study as well verify this point of view. As two basic diatom-based methods, both diatom index and IBI have advantages and disadvantages.

Researches on the diatom index have been relatively mature, and dozens of diatom indexes have been developed and tested around the world [8,10]. The applications of diatom index are becoming more and more convenient, for there are special softwares to calculate diatom index, such as Omnidia. But the application of diatom index is also limited by many factors, such as climate and water quality. Therefore, their reliability has been questioned because they are less useful when applied in regions with distinct environmental characteristics from the area they were created [23]. Compared with the diatom index, IBI is more flexible and reliable. IBI is hardly affected by regional differences, because IBI development is based on the relevance of community data and environmental factors. Furthermore, the parameters that constitute IBI can be adjusted according to the characteristics of the study area. IBI also has its drawbacks, such as the lack of standardized criteria for IBI establishment, and the highly subjective judgment for parameters selection, as well as the high requirements for professional knowledge [2].

In recent years, more and more studies on the application of diatom indexes and diatom-based IBIs in Chinese river have been conducted. But as Tornés et al. [53] stressed, diatom indexes needed to be adapted if they are to provide a reliable diagnosis for specific river systems. The same conclusion was obtained by Pignata et al. [20], who tested the applicability of the European diatom indexes in the Pearl River Basin (Guangzhou, China) and recommended adjustments to these indexes. In addition, when a study area is too different from the original intended scope, the researchers preferred to develop a new diatom index [10]. Potapova and Charles [54] created diatom metrics themselves and obtained a better evaluation effect than European diatom indexes. So far, no diatom index (multimetric indexes, such as IBI are not considered) has been developed in Mainland China. To meet the urgent need of integrating biological monitoring in the national water quality monitoring program, it is strongly suggested to strengthen the fundamental research on ecological preferences and tolerance of diatoms, and develop diatom index in Chinese rivers.

#### 4. Conclusions

Diatom-based bioassessment tools are useful for monitoring and assessing the health status of the Wutong River, but not all of the indexes responded correctly to hydrochemical characteristics. Of all the indexes selected, IBI performed best based on our evaluation. Half of the diatom indexes, especially the genus level indexes, may not be suitable in our study area. The bioassessment showed that most of the sites were not healthy. It is supported by the high concentrations of nitrogen and phosphorus and the dominance of the pollution-tolerant diatoms in the river. But it is notable that this research is based on a sampling with only 13 sites, leading to much higher uncertainties of the development process as well as the results depiction. The development of the IBI is a preliminary process, further research and supplementary data are still needed for the improvement of the index.

To make diatom-based bioassessment tools more convenient and efficient for river management, it is strongly suggested that the structure of diatom communities and the response of diatom

communities to environmental variables should be further studied. In addition, the diatom indexes should be adjusted on the basis of these studies to make them more suitable for Chinese rivers. Meanwhile, a new diatom index can be developed on the basis of data collected in Chinese rivers, which is more efficient and accurate.

**Author Contributions:** H.X. and F.M. conceived and designed the research; H.X. and P.C. performed the research; H.X. contributed to data analysis of this work; P.C. made the pictures and polished the paper; H.X., B.Z., F.M., Y.W., and L.Z. wrote the paper and approved the final version to be published.

**Funding:** This research was funded by National Major Science and Technology Program for Water Pollution Control and Treatment of China, grant number (2017ZX07302-002, 2015ZX07201-008).

**Acknowledgments:** Special and sincere thanks to Lusan Liu, Yu Wang, Wenqian Cai, Yang Xia, and Yunlong Liu of the Chinese Academy of Environmental Sciences for their help in the field sampling.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

- Dudgeon, D.; Arthington, A.H.; Gessner, M.O.; Kawabata, Z.; Knowler, D.J.; Leveque, C.; Naiman, R.J.; Prieur Richard, A.H.; Soto, D.; Stiassny, M.L. Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biol. Rev.* **2010**, *81*, 163–182. [[CrossRef](#)] [[PubMed](#)]
- Ruaro, R.; Gubiani, É.A. A scientometric assessment of 30 years of the Index of Biotic Integrity in aquatic ecosystems: Applications and main flaws. *Ecol. Indicat.* **2013**, *29*, 105–110. [[CrossRef](#)]
- Bae, M.J.; Li, F.; Kwon, Y.S.; Chung, N.; Choi, H.; Hwang, S.J.; Park, Y.S. Concordance of diatom, macroinvertebrate and fish assemblages in streams at nested spatial scales: Implications for ecological integrity. *Ecol. Indicat.* **2014**, *47*, 89–101. [[CrossRef](#)]
- Marzin, A.; Archambault, V.; Belliard, J.; Chauvin, C.; Delmas, F.; Pont, D. Ecological assessment of running waters: Do macrophytes, macroinvertebrates, diatoms and fish show similar responses to human pressures? *Ecol. Indicat.* **2012**, *23*, 56–65. [[CrossRef](#)]
- Justus, B.G.; Petersen, J.C.; Femmer, S.R.; Davis, J.V.; Wallace, J.E. A comparison of algal, macroinvertebrate, and fish assemblage indices for assessing low-level nutrient enrichment in wadeable Ozark streams. *Ecol. Indicat.* **2010**, *10*, 627–638. [[CrossRef](#)]
- Bere, T. Challenges of diatom-based biological monitoring and assessment of streams in developing countries. *Environ. Sci. Pollut. Res. Int.* **2016**, *23*, 5477–5486. [[CrossRef](#)]
- Hering, D.; Johnson, R.K.; Kramm, S.; Schmutz, S.; Szoszkiewicz, K.; Verdonshot, P.F.M. Assessment of European streams with diatoms, macrophytes, macroinvertebrates and fish: A comparative metric-based analysis of organism response to stress. *Freshwater Biol.* **2010**, *51*, 1757–1785. [[CrossRef](#)]
- Venkatachalapathy, R.; Karthikeyan, P. *Application of Diatom-Based Indices for Monitoring Environmental Quality of Riverine Ecosystems: A Review*; Springer International Publishing: Berlin, Germany, 2015; pp. 593–619.
- Lavoie, I.; Campeau, S.; Zugic-Drakulic, N.; Winter, J.G.; Fortin, C. Using diatoms to monitor stream biological integrity in Eastern Canada: An overview of 10 years of index development and ongoing challenges. *Sci. Total Environ.* **2014**, *475*, 187–200. [[CrossRef](#)]
- Rimet, F. Recent views on river pollution and diatoms. *Hydrobiologia* **2012**, *683*, 1–24. [[CrossRef](#)]
- Kelly, M.G.; King, L.; Jones, R.I.; Barker, P.A.; Jamieson, B.J. Validation of diatoms as proxies for phytobenthos when assessing ecological status in lakes. *Hydrobiologia* **2008**, *610*, 125–129. [[CrossRef](#)]
- Schneider, S.C.; Lawniczak, A.E.; Picińska-Faltynowicz, J.; Szoszkiewicz, K. Do macrophytes, diatoms and non-diatom benthic algae give redundant information? Results from a case study in Poland. *Limnologia* **2012**, *42*, 204–211. [[CrossRef](#)]
- Fetscher, A.E.; Stancheva, R.; Kociolek, J.P.; Sheath, R.G.; Stein, E.D.; Mazor, R.D.; Ode, P.R.; Busse, L.B. Development and comparison of stream indices of biotic integrity using diatoms vs. non-diatom algae vs. a combination. *J. Appl. Phycol.* **2014**, *26*, 433–450. [[CrossRef](#)]
- Stevenson, R.J.; Pan, Y.D.; Dam, H.V.; Smol, J.P.; Stoermer, E.F. Assessing environmental conditions in rivers and streams with diatoms. *Diatoms Appl. Environ. Earth Sci.* **2010**, *2*, 57–85.
- Wu, J.T. A generic index of diatom assemblages as bioindicator of pollution in the Keelung River of Taiwan. *Hydrobiologia* **1999**, *397*, 79–87. [[CrossRef](#)]

16. Watanabe, T.; Asai, K.; Houki, A. Numerical estimation to organic pollution of flowing water by using the epilithic diatom assemblage—Diatom assemblage index (DAI po). *Sci. Total Environ.* **1986**, *55*, 209–218. [[CrossRef](#)]
17. Atazadeh, I.; Sharifi, M.; Kelly, M.G. Evaluation of the Trophic Diatom Index for assessing water quality in River Gharasou, western Iran. *Hydrobiologia* **2007**, *589*, 165–173. [[CrossRef](#)]
18. Newall, P.; Walsh, C.J. Response of epilithic diatom assemblages to urbanization influences. *Hydrobiologia* **2005**, *532*, 53–67. [[CrossRef](#)]
19. Tao, T.; Cai, Q.; Liu, J. Using Epilithic Diatom Communities to Assess Ecological Condition of Xiangxi River System. *Environ. Monit. Assess.* **2006**, *112*, 347.
20. Pignata, C.; Morin, S.; Scharl, A.; Traversi, D.; Schilirò, T.; Degan, R.; Bartley, P.; Tu, M.; Liu, H.; Peres, F. Application of European biomonitoring techniques in China: Are they a useful tool? *Ecol. Indicat.* **2013**, *29*, 489–500. [[CrossRef](#)]
21. Zalack, J.T.; Smucker, N.J.; Vis, M.L. Development of a diatom index of biotic integrity for acid mine drainage impacted streams. *Ecol. Indicat.* **2010**, *10*, 287–295. [[CrossRef](#)]
22. Xiang, T.; Ma, P.; Bunn, S.E.; Zhang, Q. Development of a benthic diatom index of biotic integrity (BD-IBI) for ecosystem health assessment of human dominant subtropical rivers, China. *J. Environ. Manag.* **2015**, *151*, 286–294.
23. Tan, X.; Sheldon, F.; Bunn, S.E.; Zhang, Q. Using diatom indices for water quality assessment in a subtropical river, China. *Environ. Sci. Pollut. Res. Int.* **2013**, *20*, 4164–4175. [[CrossRef](#)] [[PubMed](#)]
24. CSEPA. *Water and Wastewater Monitoring Methods*, 4th ed.; China Environmental Science Press: Beijing, China, 2002. (In Chinese)
25. Zheng, B.H.; Zhang, Y.; Li, Y.B. Study of indicators and methods for river habitat assessment of Liao River Basin. *Acta Sci. Circumst.* **2007**, *27*, 928–936. (In Chinese)
26. Stoddard, J.L.; Larsen, D.P.; Hawkins, C.P.; Johnson, R.K.; Norris, R.H. Setting expectations for the ecological condition of streams: The concept of reference condition. *Ecol. Appl.* **2006**, *16*, 1267–1276. [[CrossRef](#)]
27. Yin, X.; Qu, X.; Li, Q.; Liu, Y.; Zhang, Y.; Meng, W. Using periphyton assemblages to assess stream conditions of Taizi River Basin, China. *Acta Ecol. Sin.* **2012**, *32*, 1677–1691. (In Chinese)
28. Kelly, M.G.; Whitton, B.A. The Trophic Diatom Index: A new index for monitoring eutrophication in rivers. *J. Appl. Phycol.* **1995**, *7*, 433–444. [[CrossRef](#)]
29. Coste, M.; Boutry, S.; Tison-Rosebery, J.; Delmas, F. Improvements of the Biological Diatom Index (BDI): Description and efficiency of the new version (BDI-2006). *Ecol. Indicat.* **2009**, *9*, 621–650. [[CrossRef](#)]
30. Muscio, C. *The Diatom Pollution Tolerance Index*; Watershed Protection and Development Review Department, Environmental Resource Management Division: Austin, TX, USA, 2002.
31. Chessman, B.; Gowns, I.; Currey, J.; Plunkett-Cole, N. Predicting diatom communities at the genus level for the rapid biological assessment of rivers. *Freshwater Biol.* **2010**, *41*, 317–331. [[CrossRef](#)]
32. Chessman, B.C.; Bate, N.; Gell, P.A.; Newall, P. A diatom species index for bioassessment of Australian rivers. *Mar. Freshwater Res.* **2007**, *58*, 542–557. [[CrossRef](#)]
33. Toporowska, M.; Pawlikowron'Ska, B.; Wojtal, A.Z. Epiphytic algae on *Stratiotes aloides* L., *Potamogeton lucens* L., *Ceratophyllum demersum* L. and *Chara* spp. in a macrophyte-dominated lake. *Oceanol. Hydrobiol. Stud.* **2008**, *37*, 51–63. [[CrossRef](#)]
34. Beck, M.W.; Hatch, L.K.; Vondracek, B.; Valley, R.D. Development of a macrophyte-based index of biotic integrity for Minnesota lakes. *Ecol. Indicat.* **2010**, *10*, 968–979. [[CrossRef](#)]
35. Breine, J.; Simoens, I.; Goethals, P.; Quataert, P.; Ercken, D.; Liefferinge, C.; Belpaire, C. A Fish-Based Index of Biotic Integrity for Upstream Brooks in Flanders (Belgium). *Hydrobiologia* **2004**, *522*, 133–148. [[CrossRef](#)]
36. Salomoni, S.E.; Rocha, O.; Callegaro, V.L.; Lobo, E.A. Epilithic diatoms as indicators of water quality in the Gravataí River, Rio Grande do Sul, Brazil. *Hydrobiologia* **2006**, *559*, 233–246. [[CrossRef](#)]
37. Jowett, I.; Biggs, B.F. Flood and velocity effects on periphyton and silt accumulation in two New Zealand rivers. *N. Z. J. Mar. Freshwater Res.* **1997**, *31*, 287–300. [[CrossRef](#)]
38. Hoagland, K.D.; Roemer, S.C.; Rosowski, J.R. Colonization and Community Structure of Two Periphyton Assemblages, with Emphasis on the Diatoms (Bacillariophyceae). *Am. J. Bot.* **1982**, *69*, 188–213. [[CrossRef](#)]
39. Passy, S.I. Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquat. Bot.* **2007**, *86*, 171–178. [[CrossRef](#)]

40. Eloranta, P.; Soinen, J. Ecological status of some Finnish rivers evaluated using benthic diatom communities. *J. Appl. Phycol.* **2002**, *14*, 1–7. [[CrossRef](#)]
41. Xiang, Z.L.; Chen, H.; Li, C.; Yin, X.W.; Xu, Z.X.; Zhang, Y. Application of diatom index in assessment of aquatic ecosystem health in Taizi River, China. *J. Dalian Ocean Univ.* **2016**, *31*, 416–425. (In Chinese)
42. Liu, L.; Xu, Z.; Yin, X.; Wu, W.; Min, W. Assessing water quality with diatom indices in the Wei River basin. *J. Beijing Normal Univ.* **2016**, *52*, 317–321. (In Chinese)
43. Feio, M.J.; Almeida, S.F.P.; Craveiro, S.C.; Calado, A.J. A comparison between biotic indices and predictive models in stream water quality assessment based on benthic diatom communities. *Ecol. Indic.* **2009**, *9*, 497–507. [[CrossRef](#)]
44. Kelly, M.G.; Cazaubon, A.; Coring, E.; Dell’Uomo, A.; Ector, L.; Goldsmith, B.; Guasch, H.; Hürlimann, J.; Jarlman, A.; Kawecka, B. Recommendations for the routine sampling of diatoms for water quality assessments in Europe. *J. Appl. Phycol.* **1998**, *10*, 215. [[CrossRef](#)]
45. Rimet, F.; Cauchie, H.M.; Hoffmann, L.; Ector, L. Response of diatom indices to simulated water quality improvements in a river. *J. Appl. Phycol.* **2005**, *17*, 119–128. [[CrossRef](#)]
46. Bellinger, B.J.; Cocquyt, C.; O’Reilly, C.M. Benthic diatoms as indicators of eutrophication in tropical streams. *Hydrobiologia* **2006**, *573*, 75–87. [[CrossRef](#)]
47. Bere, T. Are diatom-based biotic indices developed in eutrophic, organically enriched waters reliable monitoring metrics in clean waters? *Ecol. Indic.* **2016**, *62*, 312–316. [[CrossRef](#)]
48. Gomà, J.; Rimet, F.; Cambra, J.; Hoffmann, L.; Ector, L. Diatom Communities and Water Quality Assessment in Mountain Rivers of the Upper Segre Basin (La Cerdanya, Oriental Pyrenees). *Hydrobiologia* **2005**, *551*, 209–225. [[CrossRef](#)]
49. Besse-Lototskaya, A.; Verdonschot, P.F.M.; Sinkeldam, J.A. Uncertainty in Diatom Assessment: Sampling, Identification and Counting Variation. *Hydrobiologia* **2006**, *566*, 247–260. [[CrossRef](#)]
50. Karr, J.R. Assessment of biotic integrity using fish communities. *Fisheries* **1981**, *6*, 21–27. [[CrossRef](#)]
51. Baek, S.H.; Son, M.; Kim, D.; Choi, H.W.; Kim, Y.O. Assessing the ecosystem health status of Korea Gwangyang and Jinhae bays based on a planktonic index of biotic integrity (P-IBI). *Ocean Sci. J.* **2014**, *49*, 291–311. [[CrossRef](#)]
52. Lacouture, R.V.; Johnson, J.M.; Buchanan, C.; Marshall, H.G. Phytoplankton index of biotic integrity for Chesapeake Bay and its tidal tributaries. *Estuar. Coasts* **2006**, *29*, 598–616. [[CrossRef](#)]
53. Tornés, E.; Cambra, J.; Gomà, J.; Leira, M.; Ortiz, R.; Sabater, S. Indicator taxa of benthic diatom communities: A case study in Mediterranean streams. *Ann. Limnol. Int. J. Limnol.* **2007**, *43*, 1–11. [[CrossRef](#)]
54. Potapova, M.; Charles, D.F. Diatom metrics for monitoring eutrophication in rivers of the United States. *Ecol. Indic.* **2007**, *7*, 48–70. [[CrossRef](#)]

