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Research Paper

Developing a Benthic Index of Biological Integrity and Some Relationships to Environmental Factors in the Subtropical Xiangxi River, China

key words: benthic macroinvertebrate communities, index of biotic integrity, river health, Yangtze River basin

Abstract

The aim of this study is to develop a benthic index of biotic integrity (B-IBI) to help understand how the increasing anthropogenic pressure may impact the subtropical Xiangxi River in China. Benthic macroinvertebrate and environmental surveys were conducted at 77 sites in early summer 2004. Each collection site was categorized as reference or impaired based on physical, chemical, biological, and land-use information. Six non-redundant metrics from 35 metrics were used to differentiate between reference and impaired sites. We selected six metrics for the final IBI. The scoring criteria of each metric were normalized based on the quadrisection and 0–10 scaling systems. Both scaling methods were used to assess the aquatic health of each site in the Xiangxi River watershed. The results showed that most sites were in fairly poor condition. Furthermore, we identified the relationship between B-IBI metrics, water-quality, and land-use variables with a principal component analysis. A composite of nutrients and land-use intensity explained most variances. These results suggest that the B-IBI may be a suitable method for assessing river conditions within the subtropical Xiangxi River in central China.

1. Introduction

Aquatic ecosystems, and their biological assemblages, continued to be degraded globally from anthropogenic activities in their watersheds (Ganasan and Hughes, 1998; Karr, 1999). Part of the decline in water resources stems from insufficient consideration of their biological structure and function (Karr, 1999; Stoddard *et al.*, 2006).

Some factors have been reported which are linked to river degradation. Physical and chemical factors may be related to biological changes (Mullins, 1999). Water quality may only partially reflect environmental impact (Bozzetti and Schulz, 2004). Researchers increasingly include quantitative biological indicators (Joy and Death, 2004; Roset *et al.*, 2007). Biological indicators may reflect the intensity of anthropogenic stress and have been used as a tool in risk assessment and evaluation of human induced changes in freshwater ecosystem (Kamden-Toham and Teugels, 1999). The European Union's Water Framework

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Directive (EUROPEAN COMMISSION, 2000) also highlighted the central role of biological indicators to assess the ecological status of aquatic ecosystem.

There have been three basic approaches to using aquatic organisms to assess water quality. The 1st approach is based on the indicator species concept (GUZKOWSKA and GASSE, 1990). The 2nd approach involves indices of community structure (diversity, evenness, richness, similarity) which have been used extensively in monitoring the impacts of point-source pollution on rivers (BARBOUR *et al.*, 1999; HILL *et al.*, 2000). Biotic indices represent the 3rd approach to using multimetric indices to assess water quality and river ecosystem integrity (KARR *et al.*, 1986; KERANS and KARR, 1994). Over the past decade, multimetric indices have been increasingly used to quantify anthropogenic impairments (KARR *et al.*, 1986; REYNOLDSON *et al.*, 1997).

An index of biotic integrity (IBI) is commonly used to assess river ecosystem health based on multimetric indices (KARR *et al.*, 1986). The IBI usually incorporates a number of taxonomical and ecological measures (into a composite index) that can be tested for their ability to indicate anthropogenic disturbances in river ecosystems (KARR *et al.*, 1986; FAUSCH *et al.*, 1990; SIMON and LYONS, 1995; SIMON, 1998). The IBI was originally developed by KARR (1981) and has been used on all continents but Antarctica (HUGHES and OBERDORFF, 1999). In addition to using fish assemblages for bioassessment (KARR, 1981), the IBI has also been used with other aquatic organisms, such as benthic macroinvertebrates (PLAFKIN *et al.*, 1989; YODER and SMITH, 1999) and periphyton (KERANS and KARR, 1994; HILL *et al.*, 2000). Benthic macroinvertebrates are directly linked to aquatic habitats and the abundance and community structure of benthos are related to chemical and physical conditions, and to substrate type, channel morphology, and type of detritus and aquatic vegetation (RICHARDS and HOST, 1994; KLEMM *et al.*, 2003). The invertebrates are also affected indirectly by changing nutrient concentrations and shifts in primary productivity (RICHARDS and HOST, 1994; WANG and STEVENSON, 2005). This makes them useful as biological indicators. Unlike water quality measurements, which only provide an instantaneous assessment of river conditions, macroinvertebrate assemblages can be used to identify past disturbances and toxic effects that are not readily detected by chemical means (BARBOUR *et al.*, 1999; SOUTHERLAND *et al.*, 2007).

The B-IBI (benthic index of biotic integrity) is based on a series of structural and functional metrics of benthic macroinvertebrate assemblages, and thus helps quantify the impact of environmental deterioration (KERANS and KARR, 1994; SOUTHERLAND *et al.*, 2007). Structural B-IBI components include species richness, habitat guilds, trophic structure, organismal abundance and biodiversity (SIMON and LYONS, 1995). Functional components consist of feeding and trophic categories, environmental tolerance, and individual stress and condition groupings (KARR *et al.*, 1986; FAUSCH *et al.*, 1990; SIMON and LYONS, 1995).

A "reference" condition is a critical element of the B-IBI to help assess the health of the river ecosystem (KARR, 1981; KARR *et al.*, 1986). Reference sites are the segments of water bodies that reflect natural conditions and are least affected by anthropogenic activities (REYNOLDSON *et al.*, 1997). Most rivers exhibit variations in different sections especially from upstream to downstream (QADIR and MALIK, 2009). Upstream sections are generally less degraded with relatively good physical, chemical and biological conditions (QADIR and MALIK, 2009). Sites located in upstream sections are more commonly considered as reference sites and each metric of the benthic macroinvertebrate assemblage is calculated based on regional reference conditions (HUGHES and OBERDORFF, 1999) for assessment of river health (QADIR and MALIK, 2009).

Although much work on the B-IBI has already been done (SIMON *et al.*, 2000; NOVOTNY *et al.*, 2005), we felt it was important for us to continue this work in the Chinese Yangtze River basin due to its geographical importance. Our two main goals were to: (1) develop a B-IBI for a subtropical river in central China to assess the impact of anthropogenic activities on the assemblage of benthos, while also documenting the extent and degree of degradation of the Xiangxi River; and (2) investigate the spatial variations of the B-IBI in relation to physicochemical and land-use variables. We hope the results will provide a baseline for future water quality assessment in the region of the upper Yangtze River. The initiative should help highlight the need for better future protection of benthic macroinvertebrate fauna in rivers from anthropogenic activities.

2. Materials and Methods

2.1. Study Area and Site Setting

The present study was conducted on the Xiangxi River, the biggest tributary of the Three Gorges Reservoir (TGR) in Hubei province. This river is a 6th-order river that originates from the mountains of the Shennongjia Forest, and it has a total distance of 94 km before flowing into the Yangtze River. The average annual precipitation within this watershed is 900–1200 mm. The Xiangxi River has a watershed

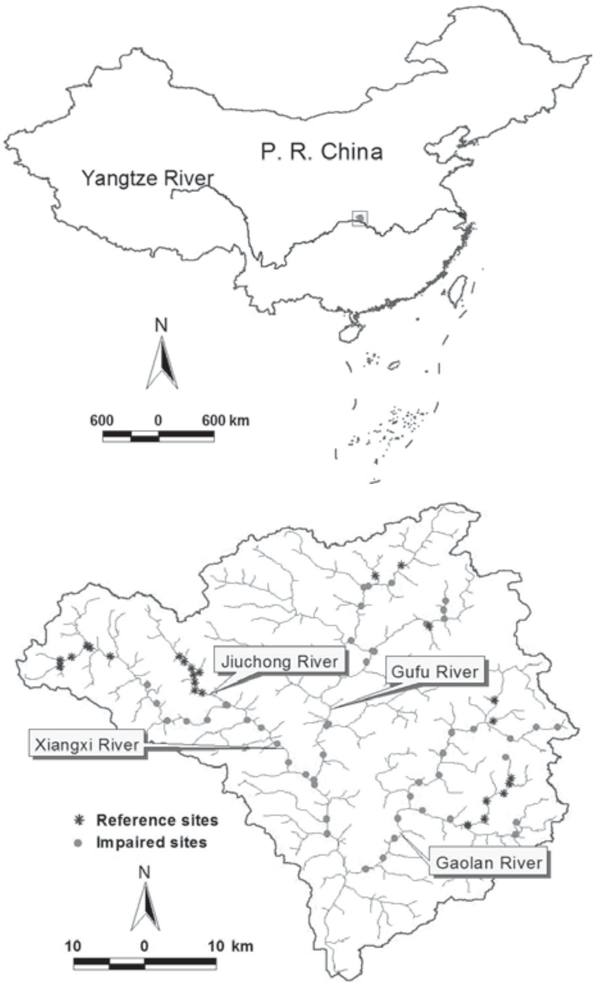

Figure 1. Location of the Xiangxi River watershed in China and the distribution of sampling sites.

area of 3099 km^2, and a descent of 1540 m from the headwaters to its confluence with the Yangtze River. The Gufu River, the Gaolan River, and the Jiuchong River are the three main tributaries (Zhou *et al*., 2008; Wu *et al*., 2009) (Fig. 1). There are complicated natural geological conditions and commercially important mineral resources in this watershed. Indeed the phosphorus reserves here among the top three in China, reach 357 million tons (Li *et al*., 2008). The soil composition and land-use patterns in this watershed are diverse. So too are the obvious changes in dominant tree species along altitudinal gradients. The forest coverage is over 60% (Jiang *et al*., 2002).

Major anthropogenic activities in the watershed that influence the river include mineral, municipal and agricultural activities and small hydropower stations. Point and non-point contaminants are major sources of pollution to the Xiangxi River. Point sources are phosphate plant effluents and municipal waste, whereas atmospheric deposition and agricultural runoff are non-point sources (Li *et al*., 2008). Recently, in this basin, the speed of local economic development is fast because small and medium industrial units are increasing along the banks of the Xiangxi River. About 3000 pollution sources are mainly located near the town of Gaoyang, the old county seat of Xingshan (Ye *et al*., 2003; Li *et al*., 2008).

As the biggest tributary of the Three Gorges Reservoir (TGR) in Hubei province, the Xiangxi River can strongly influence water quality of the TGR (Zhou *et al*., 2008; Wu *et al*., 2009). In order to represent the actual condition, we had two principles for our site selecting: (1) the distance between two sites was about 4 km; and (2) we sampled as many as possible in this watershed. However, some sections, especially the headwaters, were not sampled owing to lack of access. From these two principles, a total of 77 sites were selected to assess the health of the river ecosystem in this watershed.

2.2. Benthic Macroinvertebrate and Water Sampling

Benthic macroinvertebrate sampling was carried out during June 2004. A 0.42 mm mesh Surber sampler was employed to take samples on each occasion, for two or three times, within 100 m range of a sampling site. The sampling area was 900 cm^2. All stones within the sampler frame were scrubbed with a soft brush to remove attached organisms. In areas of unconsolidated substrata, the river bed was sampled to a depth of about 10 cm. Samples were preserved in 10% formalin. The biological samples were identified according to taxonomic references (Morse *et al*., 1994; Merritt and Cummins, 1996).

From each site where benthic macroinvertebrates were captured, surface water samples were also collected. The samples were collected in sulfuric acid-washed (pH < 2) plastic sample bottles, which were immersed at least 30 cm below the water surface (if the depth allowed), capped to exclude air. There are two water bottles for each site, one was preserved by adding sulfuric acid (analytical grade) at pH < 2, and both of them stored at 4 °C (to minimize deterioration prior to chemical analysis) for the analyses of physicochemical concentrations. For each water sample, quality parameters such as conductivity, COD, alkalinity, hardness, calcium, chloride, total nitrogen (TN), nitrate (NO_3^-–N), ammonium (NH_4^+–N), nitrite (NO_2^-–N), total phosphorus (TP), orthophosphate (PO_4^{3-}–P) and silicon were analyzed. All the analyses were carried out according to the standard methods (Huang *et al*., 2000). Additionally, altitude, water temperature, stream width, water depth and current velocity were also measured at each site.

2.3. Collection of Land-Use Data

Applying ETM images (26 Nov 2002) as data sources of the Xiangxi River, the land-use and land cover maps were obtained supported by ERDAS Imagine 8.7 by supervised classification and computer-aided screen interpretation. Watersheds were characterized as to percent of forest, agriculture, scrub/grass, urban, open water and other category (Fig. 2). The soil and water assessment tool (SWAT) was used for quantitatively simulating digitized maps of each sub watershed. When these were combined with digital elevation modes (DEM) that were derived from the relief map of 1:50 000, we were able to calculate the area for each sub watershed with Arc GIS 9.2.

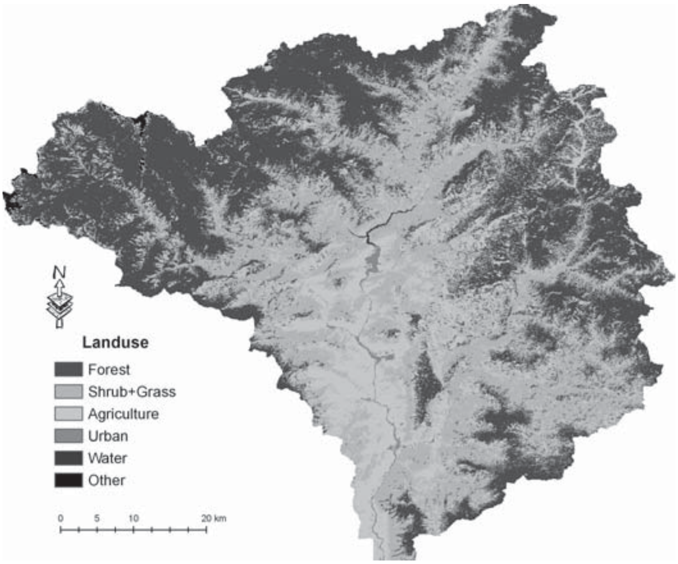

Figure 2. Land-use information in the Xiangxi River watershed.

2.4. Calculation of the B-IBI

2.4.1. Reference Sites Selection

The establishment of reference conditions was based on identification of minimally disturbed land-use sites that represented the best physical, chemical, biological conditions (Zhu and Chang, 2008; Qadir and Malik, 2008). First, we considered sites to be "reference quality" if the rivers had better riparian vegetation, no towns or human communities along the river bank, no gravel-mining activity or hydrologic modification in the watershed, and no wastewater treatment discharges (Blocksom *et al.*, 2002; Morley and Karr, 2002). Second, we referenced the physical, chemical and biological data for each site. We also added the criteria that reference sites have a 100-m forested riparian buffer zone and occur in watersheds that were >60% forested, with <20% of the total watershed consisting of agricultural and urban land-uses (Wang and Stevenson, 2005). We classified all rivers not meeting the above criteria as "impaired sites".

Reference sites possess a maximum B-IBI score which was further compared with other sites located on the same river or on sites located on other rivers of the same ecoregion. This helped to quantify the anthropogenic stress on biological integrity of the aquatic ecosystem. Our selection of least-impaired sites was further confirmed by the application of environmental data through principal component analysis (PCA).

2.4.2. Candidate the B-IBI Metrics

We calculated 35 metrics that were relevant to assess anthropogenic impacts on benthic macroinvertebrate communities. The metrics were based on the structure, composition, and assemblage pattern of benthic macroinvertebrate species (Kerans and Karr, 1994; Barbour *et al.*, 1999; Maxted *et al.*, 2000; Blocksom *et al.*, 2002; Klemm *et al.*, 2003). The B-IBI metrics were categorized into six main groups, namely richness measures, composition measures, tolerance measures, feeding measures, habitat measures and biodiversity index (Table 1).

Table 1. Metrics considered for inclusion in the B-IBI. "Percentage" metrics are calculated based on the total number of individuals collected. EPT means Ephemeroptera, Plecoptera and Trichoptera. Under expected response, an increase means that the metric value should rise with increasing human impact, whereas decrease means that the metric value should decline with increasing impact.

Category	Factor	Metric	Expected response
Richness measures	NT	No. total taxa	Decrease
	NEPT	No. EPT taxa	Decrease
	NE	No. Ephemeroptera taxa	Decrease
	NP	No. Plecoptera taxa	Decrease
	NTR	No. Trichoptera taxa	Decrease
	NC	No. Coloeptera taxa	Decrease
	ND	No. Diptara taxa	Increase
	NCH	No. Chironomidae	Increase
	NCM	No. (Crustacea + Mollusca) taxa	Decrease
Composition measures	PEPT	% EPT	Decrease
	PE	% Ephemeroptera	Decrease
	PP	% Pleccoptera	Decrease
	PT	% Trichoptera	Decrease
	PC	% Coleoptera	Decrease
	PD	% Diptera	Increase
	PCH	% Chironomidae	Increase
	PTT	% Tribe Tanytarsini	Decrease
	PON	% (Other Diptera + noninsects)	Increase
	PO	% Oligochaeta	Variable
	PCM	% (Crustacea + Mollusca)	Decrease
	PDO	% Dominant taxon	Increase
	PTD	% Three most dominant taxa	Increase
Tolerance measures	NI	No. intolerant taxa	Decrease
	PTO	% Tolerant taxa	Increase
	TV	Tolerant value	Decrease
Feeding measures	PS	% Scrapers	Decrease
	PSH	% Shredders	Decrease
	PG	% Gatherers	Increase
	PF	% Filterers	Variable
	PPR	% Predators	Variable
Habitat measures	NCL	No. clinger taxa	Decrease
	PCL	% Clingers	Decrease
	PL	% Legless taxa	Increase
Biodiversity index	SWI	Shannon-Wiener index	Decrease
	EI	Evenness index	Increase

2.4.3. Metric Selection

Metrics showing significant differences between reference and impaired data continued to be considered as candidate metrics. We used five criteria to select our final B-IBI metrics from the candidate metrics. First, we eliminated attributes with medians of 0 for both reference and impaired sites because low values would prevent identification of differences between these two groups (Barbour *et al.*, 1999).

Second, we selected metrics that differed significantly between reference and impaired sites. We performed two-tailed *t*-tests assuming equal or unequal variances, depending on *t*-test results, to determine if differences existed ($\alpha = 0.05$) (Chirhart, 2003). For this analysis, we transformed (natural log) all metrics consisting of proportional data to meet the assumptions of normality.

For metrics that met our first and second criteria, we evaluated the separation power of potential metrics using box plots. We defined separation power as the degree of overlap between boxes (*i.e.*, 25^{th} and 75^{th} quartiles) in box plots of the values of the metric for both reference and impaired sites (Barbour *et al.*, 1999). We assigned a separation power of three when boxes did not overlap between the two site groups, a value of two when interquartile ranges overlapped but did not reach medians, a value of one when only one median was within the interquartile range of the other box, and a value of zero when both medians were within the range of the other box. We then excluded metrics with CV > 1 due to their high deviations (Wang and Stevenson, 2005).

For the fifth criterion, we tested for metric redundancy by conducting Pearson correlations between all combinations of candidate metrics. High correlation coefficients ($r \geq |0.90|$) indicated redundant metrics. We expected some metrics to be highly correlated because they contain similar composition, or they may have similar functional characteristics. When metrics were similar to each other (*e.g.*, number of Trichoptera and percent of Trichoptera), we chose one based on a combination of lowest *P*-value from the *t*-test and lowest CV (Angermeier *et al.*, 2000; Dauwalter *et al.*, 2003).

2.4.4. Scaling Systems and B-IBI Calculation

We used two scaling systems to establish scoring thresholds for each metric (Table 3). First, we used the quadrisection scaling system (DeShon, 1995; Maxted *et al.*, 2000), which used a 6, 4, 2, 0 point system: 1^{st} quarter of the range = 6 points, 2^{nd} quarter of the range = 4 points, 3^{rd} quarter of the range = 2 points, and the remainder of the range = 0 points (see Fig. 3). Standard method was then used to normalize the value of metric, for metrics that decreased with impairment, we used the formula 1; for metrics that increased with impairment, we used the formula 2 (Barbour *et al*., 1999). Here, we used the 95th and 5th percentile to avoid distortion of scores by potentially extreme maximum values.

$$Vi' = Vi/V_{95\%} \qquad \text{(Eq. 1)}$$

$$Vi' = (Vmax - Vi)/(Vmax - V_{5\%}) \qquad \text{(Eq. 2)}$$

where Vi' is the normalized value of metric, Vi is the value of metric, $V_{95\%}$ is the 95^{th} percentile, $V_{5\%}$ is the 5^{th} percentile, $Vmax$ is the maximum value of metric.

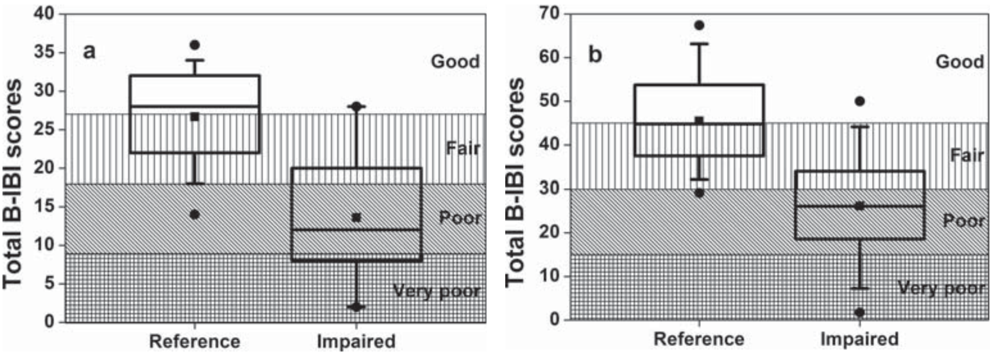

Figure 3. Box plots of total B-IBI scores of reference sites and impaired sites and their assessment. A: Quadrisection scaling system; B: 0–10 scaling system. Boxes show interquartile ranges (25^{th} and 75^{th} percentiles), middle lines are medians, middle squares are averages, whiskers are 1.5 interquartile ranges beyond the boxes, and black dots are outliers.

Second, we used a 0–10 scaling system on the ranges of metrics (Hill *et al.*, 2000). For metrics that decreased with impairment, we divided the value of the metric for each site by the 95th percentile of reference site values, and multiplied by 10 (Eq. 3). For metrics that increased with impairment, we divided the metric value by the 95th percentile of impaired site values, subtracted this value from 1, and multiplied the result by 10 (Eq. 4):

$$Vi' = 10Vi/V_{95\%R} \qquad \text{(Eq. 3)}$$

$$Vi' = 10(1 - Vi/V_{95\%I}) \qquad \text{(Eq. 4)}$$

where Vi' is the score of the B-IBI, Vi is the value of metric, $V_{95\%R}$ is the 95th percentile of reference sites, $V_{95\%I}$ is the 95th percentile of impaired sites.

The total B-IBI values were the sums of metric scores based on each scaling system.

2.5. *Relationships between Metrics and Environmental Variables*

We examined the relationship between our B-IBI metrics and selected water-quality and land-use variables at each site by using a Spearman's rank correlation. We also assessed the spatial variations of the B-IBI in relation to physicochemical and land-use variables by using a principal component analysis (PCA).

3. Results

3.1. *Reference Sites Selection*

Our reference site criteria selected 27 of 77 sites as reference sites. Sites located in upstream sections have generally been considered as reference sites. Reference sites were generally less degraded, and had relatively less variation in their physical, chemical, and biological conditions. Reference sites also showed lower average nutrient concentrations than impaired sites (Table 2).

3.2. *Metric Selection*

Of the 35 candidate metrics tested for the B-IBI, five metrics (NCM, PTT, PO, PCM and PTO) were eliminated because their medians were zero for both reference and impaired sites (Table 3). We rejected 13 metrics (NC, NCH, PP, PT, PC, PDO, PTD, TV, PSH, PG, PF, PPR and PCL) because Spearman tests indicated nonsignificant differences ($P > 0.05$) between reference and impaired sites (Table 3). We then compared the remaining metrics based on separation power, CV and redundancy. We eliminated ND, PEPT, PE, PD, PCH, PON, PL and EI because of their lower separation power ($IQ < 2$), and we removed NP because of higher CV ($CV > 1$) (Table 3). Pearson correlation coefficients between differentiating metrics resulted in three pairs of highly correlated metrics ($r \geq |0.90|$, Table 4), indicating metric redundancy. We also removed NEPT and NI because of higher CV values. Finally, the six qualified the B-IBI metrics included: NT (number of total taxa), NE (number of Ephemeroptera taxa), NTR (number of Trichoptera taxa), PS (Percent of Scrapers), NCL (number of clinger taxa) and SWI (Shannon-Wiener index). The box plots for each metric showed good separation between reference and impaired sites (Fig. 4)

Table 2. Water quality and land-use values for reference and impaired sites in the Xiangxi River.

Variables	Measured value ± *SD*		*P* value
	Reference sites	Impaired sites	
Altitude (m)	917.26 ± 231.82	537.64 ± 301.40	0.000
Water temperature (°C)	17.17 ± 4.73	19.44 ± 2.98	0.046
Water width (m)	8.10 ± 3.77	17.92 ± 20.46	0.012
Water depth (m)	0.37 ± 0.25	0.47 ± 0.58	0.421
Current velocity ($m \cdot s^{-1}$)	0.70 ± 0.45	0.82 ± 0.46	0.259
Conductivity ($\mu S \cdot cm^{-1}$)	126.72 ± 48.73	195.70 ± 51.05	0.000
COD ($mg \cdot L^{-1}$)	2.41 ± 1.29	3.56 ± 1.66	0.004
Alkalinity ($mg \cdot L^{-1}$)	115.67 ± 45.07	150.67 ± 37.34	0.006
Hardness ($mg \cdot L^{-1}$)	6.35 ± 2.67	9.33 ± 2.34	0.000
Calcium ($mg \cdot L^{-1}$)	19.08 ± 7.01	20.89 ± 10.06	0.528
Chloride ($mg \cdot L^{-1}$)	4.72 ± 1.76	4.97 ± 1.54	0.350
TN ($mg \cdot L^{-1}$)	1.56 ± 1.38	2.78 ± 1.34	0.002
NO_3^-–N ($mg \cdot L^{-1}$)	0.55 ± 0.25	0.86 ± 0.29	0.000
NH_4^+–N ($mg \cdot L^{-1}$)	0.08 ± 0.07	0.11 ± 0.21	0.636
NO_2^-–N ($\mu g \cdot L^{-1}$)	1.11 ± 3.20	2.40 ± 7.44	0.395
TP ($mg \cdot L^{-1}$)	0.03 ± 0.03	0.17 ± 0.26	0.008
PO_4^{3-}–P ($mg \cdot L^{-1}$)	0.02 ± 0.01	0.04 ± 0.08	0.187
Silicon ($mg \cdot L^{-1}$)	5.59 ± 2.57	5.57 ± 3.86	0.967
Area (km^2)	64.24 ± 54.26	464.97 ± 483.31	0.000
% Agriculture	13.18 ± 6.29	16.02 ± 4.32	0.026
% Forest	71.54 ± 13.86	60.18 ± 12.88	0.002
% Scrub/Grass	10.91 ± 9.79	23.02 ± 11.12	0.000
% Urban	0.06 ± 0.10	0.12 ± 0.15	0.038
% Other category	4.32 ± 6.18	0.65 ± 1.12	0.000

3.3. Metric Scoring

We selected six metrics for the final B-IBI (Table 5), and normalized the scoring criteria of each metric based on the quadrisection and 0–10 scaling systems (Table 5). The total B-IBI scores were then calculated, using either scaling system, and differences between reference and impaired sites were apparent (Pearson test, both $P < 0.01$; Fig. 4). There was a strong significant positive relationship between the B-IBI scores for quadrisection scaling system and 0–10 scaling system (Pearson test, both $P > 0.05$, Fig. 5). Therefore, both of them could be used for the further analysis (Fig. 5).

Both scaling methods were used to assess the health conditions for each site in the Xiangxi River watershed. The results showed that most sites were in fair to poor conditions (Fig. 4).

3.4. Metric-Environmental Variable Relationships

Correlations between metric values and environmental variables confirm prior expectations of how metrics can help assess river conditions. Pearson rank correlations indicated that most physicochemical and land-use variables were significantly correlated with the B-IBI scores and their metrics ($P < 0.05$). Land-use and watershed area appeared to be the most significant and strongly related variables to the B-IBI metrics (Table 6). The percent of the forest cover was also strongly positively correlated with the component metrics,

Table 3. Five criteria were used for metric selection. Note the results of analysis of covariance between metric values of reference and impaired sites. Metrics were considered to differentiate significantly at $P \leq 0.05$.

Variable	Median		*P* value	IQ value	*CV* value
	Reference	Impaired			
NT	15.00	8.00	0.000	3.00	0.51
NEPT	9.00	4.50	0.000	3.00	0.62
NE	6.00	3.00	0.000	3.00	0.57
NP	1.00	0.00	0.023	3.00	1.80
NTR	2.00	1.00	0.003	3.00	0.80
NC	2.00	0.00	0.060	3.00	1.10
ND	3.00	2.50	0.010	1.00	0.70
NCH	0.00	1.00	0.092	1.00	1.18
NCM	0.00	0.00	0.013	0.00	2.33
PEPT	0.86	0.77	0.000	1.00	0.39
PE	0.68	0.47	0.015	1.00	0.53
PP	0.01	0.00	0.234	0.00	2.18
PT	0.13	0.09	0.308	0.00	1.06
PC	0.03	0.00	0.586	1.00	1.40
PD	0.06	0.08	0.009	0.00	1.27
PCH	0.01	0.04	0.012	0.00	1.92
PTT	0.00	0.00	0.470	0.00	4.87
PON	0.10	0.15	0.000	1.00	1.19
PO	0.00	0.00	0.820	0.00	4.88
PCM	0.00	0.00	0.019	0.00	3.76
PDO	0.14	0.18	0.489	0.00	0.94
PTD	0.44	0.41	0.062	0.00	0.62
NI	7.00	3.00	0.000	3.00	0.65
PTO	0.00	0.00	0.022	0.00	2.50
TV	2.70	4.01	0.114	2.00	0.40
PS	0.42	0.20	0.047	2.00	0.68
PSH	0.01	0.00	0.215	0.00	2.31
PG	0.32	0.48	0.261	1.00	0.51
PF	0.04	0.04	0.364	0.00	1.37
PPR	0.13	0.09	0.082	0.00	1.01
NCL	10.00	4.50	0.000	3.00	0.56
PCL	0.58	0.55	0.886	0.00	0.45
PL	0.10	0.15	0.000	1.00	1.20
SWI	2.11	1.66	0.049	2.00	0.28
EI	0.76	0.83	0.037	1.00	0.16

Table 4. Pairwise correlations among component benthic macroinvertebrate metrics of the B-IBI. Metrics were considered to differentiate significantly at $P \leq 0.05$.

	NT	NEPT	NE	NTR	NI	PS	NCL
NEPT	0.838						
NE	0.734	0.928					
NTR	0.734	0.715	0.471				
NI	0.896	0.916	0.795	0.742			
PS	0.378	0.562	0.532	0.339	0.505		
NCL	0.860	0.875	0.807	0.662	0.906	0.507	
SWI	0.835	0.686	0.585	0.519	0.749	0.382	0.733

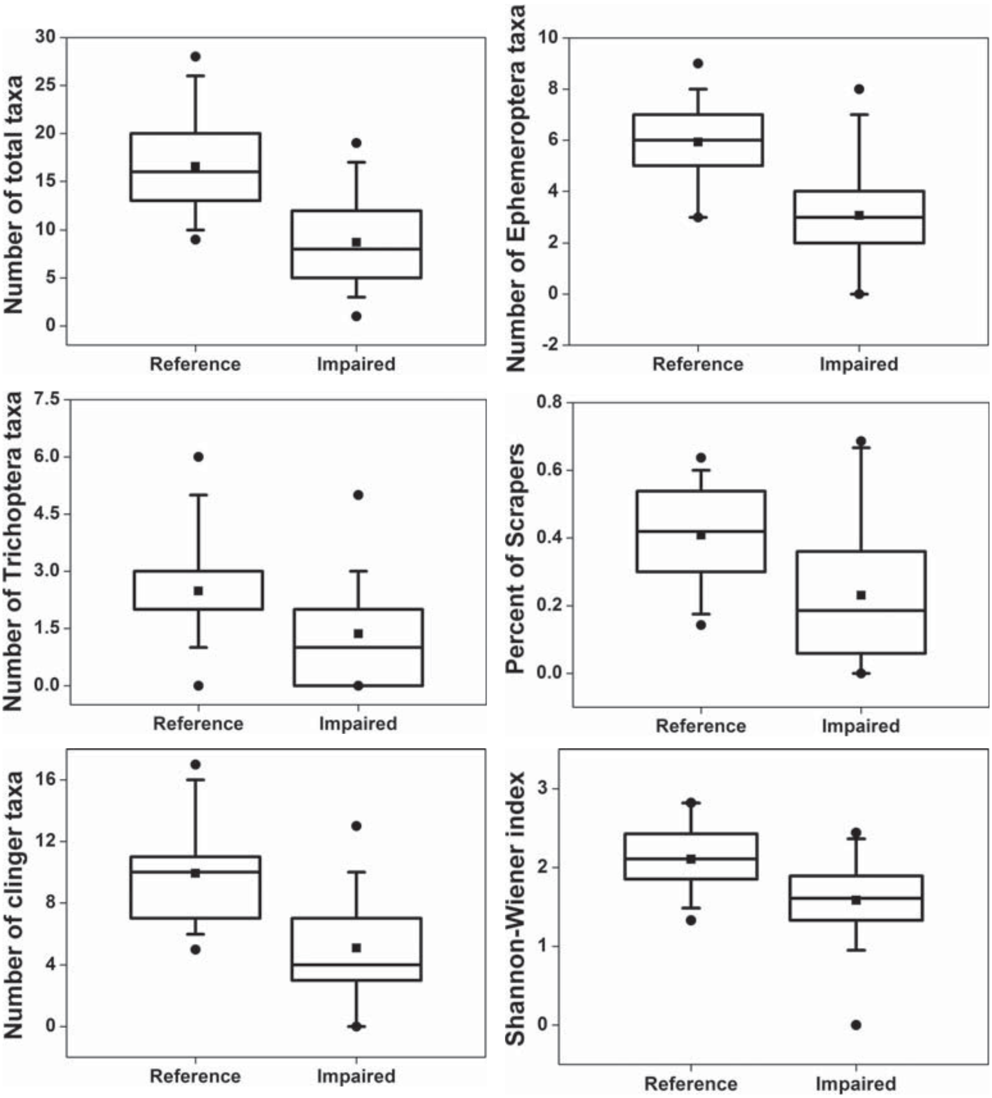

Figure 4. Box plots of component benthic macroinvertebrate metrics for reference and impaired sites. Boxes show interquartile ranges (25th and 75th percentiles), middle lines are medians, middle squares are averages, whiskers are 1.5 interquartile ranges beyond the boxes, and black dots are outliers.

especially NE and NCL (Table 6). In contrast, the B-IBI and its component metrics were strongly negatively correlated with percent agriculture, percent urban and percent scrub/grass land-use (Table 6). Although the water physicochemical variables had significant differences with the B-IBI scores and their metrics ($P < 0.05$), compared with land-use, they were less important.

Water-quality and land-use data for 24 variables from 77 sample sites were included in the PCA (Table 7). The PCA 1–5 had eigenvalues >1 and suggested meaningful results.

Table 5. Metric values of six components and their scores. These were based on the quadrisection scaling system, and 95th percentile values of reference and impaired sites were used to calculate scores in the 0–10 scaling system.

Metric	Quadrisection scaling system				0–10 scaling system (95th percentile)	
	0	2	4	6	Reference sites	Impaired sites
NT	<5.29	5.29–10.57	10.58–15.85	≥15.86	25.00	17.55
NE	<2.00	2.00–3.99	4.00–5.99	≥6.00	8.00	6.55
NTR	<1.25	1.25–2.49	2.50–3.74	≥3.75	5.00	3.55
PS	<0.15	0.15–0.30	0.31–0.45	≥0.46	0.60	0.56
NCL	<3.29	3.29–6.57	6.58–9.85	≥9.86	15.75	10.00
SWI	<0.63	0.63–1.26	1.27–1.89	≥1.90	2.78	2.31

This accounted for 28.36%, 19.17%, 12.94%, 7.57% and 4.95% of the among-site variance, respectively. The PC 1 represented land-use intensity variables, and the PC 2 and 3 represented composite physicochemical variables. A biplot of PC 1 versus PC 2 indicated that reference sites were grouped (Fig. 6a, b). However, the impaired sites, with high land-use intensity and/or physicochemical concentrations, were grouped (Fig. 6a). The biplot of PC 1 versus PC 3 indicated that most reference sites were grouped but several of the more distant sites showed only a weak differentiation of alkalinity, hardness and silicon (Fig. 6b).

The PCA revealed some predictable relationships among physicochemical and land-use variables. For example, both altitude and land-use were strongly correlated with the PC 1, and accounted for 28.36% of the data variance. The PC 1 indicated a positive association with percent agriculture, percent urban, percent scrub/grass land-use, and watershed area and a negative association with altitude and percent forest land-use. In addition, water quality variables should also be positively related to the PC 2.

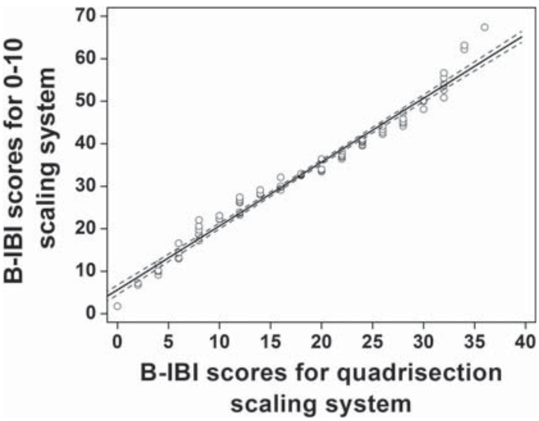

Figure 5. Relationship between B-IBI scores for quadrisection scaling system and 0–10 scaling system with 95% confidence intervals.

Table 6. Significant ($a = 0.05$) Pearson rank correlations between B-IBI scores, core metrics and tested physicochemical and land-use variables. B-IBI score 1 indicates that they are from quadrisection scaling system, while B-IBI score 2 indicates that they are from 0–10 scaling system.

Variable	B-IBI score 1	B-IBI score 2	NT	NE	NTR	PS	NCL	SWI
Altitude	0.152	0.130	0.130		0.314			
Water temperature	–0.301	–0.266	–0.292	–0.232			–0.229	–0.388
Water width	–0.287	–0.280		–0.265	–0.104	–0.135	–0.262	
Water depth	–0.326	–0.297		–0.253	–0.316		–0.260	
Current velocity					–0.277			
Conductivity			–0.123	–0.114				–0.130
COD	–0.185	–0.152	–0.188	–0.167		–0.179	–0.127	–0.220
TN	–0.100			–0.119	–0.169			
Area	–0.625	–0.606	–0.625	–0.601	–0.484		–0.602	–0.472
% Agriculture	–0.383	–0.384	–0.335	–0.411		–0.292	–0.405	–0.409
% Forest	0.430	0.424	0.405	0.510			0.510	0.443
% Scrub/Grass	–0.471	–0.453	–0.474	–0.538			–0.535	–0.490
% Urban	–0.341	–0.327	–0.248	–0.327		–0.232	–0.361	–0.333
% Other category	0.394	0.367	0.429	0.351		0.253	0.338	0.440

Table 7. Principal component loadings of physicochemical variables and land-use variables from 77 Xiangxi River sites.

Variable	PC 1	PC 2	PC 3
Altitude	**–0.712**	–0.224	–0.118
Water temperature	0.590	–0.563	–0.292
Water width	0.517	0.530	0.139
Water depth	0.335	0.602	0.119
Current velocity	0.129	0.470	0.350
Conductivity	–0.517	0.501	0.415
COD	–0.465	**0.689**	–0.037
Alkalinity	0.272	–0.220	**0.877**
Hardness	0.411	–0.438	**0.732**
Calcium	0.198	0.393	–0.161
Chloride	0.143	0.044	–0.077
TN	–0.395	**0.748**	0.048
NO_3^-–N	0.693	–0.363	0.097
NH_4^+–N	0.057	0.272	0.124
NO_2^-–N	0.446	**0.647**	0.029
TP	0.587	0.476	0.098
PO_4^{3-}–P	0.455	**0.638**	0.057
Silicon	0.258	–0.253	**–0.728**
Area	–0.682	0.609	0.106
% Agriculture	0.593	–0.161	–0.556
% Forest	**–0.731**	0.093	0.507
% Scrub/Grass	**0.809**	–0.188	–0.256
% Urban	**0.712**	0.388	–0.220
% Other category	–0.629	0.421	–0.282
% PCA variance	28.36	19.17	12.94
$\sum$ % PCA variance	28.36	47.53	60.47

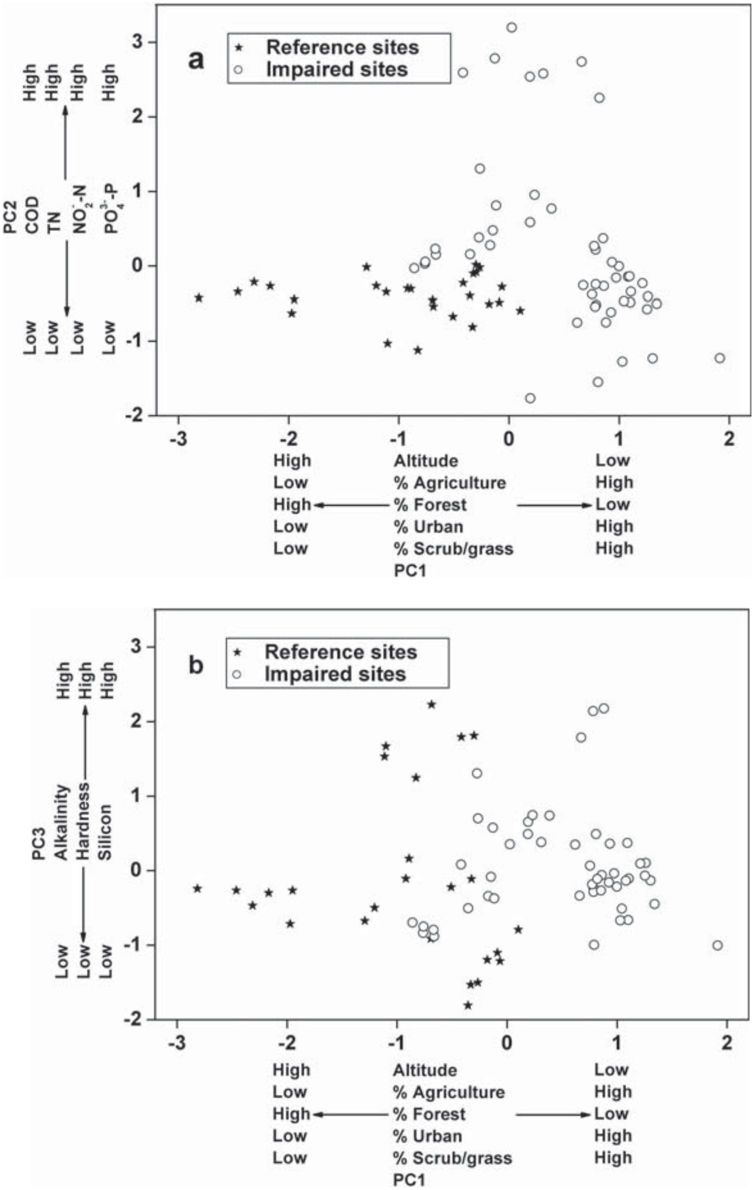

Figure 6. Plot of composite land-use intensity variables versus composite physicochemical variables from a PCA comparing variables from 77 Xiangxi River sites in central China: (a) PC 1 versus PC 2; (b) PC 1 versus PC 3.

4. Discussion

We developed the B-IBI for the subtropical Xiangxi River in central China. Our selection yielded metrics similar to those commonly found useful for detecting river conditions elsewhere. We included taxonomic richness and composition, tolerance composition, feeding groups, habitat and diversity composition. We selected six metrics from 35 candidates. Although we only selected six metrics, we believe our reference-site classification and metric-selection processes selected the B-IBI metrics with proven abilities to differentiate river-site quality. For these reasons, and the fact that the data used represented all three major tributary drainages in this ecoregion, we believe that the Xiangxi River B-IBI was a good indicator to assess the impairment caused by human disturbance.

4.1. Reference Selection

Criteria for distinguishing reference and impaired sites, or contrasting biological conditions at sites (*e.g.*, good, fair, poor, very poor) using the B-IBI or single metrics, may be established with many methods. These may include levels desired by the public, percentiles of frequency distributions of conditions at all impaired or reference sites (Barbour *et al.*, 1999). The B-IBI that we developed for the Xiangxi River separated reference from impaired sites for all sites where collections were made.

Some procedures used for the B-IBI development, such as the *t*-tests we used, rely heavily on *a priori* categorizations of sample sites. Although our site selections also included subjective goals, we think that good reference sites were chosen. Water quality and land-use distributions revealed that our reference sites, and many impaired sites, tended to have low nutrient levels and land-use intensity. Subsequently, impaired sites were categorized as such because of visible disturbances (*e.g.*, point sources or hydropower plant), even if water quality and land-use suggested no impairment. Consequently, some impaired sites were interspersed among reference sites in Figure 6a.

4.2. B-IBI Development

Our metric-selection criteria for the Xiangxi River B-IBI reflected a combination of techniques used to develop past B-IBI. Indices composed of different metrics may be sensitive to different impacts (Barbour *et al.*, 1999). Indices also differ by their types of scaling (discrete or continuous scoring), the manner in which expectations are set for each metric, and how metric scores are aggregated into an overall score. Different scoring methods affect indicator variability and may affect the ability of an index to categorize condition (Blocksom *et al.*, 2002).

We included both order- and family-based attributes in our metrics. Species metrics, however, may be much better for the development of the B-IBI owing to their different environmental optima, sensitivities, and tolerances (Hill *et al.*, 2001). However, species metrics may be more suitable for regional use in specific aquatic habitats in small geographic areas, rather than for broader regional use. This is because the number of species is likely to be correlated with the size and habitat diversity of the region. We used our evaluation system to assess the health of river ecosystem in the upper Yangtze River, and we also recommend its use elsewhere. Because of its fine scale (Hill *et al.*, 2000), we recommend using the 0–10 scaling system for the B-IBI scoring. We observed no distinct difference, in correct assessment of sites using the quadrisection *vs*. 0–10 scaling systems, but the 0–10 system is analytically simpler to compute thus, easier for naive users to implement accurately.

4.3. Comparison between Multimetric B-IBI and Single Metrics

The advantages of using a multimetric system over a univariate assessment include: (1) the assumed greater certainty of multimetrics in detecting impairment, and (2) the transferability of multimetrics among habitats both within and among regions (Barbour *et al.*, 1999). Our multimetric B-IBI had a higher separation power and lower CV than single metrics. Ideally, multimetric indices should be constructed to reflect multiple types of stressors occurring within the region of interest. Multimetric systems can provide more thorough integration of the overall system condition because no single metric is sensitive to all types of stressors (Karr, 1999). Moreover, multimetrics can compensate for erroneous responses of a few metrics, and may incorporate information related to multiple ecological attributes that are valued by both decision makers and stakeholders.

A potential consequence of using a multimetric B-IBI is that it can mask responses of individual metrics because of averaging (Suter, 1993). Meaningful metrics that represent different ecological conditions should be examined. An individual criterion could be based on a metric indicating a valued ecological attribute of sufficient public or ecological concern that merits specific management attention (Campbell, 2001). For example, the number of Ephemeroptera taxa and number of clinger taxa, which are highly correlated with percent of forested land-use in this watershed, could be used to set the minimum criteria for water-quality standards.

4.4. Relationships between the B-IBI and Environmental Variables

All six metrics were shown to have significant correlations with at least two of the selected physicochemical and land-use variables. Because of the many pairwise comparisons, some of these correlations may have reflected chance alone. However, such established relationships may help managers discern causes of river impairment. For example, altitude, land-use, nutrient and COD were most consistently correlated with the B-IBI metrics. Similar relationships have been shown in other regions and are thought to be the most important factors contributing to river degradation in the world (Lammert and Allan, 1999; Waite and Carpenter, 2000; Dauwalter *et al.*, 2003).

Nutrient, and most land-use variables, showed positive relationships with the PCA. This may reflect the heavy agricultural land-use identified in the Xiangxi River watershed. Nutrients from fertilizers or livestock excretory products may flow directly into the river during episodic events such as runoff from storms. A plot of PCA scores showed that "reference sites" grouped together (Fig. 6). The observed grouping indicated that land-use intensity and chemical variables were important attributes of analyzed reference sites. This finding is characteristic of a useful B-IBI. The groupings also indicated that many sites were in relatively good condition, which was concordant with the distribution of B-IBI scores for the Xiangxi River sites (Fig. 6). As expected, sites judged to have the least human impacts based on riparian and watershed land-uses had the best B-IBI scores. Agricultural sites had scores lower than the least-impacted sites but higher than those of urban sites, which had the worst scores. This result corresponds with the findings concerning the relative impacts of agricultural and urban land-use on Wisconsin perennial rivers (Wang *et al.*, 2003; Lyons, 2006).

We considered that if there were big physical, chemical and biological differences between the upper and lower reaches, that stream could be regarded as having some "stratification". However, all streams flow downhill from some upper elevation to some lower elevation, so there is commonly some increase in physiochemical factors and volume of flow as one travels downstream. If there were no sudden or dramatic differences in physical, chemical or biological conditions along the stream, then the stratification may not be an appropriate term to classify streams. Additionally, we also considered that stratification by altitude should be classified into several categories, such as the lower altitude (<1000 m), the medium altitude (1000–2000 m), the medium to strong altitude (2000–3000 m), and the higher altitude (>3000 m). Both sites in the Xiang River belonged to lower altitude as the classification criteria, so we recommend no further stratification of altitude for the B-IBI in this watershed.

Although we think that disturbance sensitive metrics were selected, and that the B-IBI presented herein can differentiate relative river conditions, we also think that the B-IBI criteria may need further adjustment for easier application by managers. Also, validation of categorizations must be conducted later on independent river sites with independent judgment criteria for both small (<100 km^2 watersheds) and large (≥100 km^2) rivers. These tasks will be challenging given that thresholds are only arbitrary when considering a continuous river disturbance gradient, and that the B-IBI is generally considered one of the best ways to measure river conditions. Thus, validation may only be possible for reference categorizations by using minimally disturbed river sites.

In summary, Xiangxi River has a characteristic benthic macroinvertebrate fauna that responds in a consistent and predictable manner to human environmental degradation. A B-IBI with six metrics portrays the pattern of benthic macroinvertebrate assemblage change in response to human degradation. As such it is an accurate and reasonably precise measure of river environmental quality. Zhu and Chang (2008) used a modified Karr's Fish IBI for their work on the upper Yangtze River. We used an assemblage of benthic macroinvertebrates to develop the B-IBI in the Xiangxi River, the largest tributary of the Three-Gorges Reservoir in Hubei Province. However, the IBI still needs further testing and validation in other ecological areas with different fauna.

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