



Development of a benthic diatom index of biotic integrity (BD-IBI) for ecosystem health assessment of human dominant subtropical rivers, China



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ABSTRACT

As efforts intensify to address the issues of declining water quality and biodiversity losses in freshwater ecosystems, there have been great demands for effective methods of evaluating aquatic ecosystem health. In this study, benthic algae assemblages and water quality variables were analyzed to develop a benthic diatom-based index of biotic integrity (BD-IBI) for assessment of the aquatic environment in the upper Han River (China). Through the use of multivariate and multimetric approaches, four metrics – % prostrate individuals, % Amphora individuals, % polysaprob species, and diatom-based eutrophication/pollution index (EPI-D) – were identified from 98 candidate metrics to develop a BD-IBI. Application of the index revealed that water quality in 11% of the 31 sampled sites could be described as excellent condition, in 43% of the sites it could be described as good condition, in 25% as moderate condition, and in 21% as poor condition. The assessment further revealed that the main reason for degradation of the Han river ecosystem was nutrient enrichment through agricultural land use.

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1. Introduction

Freshwater ecosystems are considered to be the most threatened on earth (Vörösmarty et al., 2010), and many streams, rivers and lakes experience declining water quality, including heavy metal contamination (Roig et al., 2011), sedimentation and turbidity (Thompson et al., 2014), nutrient enrichment and algal blooms (Vitousek et al., 1997; Raymond et al., 2008). As a consequence of these and other threats associated with flow alteration and habitat modification, freshwater systems have experienced significant decline in biodiversity and damage to ecosystem integrity (Dudgeon et al., 2006; Balian et al., 2008). Growing concerns about declining water quality and ecosystem health have led to increased interest in integrative assessment of freshwater systems (Friberg et al., 2011).

Freshwater monitoring has traditionally relied on water quality and structural measures of the biota, especially fish and

macroinvertebrates, though algal communities, protozoa, and macrophytes have also been used widely (e.g., Hill et al., 2000; Norris and Hawkins, 2000; Pignata et al., 2013; Almeida et al., 2014). Many monitoring programs have adopted a reference condition approach deriving expected values for bio-indicators by sampling unimpaired or minimally impaired sites, using either predictive models (e.g., Norris and Hawkins, 2000) or multimetric measures (e.g., Karr and Chu, 2000). A number of indices of biotic integrity have been developed and trialed, particularly based on macroinvertebrate and fish assemblage data (e.g. Baptista et al., 2007; Casatti et al., 2009). Such assessments based on the structural attributes of organisms can provide a holistic perspective and general indications for the ecological status of rivers (Mistri et al., 2008).

Benthic diatoms are widely distributed in varying habitats and they are sensitive to a range of environmental variables such as temperature, nutrients, hydrologic regime, sediment, land use, etc. (Snyder et al., 2002; Chessman et al., 2007). By using assemblage data, benthic diatom-based indices of biotic integrity (BD-IBI) have been developed and applied for ecosystem health assessment in some regions including the Mid-Appalachian region of the USA

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(Wang et al., 2005; Hill et al., 2000), Eastern Canada (Lavoie et al., 2014), and coastal rivers in NW Spain (Delgado et al., 2010). Previous studies have indicated that BD-IBI's are particularly useful to assess the impacts of nutrient pollution and landscape modification (Wang et al., 2005). However, relatively few studies have developed or applied a biotic integrity index based on diatoms to evaluate river health in subtropical rivers, especially in Asia (Pignata et al., 2013).

The upper Han River is the source area for the Middle Route of China's South to North Water Transfer Project, and thus its declining water quality is a great concern for the transboundary water diversion project (Zhang, 2009). The diatoms might be particularly suitable river health indicators in this study area given we know the types of pressures/threats prevalent in this region are pollution and nutrient enrichment based on our previous studies. (Li et al., 2008, 2009; Tan et al., 2013, 2014a, 2014b). The main objectives of this study were: 1) to develop an integrative biotic integrity index based on benthic diatoms; 2) to apply this index to evaluate comprehensive river conditions in the upper Han River; and 3) to relate diffused pollution and nutrient enrichment to bio-indicators (epilithic diatoms) in order to explore and quantify the human perturbations of primary producers in river ecosystems.

2. Materials and methods

2.1. Study area

The upper Han River is located in the region 31°–34° N, 106°–112° E. It has a length of 925 km with a drainage area of 95,200 km² (Fig. 1). The region has a subtropical humid climate with an annual precipitation ranging from 950 to 1200 mm, and most precipitation falls during the wet season from July to October. The mean temperature varies from –0.3 °C in January and to 21.9 °C in July with a mean annual temperature of 11.8 °C. Small towns and villages with small populations reside in the headwaters while several larger cities such as Hanzhong, and Ankang with large populations are found along the Han River corridor. There are large areas of cultivation (e.g., about 15% of the total basin area) including maize, wheat, rice, cassava, vegetables, and citrus (Li et al., 2008, 2009).

The Jinshui River (33° 16'–33° 45'N, 107° 40'–108° 10'E), 87 km

long, is a tributary of the Han River with a drainage area of 720 km² and is located in Foping and Yang counties in Shaanxi Province. The region is a subtropical monsoon zone with annual rainfall ranging from 924 to 1244 mm, of which most falls also in the period from July to October. In the catchment, forest vegetation coverage is extensive and accounts for up to 96.4% of the land area, while agricultural land accounts for only about 2% (Zhang et al., 2010). Streams and creeks upstream of the Jinshui River are within a national natural reserve for giant panda conservation and are in pristine condition. There is little human activity upstream and only a few small villages and towns are located along the lower reaches of the riverine system. There are no industrial activities in the Jinshui River catchment.

2.2. Diatom sampling and identification

Diatom sampling was carried out in the upper Han River in November 2007, 2008, 2009 and in the Jinshui River (a tributary of the Han River) in November 2008, 2009. Diatoms on rocks were collected from 31 sites (H1–31) in the upper Han River and 16 sites (J1–16) in the Jinshui River (Fig. 1). Three cobbles (diameter < 25 cm) were collected randomly along a transect of left, middle and right across the creek (if possible subject to depth). A fixed circular area (diameter 7 cm) was scrubbed from each of the three rocks and periphyton was rinsed with distilled water into one container as a replicate (Tan et al., 2013). A 100 ml sample was then preserved in plastic bottles in 4% formaldehyde. A total of 125 samples were collected for benthic algae analysis. Diatoms were mounted with Naphrax™ after organic material was removed with acid (HNO₃ and H₂SO₄). Species were identified and a minimum of 400 valves were counted per slide at 1000 × magnification under Microscope (Olympus B×51) (Tan et al., 2014a, 2014b).

2.3. Physical and chemical analyses

Water samples were collected from the same sites simultaneously with benthic algae samplings in the river network (Fig. 1). Water temperature (*t*), pH, electrical conductivity (EC), dissolved oxygen (DO), ammonium nitrogen (NH₄-N), nitrate nitrogen (NO₃-N) and turbidity were measured using a multimeter YSI 6920. Ca²⁺ and SiO₂ were determined using Inductively Coupled

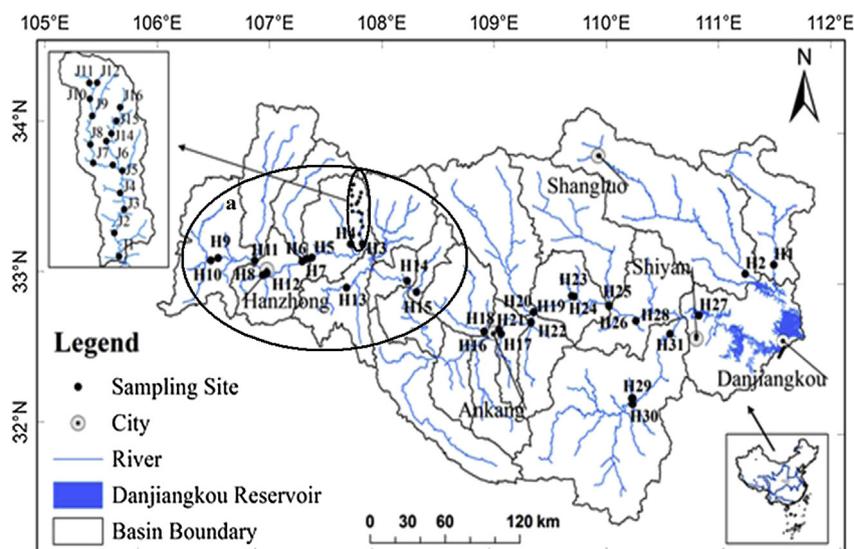


Fig. 1. Sampling sites in the upper Han River (H1–H33) and one of its tributaries, the Jinshui River (J1–J16), China.

Plasma Atomic Emission Spectrometry (ICP-AES) (IRIS Intrepid II XSP DUO, USA). Anions (Cl^- and SO_4^{2-}) were detected using a Dionex Ion Chromatograph (IC) (Dionex Corporation, Sunnyvale, USA). DOC was determined using a TOC analyzer (TOC-V CPH, Shimadzu Corporation, Japan), while TP, SRP and TN were measured using the spectrophotometric method.

2.4. Development of BD-IBI

The entire procedure for constructing BD-IBI followed the literature (Wang et al., 2005; Wu et al., 2012) with modifications to the selection of the reference sites.

2.4.1. Selection of impaired sites and reference sites

Samples from the upper Han River (November 2007 and November 2008) and those in the Jinshui River (November 2008) were selected to develop a BD-IBI. Reference sites were selected based on previous data on water quality in the entire upper Han River (Li et al., 2009), and geomorphologic attributes such as river sinuosity and substrates type by calculating the Qualitative Habitat Evaluation Index (QHEI) (Ohio EPA, 2006) (Table 1). Afterwards, nonmetric multidimensional scaling (MDS) was carried out based on benthic diatom communities (Fig. 2). Sampling sites J1 and H3 were eliminated because they were located at the boundary of two groups. H4 was sampled in the Han River but fell in the group of the Jinshui River, so it was also excluded from the impaired sites. Thus, the impaired sites – H5–H15 from the upper Han River in November 2007 and 2008 (total 22 samples) and the reference sites (J2–J16) in November 2008 located geographically in the Jinshui River – were used to develop the BD-IBI.

The difference in the environmental variables between the reference and impaired sites in the training-site data set (sampled in November 2007 and November 2008 for reference sites, and November 2009 for impaired sites) was tested by a non-parametric test, the Mann–Whitney test (Table 2). The Spearman correlation was used to explore the relationships among individual metrics or

BD-IBI, water quality, and land use variables. Spearman rank correlation, Mann–Whitney test, and box plots were prepared using SPSS 16.0.

2.4.2. Calculation of metrics

BD-IBI was developed based on benthic diatom from 37 samples (11 samples from impaired sites (H5–H15) sampled in November 2007 and 11 samples from H5–H15 in November 2008 and 15 samples in 15 reference sites J2–J16) sampled in November 2008). 98 metrics were calculated (Wang et al., 2005; Hill et al., 2003; Wu et al., 2012), and classified into 6 categories, i.e., biotic diatom indices, ecological values, diversity indices, growth form, sensitive species, and taxonomic composition (Appendix 1) (van Dam et al., 1994; Wang et al., 2005). Sixteen widely used diatom indices (CEE, DESCY, DI-CH, EPI-D, IBD, IDAP, IDP, SHE, SID, SLA, TDI, TID, and WAT) and ecological values were also calculated using Omnidia 7 software V 4.2. Other metrics were computed according to the definition. Their details are listed in Appendix 1.

2.4.3. Selection of metrics

Initially, metrics with medians of 0 were excluded from the 98 candidate metrics because they would decrease the separating capacity (Wang et al., 2005). Since most of the metrics were not distributed normally (Kolmogorov–Smirnov test, $p < 0.05$), the remaining metrics were evaluated using non-parametric Mann–Whitney tests. A metric was excluded if there was no significant difference between the impaired and reference groups.

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 (Wang et al., 2005). 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, and a value of 0 was assigned when both medians were within the range of the other box.

Metrics with large variations were excluded since such metrics would fluctuate when they were used for the assessment. We selected the ratio $(q^{0.75} - q^{0.25}) / (q^\alpha - q^\beta)$ to indicate the variation of the metrics (Barbour et al., 1999), where $q^{0.75}$ and $q^{0.25}$ were the 25th and 75th quartiles, and q^α and q^β represented the α and β

Table 1
QHEI marks based on the Han River in November 2007 and Jinshui River in November 2008 (Ohio EPA, 2006).

Han	Mark	Jinshui	Mark
H1 Laoguan River	54	J1	57.5
H2 Danjiang River	45	J2	69.5
H3 Jinshui River	45	J3	66.5
H4 Youshui River	59	J4	63.5
H5 HanRiver (YangCounty)	47.5	J5	60
H6 Xushui River	55	J6	60
H7 HanRiver (Chenggu city)	55	J7	64.5
H8 Lianshui River	54.5	J8	80
H9 Jushui River	50	J9	65
H10 HanRiver (Mian County)	54.5	J10	78
H11 Bao River	54.5	J11	80
H13 Muma River	47	J12	75
H14 HanRiver (Shiquan County)	41.5	J13	66.5
H15 Chi River	24	J14	65
H16 Yue River	53	J15	63.5
H17 Huangyang River	51.5	J16	75
H18 Han River (Ankang)	50		
H20 Xun River	48		
H21 Ba River	59		
H22 Han River (Joint with Ba River)	37		
H23 Shu River	46		
H24 HanRiver (ShuRiver town)	38		
H25 Jia River	45		
H26 Han River (JiaRiver town)	35		
H28 Jiangjun River	42		
H29 Du River	47		
H31 Huanglongtan	45		

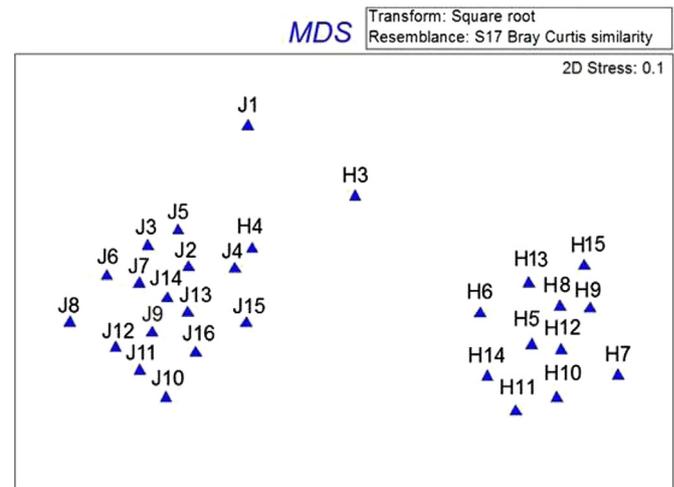


Fig. 2. Non-metric multidimensional scaling (MDS) based on benthic diatom communities sampled in November 2007.

Table 2

The descriptive statistics of environmental variables for reference and impaired sites in the training-site data set, and the results of non-parametric test between the reference and impaired sites. Bold means significant ($p < 0.01$).

Environmental Variables	Reference (n = 15)			Impaired (n = 22)			Mann–Whitney test	p-Values
	Percentile			Percentile				
	25th	50th	75th	25th	50th	75th		
Depth (m)	0.27	0.42	0.51	0.20	0.51	0.52	25.00	0.958
Width (m)	5.00	8.00	16.00	30.00	40.00	57.50	2.00	0.004
Velocity (m/s)	0.29	0.43	0.68	0.10	0.33	0.69	89.00	0.339
t (°C)	7.08	8.23	9.57	11.31	13.91	17.81	100.00	0.000
EC (μS/cm)	160	170	240	278.00	310.00	336.80	0.00	0.000
DO (mg/L)	10.10	10.80	11.48	1.58	12.12	13.60	273.00	0.282
pH	7.53	7.70	7.85	7.98	8.31	8.40	86.00	0.000
Turbidity (NTU)	1.00	1.50	1.80	7.65	12.40	20.60	128.50	0.000
TP (mg/L)	0.002	0.009	0.027	0.042	0.101	0.182	1.00	0.000
SiO ₂ (mg/L)	8.05	9.60	9.95	8.63	10.49	14.11	61.50	0.000
TOC (mg/L)	0.86	0.96	1.12	0.64	1.09	1.54	238.00	0.015
TN (mg/L)	0.43	0.80	1.01	1.39	2.40	3.72	336.50	0.392
HCO ₃ ⁻ (mg/L)	36.50	53.00	61.00	150.30	170.80	203.50	3.00	0.000
Cl ⁻ (mg/L)	2.60	6.27	10.71	4.08	5.09	6.73	378.50	0.854
Ca ²⁺ (mg/L)	13.29	15.18	17.19	37.76	43.32	47.52	2.00	0.000

quartiles, respectively. When the value of a metric decreased with the increase of ecosystem degradation, $\alpha = 0.25$, $\beta = 0$; if the value of a metric increased with the increase of ecosystem degradation, $\alpha = 1$ (maximum value), $\beta = 0.75$. We excluded the metrics with $(q^{0.75} - q^{0.25}) / (q^\alpha - q^\beta) > 1$ because they would have a large variation and weak power to indicate situations of ecosystem degradation. The metrics were selected based on separation power > 3 and $(q^{0.75} - q^{0.25}) / (q^\alpha - q^\beta) < 1$. Lastly, when more than 2 metrics were included and they were highly correlated ($r_s > 0.80$), the metric with the highest separation and lowest variation indicated by lower $(q^{0.75} - q^{0.25}) / (q^\alpha - q^\beta)$ was selected. The ranges of the selected metrics were normalized in a scale of 1–10 (Wang et al., 2005; Hill et al., 2000).

2.5. Testing of the metrics and BD-IBI

The BD-IBI was tested using 37 samples from 33 sites (15 references sites and 18 impaired sites) from a different dataset. The reference sites (J2–J16) were samples from the Jinshui River in November 2009 and impaired sites were the samples (H1, H2, H16–H31, downstream of the upper Han River, Fig. 1) in the upper Han River collected in November 2007 and November 2008. The calculation and scaling system (0–10 scaling) of the final metrics for the BD-IBI were used in the same way as that for the training sites.

Separation power was first used to test the differences between reference and impaired sites. Afterwards, we used correlation index (*Col*) and *Cumulative_R²* according to Blanco et al. (2007) and Wu et al. (2012):

$$\text{Cumulative_R}^2 = S(r_s^2) \quad (1)$$

where $R^2 = \text{sum of } r_s^2$, and $r_s = \text{Spearman's correlation coefficient between BD-IBI and a given environmental variable}$.

$$\text{Col} = \frac{\text{Cumulative_R}^2 S}{n^2} \quad (2)$$

where *Col* was the correlation index for the BD-IBI, *S* was the number of r_s statistically significant at $p < 0.05$, and *n* was the number of environmental variables evaluated. *Col* ranged from 0 to 1, while *Cumulative_R²* from 0 to *n*, indicating the theoretical minimum and maximum relationship between the BD-IBI and environmental variables. The BD-IBI was accepted if there was no

difference between values of the above criteria at testing-site and training-site data set (examined by paired *t*-test, here nonparametric Kruskal–Wallis tests).

2.6. The application of the BD-IBI

The derived BD-IBI was applied to evaluate the river conditions in the upper Han River using the different benthic diatom samples collected in the upper Han River (H1–H31) in November 2009.

3. Results

3.1. Development of the BD-IBI

A total of 68 metrics were selected from 98 candidate metrics (Appendix 1) after the first step evaluation (i.e., medians > 0 , Mann–Whitney U test). We removed an additional 20 metrics including density, % erect individuals, and % stalked individuals from 68 metrics because there were no significant differences between the reference sites and the impaired sites based on their separating capacity, which was evaluated using nonparametric Mann–Whitney tests ($p < 0.05$).

The remaining 48 potential metrics were assessed based on separation power > 3 and redundancy, and 32 metrics were removed from further consideration. Another 8 metrics, i.e., IDAP, hypereutraphentic, eutraphentic, meso- α -mesosaprob, saprophile species, % unattached individuals, % motile individuals, and % Nitzschia individuals were also removed because their ratios of $(q^{0.75} - q^{0.25}) / (q^\alpha - q^\beta)$ were greater than 1. We selected polysaprob over meso-eutraphentic and α -messaprob because polysaprob had a lower value of $(q^{0.75} - q^{0.25}) / (q^\alpha - q^\beta)$ and all of them indicated community structure.

Finally, 4 metrics (% prostrate individuals, % Amphora individuals, EPID, and polysaprob) were selected for the BD-IBI development (Table 3). Their values were normalized to the scale of 0–10 so the total BD-IBI scores could be calculated. Box plots for each metric showed great separation capacity between reference and impaired sites. EPID and % prostrate individuals decreased with impairment, whereas the other two metrics increased with impairment (Fig. 3). The total score of BD-IBI also demonstrated its power to separate the reference and impaired sites (Fig. 4).

3.2. Testing the BD-IBI

Both the scores of the 4 individual metrics and the BD-IBI (i.e., the total score of the 4 metrics) from the testing dataset showed a difference between the reference and impaired sites using box plots (Figs. 5 and 6). The Spearman correlations among the metrics, physical and chemical variables, and land use (i.e., %agriculture, % forest) are displayed in Table 4. There were no significant differences in values of separation powers, correlation index (*Col*), and Cumulative R^2 of the BD-IBI between the testing site dataset and training site dataset (Table 5).

3.3. Application of the BD-IBI

The derived BD-IBI was subsequently applied to evaluate the ecological conditions using a different dataset collected in November 2009. The total BD-IBI scores for the upper Han River had a large range from 10.47 to 37.61 (Fig. 4). The sampled sites were classified as excellent, good, moderate, or poor condition according to the percentile of their BD-IBI scores, i.e., >34.07 (90th percentile), >29.38 (50th percentile), >24.01 (25th percentile), and >0. The results according to the BD-IBI score at each site showed that about 11% of the 31 sampling sites were in excellent condition, and about 43%, 25%, and 21% of the sampling sites were in good, moderate and poor condition, respectively.

4. Discussion

Bioassessment has been a common practice for evaluation of ecological or trophic status by monitoring biological community structure characteristics such as indicator species and diversity or indices developing from bio-indicators (Norris and Hawkins, 2000; Morin et al., 2008). Since this methodology for river system assessment has been routinized and markedly cost-effective, it is applied world-wide (Norris and Hawkins, 2000). The periphyton community, specifically benthic diatoms, has been regarded as an effective bio-indicator of stream health/biotic integrity (Potapova et al., 2004; Lavoie et al., 2014) because the community's characteristics (e.g., dominant species, abundance, and benthic metabolism) respond sensitively to various environmental stressors (Bunn et al., 1999; Leland, 1995; Snyder et al., 2002).

However, the efficiency of bio-indicators including accuracy in diagnosing impairment, methods and presentation of results varies dramatically in different studies (Norris and Hawkins, 2000). For example, Trophic Diatom Index (TDI) and Watanabe's Index (WAT) have been developed based on the indicator species concept and has proved to be powerful in assessing the water quality (Kelly and Whitton, 1995; Hill et al., 2000; Pignata et al., 2013), but it is not reliable in the upper Han River (Tan et al., 2013). Another approach of biological monitoring is based on the assumption that diversity (i.e., diversity index, evenness and richness) will decrease with the degradation of the aquatic environment (Hill et al., 2000; Norris and Hawkins, 2000; Wang et al., 2005). Yet, their reliability has been questioned because the responses of indices are dependent on

the initial structure of the community and perturbation types (Boyle et al., 1990). Multimetric indices such as IBI are developed on the basis of correlation of community data with current and past environments and they are presumably much more rational in characterizing ecological conditions in the aquatic ecosystems (Reynoldson et al., 1997).

In this study, the derived DB-IBI included 4 individual metrics and effectively separated reference sites from impaired sites (Figs. 4 and 6), while it also had strong relationships with water quality parameters such as phosphorus, nitrogen, ions (Cl^- , SO_4^{2-} , Ca^{2+}), and catchment land use (e.g., %forest, and %agriculture) (Table 4). Further testing using a separate dataset from different sampling sites also demonstrated its robustness and effectiveness on separating sites with varying ecological conditions and enabled the identification of sites that were subject to intensive anthropogenic activities such as higher percentage of agricultural land (Table 4; Figs. 5 and 6). Thus, the developed BD-IBI is able to diagnose the likely cause of degradation of a river's ecosystem.

Of the four selected metrics, %prostrate individuals is based on a diatom's growth form (e.g., motile, erect, stalked, and prostrate), which indicates the strategy for diatoms adapting to flow environmental conditions (Wang et al., 2005). Prostrate diatoms indicate high grazing pressure, hydraulic disturbance or early diatom succession in benthic diatom communities to some extent. The % Amphora individuals is a metric based on genus of organisms. Compared to diatom indices based on species (e.g., EPI-D), the genus-based metric is easier to estimate but could ignore the differential responses of individual species to nutrients, carbon, ions, and pH (Hill et al., 2001). In the study area, *Amphora inariensis* and *Amphora pediculus* are the most dominant species, and both have high optima values. *A. inariensis* has the optima of 1.89 mg/L for total nitrogen, 0.03 mg/L for total phosphorus, and 1.50 mg/L for DOC, respectively. *A. pediculus* has the optima for total nitrogen, total phosphorus and DOC of 1.99 mg/L, 0.05 mg/L, and 1.46 mg/L, respectively (Tan et al., 2014a). This implies that dominant *Amphora* species could indicate habitats with high nutrient conditions.

The diatom-based eutrophication/pollution index (EPI-D) could predict the environmental factors DO and ion concentration (e.g., Cl^-) during the dry season (Dell'Uomo, 1996; Tan et al., 2013). Meanwhile, the chloride (Cl^-) concentration also implies pollution or contamination from human activities such as paper mills, textile manufacturing, and pharmaceutical manufacture (Sawyer et al., 2002). Therefore, the appearance of elevated chloride could be considered as an indication of increased risk of chemical pollutants in natural water courses. On the other hand, % Polysaprob indicating the saprobility system has been used to estimate ecosystem status by the relative probability of species occurrences across saprobic zones (Watanabe et al., 1986; Álvarez-Blanco et al., 2013). A larger value of %polysaprob means that water is heavily polluted (e.g., oxygen saturation) is less than 10% and Biochemical Oxygen Demand (BOD₅) is more than 22 mg/L (van Dam et al., 1994). Therefore, these two metrics have incorporated potential responses to chemical pollutants from anthropogenic activities in the derived BD-IBI.

The entire process for the BD-IBI development in this study follows the multimetric approach in general (Reynoldson et al., 1997; Norris and Hawkins, 2000; Wang et al., 2005). The multimetric indices have been criticized for reducing data into one number and many metrics into one metric, which means loss or underestimation of some information. However, the multimetric approach can produce a single final score involving the ecological information and the score is easily compared with a target value for management practices (Reynoldson et al., 1997; Norris and Hawkins, 2000). Also, the additive multimetric indices that are created specifically for environmental quality evaluation are

Table 3

Four component diatom metrics of BD-IBI, minimum and 90th percentile values of each metric used to calculate scores in the 0–10 scaling system (see text for details). Values were obtained from the training-site data set.

	Minimum	90th Percentile
% Prostrate individuals	68.18	97.01
% Amphora individuals	0	5.70
EPI-D	15.9	17.80
% polysaprob	0	15.75

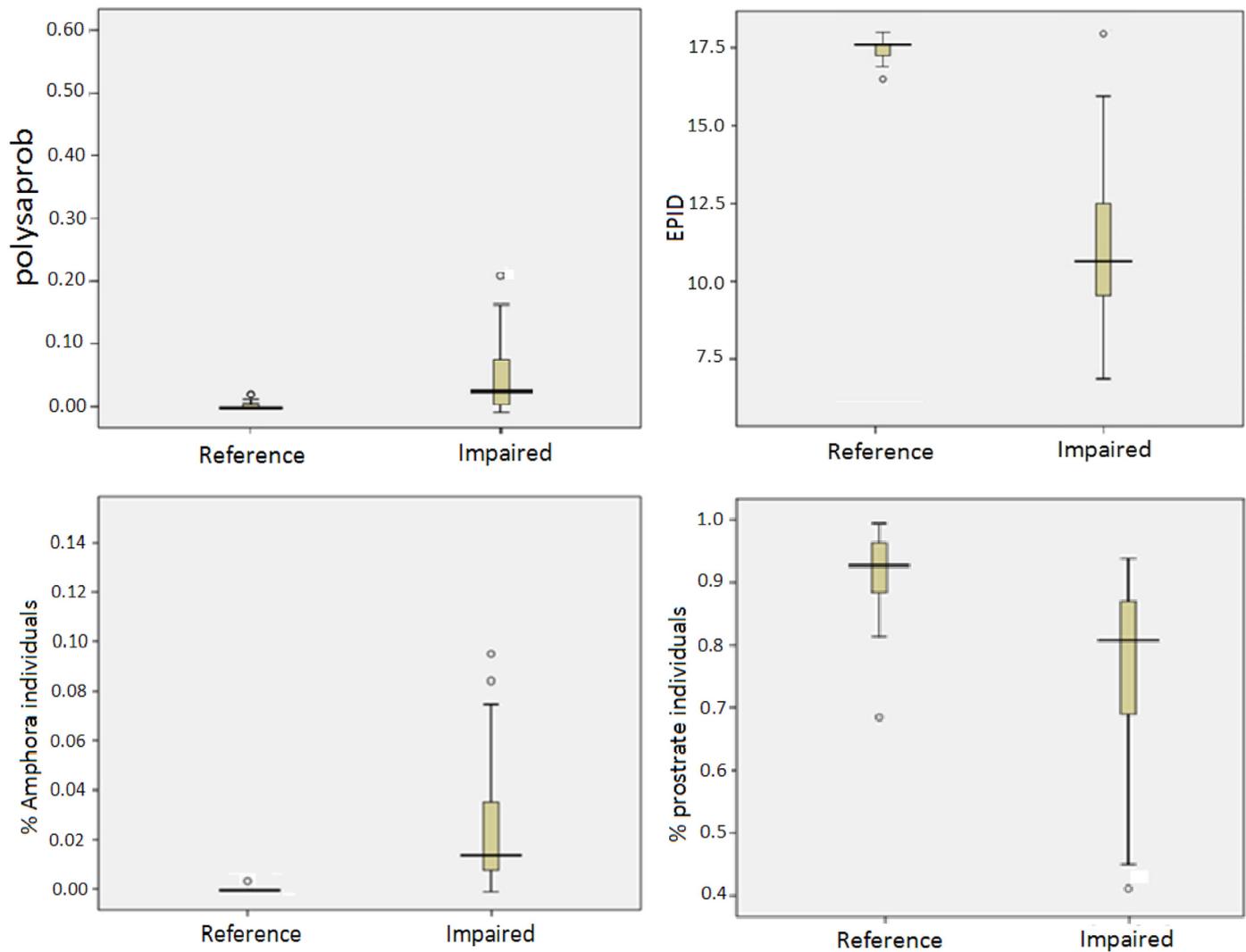


Fig. 3. Box plots of four component diatom metrics of BD-IBI, i.e., % Prostrate individuals, % Amphora individuals, EPI-D and % polysaprob from the training dataset.

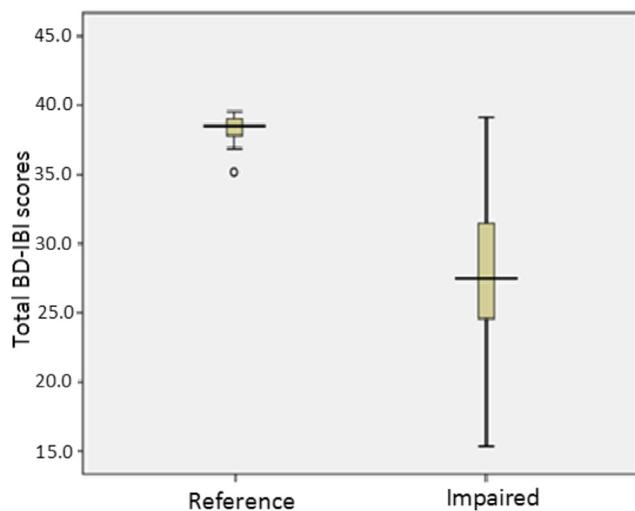


Fig. 4. Box plots of BD-IBI from the training dataset.

effective in identifying degradation at a biological level and perform well when developed from reference data bases (Gerritsen, 1995).

Nevertheless, involvement of the multivariate used in selecting the reference sites in this study has to a large extent avoided some deficiencies when the reference sites were subjectively selected based on some features like water quality. Traditionally, reference conditions in a BD-IBI development are predefined using geomorphological features such as physiography and geology (Reynoldson et al., 1997). In this study, we have introduced the multivariate approach (i.e., MDS) to define the reference and impaired sites in the BD-IBI development. MDS is an iterative ordination technique that can be used until an acceptable solution has been achieved based on the similarity of the species composition. Obviously, this is much more objective for the selection and definition of the reference sites. Therefore, the combination of multivariate and multi-metrics approaches is able to produce a reliable index for bio-integrity evaluation.

The BD-IBI correlated strongly with most of the physical and chemical variables (e.g., nitrogen, phosphorus, and turbidity) (Table 4), while EPI-D was able to predict the water pollution implied by DO, ionic concentration or nutrient in the same region (Tan et al., 2013). However, The BD-IBI is an integrated

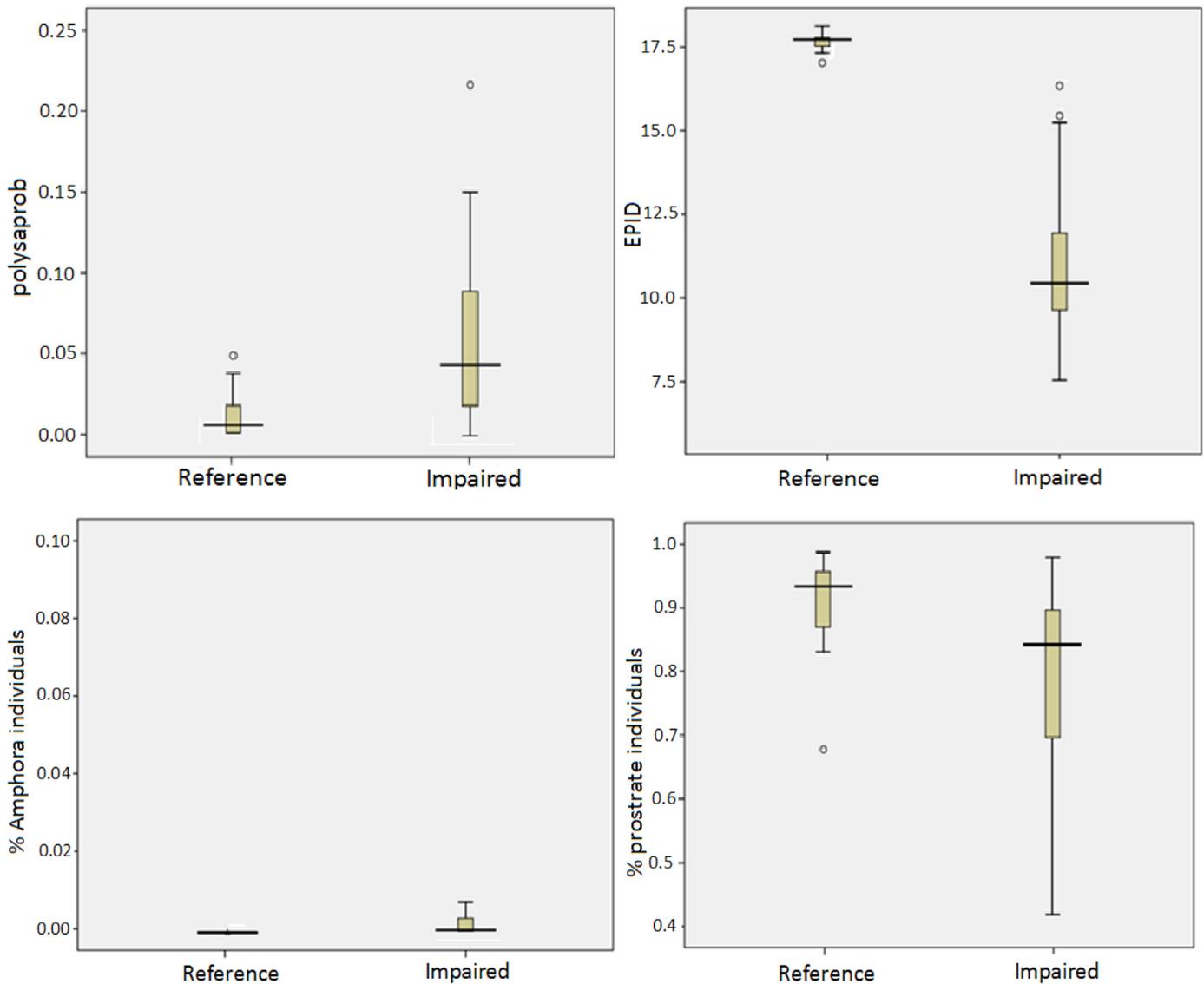


Fig. 5. Box plots of four component diatom metrics of BD-IBI, i.e., % Prostrate individuals, % Amphora individuals, EPI-D and % polysaprob from the testing dataset.

assessment that compares the distance from impairment degree to reference (pristine, intact) conditions on the aquatic environment. The BD-IBI in this study was correlated with the land use including agriculture or forest in the upstream of rivers (Table 4), implying that the derived BD-IBI is capable of indicating the influences from anthropogenic activities. The previous studies also concluded that BD-IBI was a powerful indicator to respond to land use category such as watershed area occupied by farming or forest upstream (Wang et al., 2005; Lavoie et al., 2014). This significant correlation with agriculture land upstream can be explained by agriculture practices that export considerable quantities of nutrients such as nitrogen and phosphorus or organic matter into the waterways (Lavoie et al., 2014).

5. Conclusion

The procedure combining multimetric and multivariate approaches provides a novel way to derive BD-IBI which is applicable to other regions. The derived BD-IBI score can reflect the health status of the rivers, determine the main cause of aquatic

ecosystems deterioration (e.g., nutrient enrichment and landscape alteration), and consequently provide information suitable for the needs of different groups such as policy makers, scientists, and other stakeholders. Finally, the BD-IBI is a powerful tool for evaluation of the biological integrity of river ecosystems subject to anthropogenic impacts and determining their degree of deterioration and degradation in comparison with a pristine condition.

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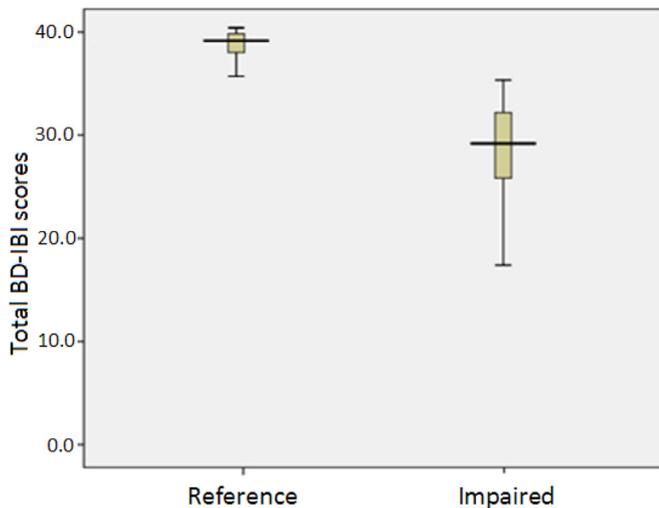


Fig. 6. Box plots of BD-IBI from the testing dataset.

Table 4

The spearman correlation among metrics (or BD-IBI), water quality and land use. (*means significant at $p < 0.05$; **means significant at $p < 0.01$).

Variables	Metrics or BD-IBI				
	%Prostrate	%Amphora	EPI-D	Polysaprob	BD-IBI
depth	.118	.155	-.134	.093	.061
velocity	.020	-.455*	.489*	-.250	.169
t	-.443**	.591**	-.512**	.419**	-.516**
EC	-.385**	.544**	-.586**	.503**	-.575**
DO	.178	-.161	.136	-.158	.179
pH	.401**	.759**	-.109	.080	.020
NH ₄ -N	-.347*	.669**	-.586**	.392**	-.518**
TURB	-.443**	.556**	-.602**	.445**	-.613**
SRP	-.252	.659**	-.421**	.373**	-.454**
TP	-.265	.641**	-.548**	.379**	-.474**
TN	-.372**	.539**	-.493**	.410**	-.475**
DOC	-.295*	-.345*	-.109	.069	-.121
SiO ₂	.347*	.416**	.143	-.177	.261
Cl ⁻	-.414**	.588**	-.567**	.463**	-.556**
SO ₄ ²⁻	-.376**	.568**	-.626**	.422**	-.569**
Ca ²⁺	-.376**	.590**	-.594**	.400**	-.545**
%agriculture	-.433**	.402**	-.591**	.435**	-.584**
%forest	.259	-.440**	.586**	-.507**	.483**

Table 5

The separation powers, correlation index (Col) and Cumulative R² of the benthic diatom index of biotic integrity (BD-IBI) based on the 0-10 scaling system from both training-site and testing-site data sets.

	Training dataset	Testing dataset
Separation powers	3	3
Col	0.15	0.13
Cummulative_R ²	4.10	3.40
Paired t-test	df = 2, t = 1.043, p = 0.406	

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jenvman.2014.12.048>.

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