Application of multiple biological indices for river health assessment in northeastern China

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Abstract – Bioassessment has become a hot topic and could provide important information for river management and restoration. In this study, we developed multiple biological indices using three groups of aquatic organisms – benthic algae, macroinvertebrates and fish – for river health assessment. The main steps for development of multiple biological indices were selection of sensitive indices, optimization of core indices, setting target and critical values for core indices, and calculation of final scores of health condition. In order to evaluate the 19 candidate indices, disturbance gradients were constructed for water contamination, sediment contamination and land use change using principal components analysis. Thirteen indices showed significant and relatively high coefficients of determination with the disturbance gradients using multiple regression analysis ($P < 0.05$). Six indices – two indices each for benthic algae, macroinvertebrates and fish – were finally selected as the core indices using correlation analyses. River health condition scores were assigned to five categories: critical, poor, fair, good and excellent. One site was classified as critical and 10.5% of sites were in poor condition. Meanwhile, 45.6% of the sampling sites were classified as fair and 42.1% were either good or excellent. Regression analysis between the final scores and disturbance gradients confirmed the usefulness of multiple biological indices as a confident bioassessment method. Thus, we highly recommend using multiple biological indices to assess river health that will inform river management and restoration strategies.

Key words: River health assessment / multiple biological indices / bioassessment / target and critical values

Introduction

Rivers and streams are valuable ecosystems that sustain the biodiversity of freshwater organisms and provide different goods and services for human beings (Karr and Chu, 1999; Millennium Ecosystem Assessment, 2005; Arthington *et al.*, 2006). River health assessment using biological indices continues to be widely used since its initial development several decades ago (Hynes, 1960; Norris and Hawkins, 2000). Benthic algae, macroinvertebrates and fish are commonly used in bioassessment due to their importance for riverine ecosystems, representation of river health condition and convenience for sampling, identification and calculation of biological indices (Rosenberg and Resh, 1993; Chessman and Royal, 2004; Kennard *et al.*, 2006).

By integrating different kinds of biological indices, multimetric approaches of bioassessment have been shown to be comprehensive methods reflecting different kinds of human disturbances, and have therefore been applied widely for freshwater ecosystems (Karr, 1981; Hawkins, 2006). Different taxonomic groups respond differently to environmental stressors (Hering *et al.*, 2006). Biological indices of macroinvertebrates usually show higher sensitivities for organic pollution (Álvarez-Cabria *et al.*, 2011) and heavy metal contamination (Clements *et al.*, 2000; Qu *et al.*, 2010) than fish and benthic algae. The close relationship between in-stream heterogeneity of substrate and macroinvertebrate biodiversity make macroinvertebrate indices more suitable to reflect in-stream habitat disturbances, such as dredging, sedimentation, and sand and gravel extraction (Bae *et al.*, 2011; Zheng *et al.*, 2011). Benthic diatoms usually show higher sensitivity and stronger response at low levels of nutrient

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concentrations, often before the effects on fish and macroinvertebrate communities are observed (Allan and Castillo, 2007). Thus, benthic algae may be a better indicator of eutrophication than other organisms (Stevenson and Pan, 1999). Fish usually show higher sensitivity to hydraulic alteration in rivers and as such are suitable indicators of flow regulation (Arthington *et al.*, 2006). Dam construction usually causes severe degradation of fish communities; however, the effects may not be clearly identified in the short-term by benthic algae (Wu *et al.*, 2009).

In the past, most approaches often used one kind of aquatic organism to assess river health, based on expert opinion and budget limitations (Barbour *et al.*, 1999; Boulton, 1999). In recent years, improved understanding of the relationships among the physical, chemical and biological aspects have led to comprehensive assessments that use a wider range of aquatic organisms and ecosystem processes (Flinders *et al.*, 2008; Meng *et al.*, 2009; Bunn *et al.*, 2010; Bae *et al.*, 2011, 2014). Considering the long-term history of development and severe stresses to Chinese rivers, multiple biological indices have been applied by the National River and Lake Health Program (NRHLP) for river management and restoration. The NRHLP aims to evaluate the current condition of rivers and lakes and was initiated by the Ministry of Water Resources of China. It developed a comprehensive river health assessment based on five components of biological, physical, chemical, hydrological integrity and ecological services. Multiple indices of benthic algae, macroinvertebrate and fish were recommended to assess river health for the biological integrity component of the NRHLP.

In this study, we describe how we selected the suitable biological indices by deriving abiotic stressor gradients to determine target and critical values for biological integrity. The first objective was suitable indices selection, those which responded to human disturbances and environmental stressors. The second objective was health score calculation that combined several indices from different categories of aquatic organisms.

Materials and methods

Study area

The Taizi River is located in southeastern Liaoning Province, China, bordering the Yellow Sea and the Bohai Gulf to the south. The river is 413 km long with a watershed area of 1.39×10^4 km². The research area is located at middle and high latitudes within the temperate continental monsoon climate zone. The Taizi River supplies the main water resources for domestic, industrial and irrigation purposes for the surrounding cities and farmland in the watershed. Industrial development and urbanization have caused severe water contamination in the Taizi River during the past few decades.

Sample collection

Benthic algae, macroinvertebrates and fish were collected from 57 sampling sites in the Taizi River from August to September 2014 (Fig. 1). The sampling region was classified into three stream types of highland, midland and lowland based on patterns of altitude, precipitation and macroinvertebrate communities (Leigh *et al.*, 2012; Kong *et al.*, 2013). Among the 57 sampling sites, 23 sites were located in the highland, 20 sites were located in the midland and 14 sites were located in the lowland (Fig. 1). A 300-m section of stream channel was selected to sample the aquatic organisms at each site.

Fish were sampled by electrofishing for 30 min. Due to the high mobility of fish, the upstream and downstream sections of the sampling reach were blocked with a gill net (1×1 cm² net size) during the sampling period. All collected fish were identified, enumerated and weighed *in situ*. Identifications were to the species level according to Zhang (1995) and Xie (2007). Rare and/or unknown species were preserved with 4% formalin for identification in the laboratory. The other individuals were returned to the stream where they were captured. No fish were captured at one sampling site.

Both diatoms and macroinvertebrates were sampled using quantitative methods. Nine stones (diameter ~ 25 cm) were randomly picked within the 300-m sampling reach. A 3.5-cm diameter PVC pipe, rubber corer and toothbrush were used to thoroughly scrub and rinse benthic algae from each stone. Each sample and rinsed water were combined together and preserved using a 4% formalin solution in a 50-mL plastic bottle. In the laboratory, diatom slides were kept in a glass jar to oxidize the organic material with acid disposal. Within each diatom slide, a minimum of 300 valves were counted under high magnification oil emersion. The "soft" algae were identified directly using a 0.1-mL counting chamber. Most of the benthic algae were identified to the species level using the classic manuals of Hu *et al.* (1980) and Zhu and Chen (2000).

Benthic macroinvertebrates were collected using a Surber net with three replicates (30×30 cm² and 500-μm mesh size). The substrate was kicked or disturbed using a shovel into the net. The sample was then transferred from the net to a 10-L plastic container. After rinsing all the samples through stainless steel frames with 40-mm mesh, all the samples were put into a 500-mL jar and preserved with 70% alcohol. In the laboratory, macroinvertebrates were identified to the lowest level, mainly to the genus level according to the manuals (Morse *et al.*, 1994; Merritt and Cummins, 1996).

Environmental parameters: land use, water quality and sediments

The proportion of land use types at both watershed and riparian scales were used as the primary disturbance gradient to evaluate the sensitivity of biological indices. Three main types of land uses, including forest, farmland
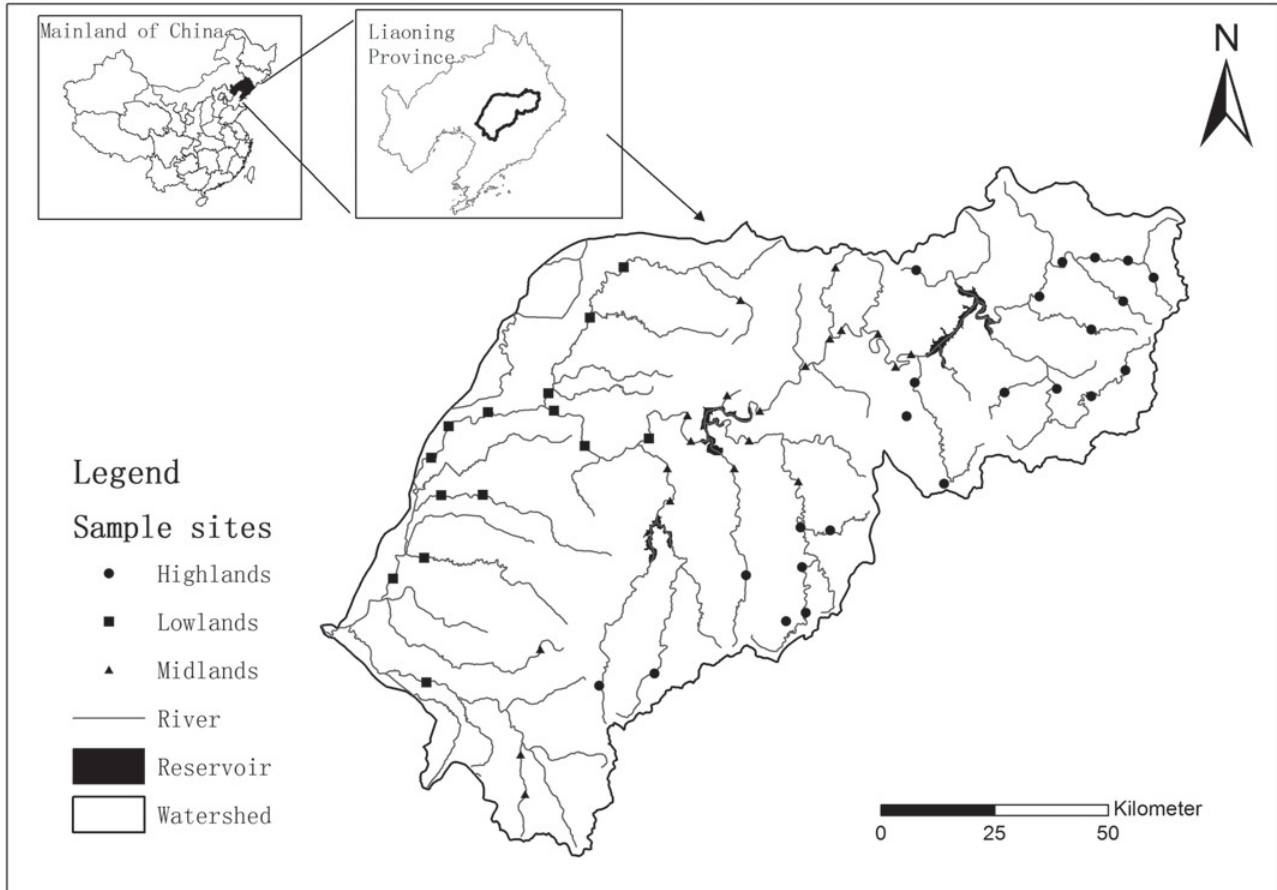
Fig. 1. Sampling site distribution with different symbols representing the three river type categories of highland, midland and lowland in the Taizi River basin.

and impervious areas of urban land, were extracted from 2010 Landsat-5 TM and Landsat-7 ETM + images using ArcGIS 9.0 Desktop GIS software. The proportion of each land use within the catchment upstream from each site was calculated. Meanwhile, the proportion of each land use within a riparian zone 10-km long and 1-km wide on either side of the riverbank upstream of the sampling site was also calculated.

At each sampling site, dissolved oxygen (DO), conductivity, total dissolved solids (TDS) and pH were measured using an multiparameter instrument (HQ40, HACH company, USA) *in situ*. In addition, stream water and sediments were sampled at each study site. The water and sediment samples were stored over ice and moved to the laboratory within 2 days. In the laboratory, concentrations of total suspended solid, alkalinity, 5-day biological oxygen demands (BOD_5), chemical oxygen demand (COD), permanganate, ammonia nitrogen, nitrite, nitrate, total nitrogen, active phosphate and total phosphorus were measured according to the Chinese Standard Methods for Examination of Water and Wastewater (MEP, 2009). Total dissolved carbon and dissolved organic carbon of sediment were also measured according to Chinese Standard Methods for Sediment (ISO-10694-1995). Sediment samples were further freeze-dried, powdered and sieved through a 100-μm mesh nylon sieve. Then 0.1 g of

sediment subsample was collected randomly and digested in Teflon vessels with 10 mL HNO_3 in a microwave oven (MARSX-press, CEM company, USA). Each sample solution was adjusted to 50 mL with twice-deionized water and filtered through a 0.45-μm membrane for further analysis. The sediment extracts were measured for iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb) using inductively coupled plasma–mass spectrometry (Agilent 7500CX).

Biological indices of aquatic organisms

Eight benthic algae indices, four fish indices and seven macroinvertebrate indices were selected as the main biological indices. Biological indices were mainly selected based on community composition, biodiversity, sensitivity and tolerance. Total abundance, taxa richness and Shannon–Wiener (H) biodiversity were used for algae, fish and macroinvertebrates, and Berger–Parker (BP) dominance (Magurran, 2004) was selected for fish and macroinvertebrates. Five diatom-based indices were selected, including Biological Diatom Index (IBD), Schiefele and Schreiner's Index (SHE), Specific Pollution Sensitivity Index (IPS), Descy Index (DES), and Rott Saprobic Index (ROTT). The diatom-based indices (Table 1) were

Table 1. The average, standard division and range of stream water, sediments, land uses and biological indicators in the sampling period of Taizi River.

Environmental and biological indicators	Acronyms	Unit	Number of samples	Mean ± SD	Min–max
Water quality					
Basic parameters					
pH	pH		57	8.11 ± 0.47	7.0–8.8
Conductivity	EC	$us.cm^{-1}$	57	394 ± 264	74–1230
Total suspended solid	TSS	$mg.L^{-1}$	57	165.1 ± 189.4	8.5–978.5
Total dissolved solid	TDS	$mg.L^{-1}$	57	339.6 ± 186.6	51.0–975.5
Alkalinity	Alk	$mg.L^{-1}$	57	72.9 ± 31.0	15.3–158.0
Organic matters					
Dissolved oxygen	DO	$mg.L^{-1}$	57	6.6 ± 1.8	0.4–13.5
5 days biological oxygen demands	BOD_5	$mg.L^{-1}$	57	8.0 ± 15.3	1.9–117.5
Chemical oxygen demands	COD	$mg.L^{-1}$	57	22.9 ± 24.9	5.1–127.5
Permanganate	Per.	$mg.L^{-1}$	57	4.36 ± 3.64	1.4–22.1
Nutrients					
Ammonia nitrogen	NH	$mg.L^{-1}$	57	1.25 ± 2.56	0.0–13.2[a]
Nitrite	NO_2	$mg.L^{-1}$	57	0.06 ± 0.09	0.0–0.41[a]
Nitrate	NO_3	$mg.L^{-1}$	57	1.75 ± 1.04	0.195–7.3
Total nitrogen	TN	$mg.L^{-1}$	57	3.46 ± 2.94	0.7–17.0
Active phosphate	PO_4	$mg.L^{-1}$	57	0.09 ± 0.22	0.01–1.21
Total phosphorus	TP	$mg.L^{-1}$	57	0.21 ± 0.39	0.03–2.63
Sediments					
Organic matters					
Total dissolved carbon	TC	$mg.kg^{-1}$	57	58.1 ± 21.0	23.0–132.2
Dissolved organic carbon	DOC	$mg.kg^{-1}$	57	21.30 ± 11.00	8.9–67.9
Heavy metals					
Iron	Fe	$mg.kg^{-1}$	57	$3.2 \times 10^5 \pm 1.3 \times 10^5$	0.8×10^5–8.8×10^5
Nickel	Ni	$mg.kg^{-1}$	57	22.8 ± 8.1	5.0–46.5
Copper	Cu	$mg.kg^{-1}$	57	35.1 ± 36.5	6. 6–254.0
Zinc	Zn	$mg.kg^{-1}$	57	97.2 ± 114.6	13.1–583.8
Chromium	Cr	$mg.kg^{-1}$	57	27.6 ± 9.6	7.6–55.7
Manganese	Mn	$mg.kg^{-1}$	57	531.4 ± 282.5	155.3–1480.6
Cadmium	Cd	$mg.kg^{-1}$	57	0.3 ± 0.4	0.04–2.2
Lead	Pb	$mg.kg^{-1}$	57	3.1 ± 4.7	0.03–18.1
Land uses					
Riparian scale					
Farmland	Farm-R	%	57	38.0 ± 21.4	1.8–78.2
Forest	FT-R	%	57	46.7 ± 30.2	0.0–96.5
Impervious surface areas	IS-R	%	57	14.4 ± 19.6	0.0–89.9
Catchment scale					
Farmland	Farm	%	57	17.3 ± 9.9	0.5–51.2
Forest	FT	%	57	74.4 ± 15.3	23.2–95.8
Impervious surface areas	ISA	%	57	3.18 ± 4.2	0.0–21.0
Biological indicators					
Macroinvertebrate					
Individuals	Ind.-M	Ind.	57	2439 ± 6103	6–36 075
Taxa richness	TR-M		57	19 ± 12	1–42
Family Taxa richness of EPT[b]	EPT-M		57	5 ± 4	0–13
Berger–Parker index	BP-M		57	0.5 ± 0.3	0.1–1.0
Shannon–Wiener diversity	H-M		57	2.2 ± 1.3	0.0–4.5
Biological monitoring working party	BMWP		57	52 ± 35	1–135
Family level of biological index	FBI		57	3.6 ± 2.5	0.1–8.0
Fish					
Individuals	Ind.-F	Ind.	56	103 ± 101	2–505
Species richness	SR-F		56	6 ± 3	2–13
Berger–Parker index	BP-F		56	0.6 ± 0.2	0.3–0.97
Shannon–Wiener diversity	H-F		56	1.6 ± 0.6	0.3–3.1
Benthic algae					
Density	Ind.-A	$Ind.cm^{-2}$	57	$1.5 \times 10^6 \pm 3.6 \times 10^6$	1.7×10^3–2.7×10^7
Taxa richness	TR-A		57	32 ± 15	2–66
Shannon–Wiener diversity	H-A		57	3.1 ± 1.0	0.2–4.9
Biological diatom index	IBD		57	12.3 ± 3.7	3.7–19.2
Schiefele and Schreiner's index	SHE		57	14.1 ± 2.0	7.1–20
Specific pollution sensitivity index	IPS		57	11.05 ± 2.65	4.9–15.6
Descy index	DES		57	15.13 ± 2.34	2.2–20
Rott saprobic index	ROTT		57	9.73 ± 2.82	5.3–17.6

[a] Lower than the detectable level: 0.025 mg.L − 1 for Ammonia nitrogen and 0.03 mg.L − 1 for Nitrite.
[b] EPT: Ephemeroptera, Plecoptera and Trichoptera.

calculated using OMNIDIA software (Version 8.0, Lecointe *et al.*, 2003). The other three macroinvertebrate indices were family-level taxa richness of Ephemeroptera, Plecoptera and Trichoptera (EPT; Barbour *et al.*, 1999), Biological Monitoring Working Party (BMWP; Hellawell, 1986), and family-level biological index (FBI; Hilsenhoff, 1988). Biological indices were selected due to their wide use in biological assessments and simple calculation for river health. All indices are summarized in Table 1.

Data analysis

Associations between environmental factors and biological indices were analyzed separately using Spearman rank correlation analysis. This non-parametric method identifies the most significant correlations (Table 2). Before the analysis, the distributions of data were checked to identify outliers. Principal component analysis (PCA) was performed to detect principal patterns of variation within the environmental variables. Before PCA, the environmental factors with high partial correlation coefficients ($r > 0.75$) were eliminated. Then the environmental data matrix of the remaining variables was re-analyzed by PCA to reduce the dimensionality of the original data into a few easily interpretable principal components. After generalization by PCA procedures, the first four PC axes representing the general environmental and stressor gradients were used to evaluate sensitivities of biological indices using multiple linear regression analysis. A forward stepwise procedure of linear regression analysis was followed to find the models that gave the best fit. Residuals were also checked to ensure model assumptions were not violated and data were transformed as necessary. Finally, the sensitivity and suitability of the generalized river health scores to environmental gradients were evaluated using linear regression analysis. All the data analyses were conducted using STATISTICA 7.0 or Microsoft Excel 2013.

Integrated river health index

For the purpose of constructing an integrated river health index, core indices showed relatively higher sensitivities to disturbance gradients, better representations of the health condition and lower intra-correlations. These core indices were recommended according to regression analysis using approximate proportion of variation (R^2 value) and significance ($P < 0.05$). Intra-correlation among different biological indices was also considered for core indices selection. When two indices had significant ($P < 0.05$) and relatively high correlation coefficients ($r > 0.75$), one of the indices was eliminated from further analysis. Finally, the modeled stressor gradients and biological indices were verified such that they responded in the expected direction. In general, models were rejected if predictors were not significantly correlated with the stressor gradients ($P > 0.05$), or if modeled responses were in the opposite direction to that expected.

The core indices were then standardized using the target and critical values. Target values represent good river health under the best attainable condition (BAC), and critical values represent the unacceptable or degraded river health condition in this study. These target and critical values were mainly cited from published papers or referred from similar research in the same region. All core indices were standardized from 0 to 1 using the target and critical values, where 1 represents the BAC and 0 represents the unacceptable condition. Then the scores were averaged within each aquatic fauna category. The generalized river health score for each sampling site was calculated by averaging the scores across aquatic fauna categories. Thus, the final score ranging from 0 to 1 was used to represent the river health condition.

Five levels of health condition were established to represent river health, including critical, poor, fair, good and excellent. The "critical" health condition ranged from 0 to 0.2 representing severe impact from human activities with high homogeneity of aquatic organisms or even no aquatic organisms. The "poor" health condition ranged from 0.2 to 0.4 with severe to moderate human disturbances. The aquatic community was dominated by taxa tolerant of poor water quality. The "fair" health condition ranged from 0.4 to 0.6 and had moderate human disturbances. The aquatic community had several dominant, moderately tolerant taxa. The "good" health condition ranged from 0.6 to 0.8, which was indicative of a low level of human disturbance. These sites had relatively high taxa richness and abundance dominated by intolerant or sensitive taxa. The "excellent" health condition ranged from 0.8 to 1.0 representing the least human disturbance, where the aquatic community was dominated by diverse and sensitive taxa and the indicator species for habitat and water quality were present. Five levels of health condition are commonly used in river health assessment for the US Environmental Protection Agency (Barbour *et al.*, 1999), EU Water Framework Directive (Hering *et al.*, 2010) and other countries (Park *et al.*, 2007; Bunn *et al.*, 2010).

Results

Environmental characteristics

All water quality factors except pH showed a wide range of values from the least polluted condition to high organic pollution, or nutrient enrichment (Table 1). The pH values reflected low acidification according to the average value of 8.11 and low standard deviation. Total suspended solids (TSS) and TDS had relatively high average and standard deviation values, potentially due to sand extraction in some areas of the stream channel, even though the sampling team avoided collecting samples after rain. Extremely low DO values (*e.g.*, 0.4 mg.L^{-1}) and relatively high BOD$_5$ and COD values (*e.g.*, > 100 mg.L^{-1}) were observed, potentially indicating high

Table 2. Spearman rank correlation coefficients of the water quality parameters, sediment variables and land uses.

	pH	EC	TSS	TDS	Alk	DO	BOD	COD	Per.	NH3	NO2	NO3	TN	PO4	TP	TC	DOC	Fe	Ni	Cu	Zn	Cr	Mn	Cd	Pb	FM	FT	ISA	FM-R	FT-R
EC	−0.25																													
TSS	−0.26	0.77																												
TDS	−0.30	0.71	0.91																											
Alk	−0.16	0.80	0.48	0.48																										
DO	0.17	−0.41	−0.43	−0.49	−0.33																									
BOD	−0.34	0.39	0.47	0.49	0.30	−0.27																								
COD	−0.19	0.55	0.64	0.67	0.38	−0.38	0.76																							
Per.	−0.14	0.62	0.76	0.77	0.44	−0.47	0.69	0.93																						
NH	−0.30	0.87	0.79	0.71	0.65	−0.27	0.39	0.55	0.61																					
NO2	−0.06	0.62	0.65	0.52	0.30	−0.27	0.21	0.47	0.54	0.63																				
NO3	0.23	0.10	0.14	0.11	−0.09	0.16	−0.29	−0.09	−0.04	0.02	0.29																			
TN	−0.18	0.73	0.71	0.69	0.48	−0.30	0.29	0.53	0.57	0.69	0.51	0.47																		
PO4	−0.15	0.54	0.49	0.53	0.46	−0.40	0.35	0.43	0.52	0.46	0.18	−0.04	0.46																	
TP	−0.06	0.63	0.63	0.66	0.50	−0.46	0.27	0.51	0.62	0.57	0.33	0.05	0.55	0.82																
TC	0.10	−0.04	0.13	0.09	−0.02	−0.17	−0.06	−0.06	−0.01	−0.03	0.003	−0.11	−0.03	−0.05	−0.07															
DOC	0.03	0.36	0.29	0.29	0.44	−0.18	0.25	0.29	0.32	0.26	0.07	−0.01	0.27	0.18	0.23	0.52														
Fe	0.01	0.23	0.33	0.27	0.02	−0.21	0.07	0.17	0.26	0.18	0.26	0.17	0.20	0.26	0.25	0.06	−0.04													
Ni	0.18	−0.26	−0.14	−0.19	−0.21	−0.11	−0.11	−0.21	−0.15	−0.33	−0.02	−0.15	−0.11	−0.03	0.18	0.19	0.03	0.43												
Cu	0.06	−0.02	0.19	0.17	−0.20	−0.11	−0.05	0.09	−0.17	−0.09	0.25	−0.02	0.18	0.26	0.26	0.25	0.11	0.48	0.68											
Zn	−0.36	0.17	0.16	0.26	0.04	−0.32	0.29	0.32	0.27	0.16	0.07	0.14	0.35	0.17	0.14	0.16	0.02	0.23	0.04	0.31										
Cr	0.07	0.05	0.15	0.12	−0.02	−0.19	−0.06	0.05	0.10	0.03	0.27	0.32	0.22	0.14	0.15	0.10	0.03	0.38	0.76	0.67	0.11									
Mn	0.25	−0.37	−0.35	−0.37	−0.35	0.24	−0.10	−0.21	−0.23	−0.35	−0.20	0.16	−0.18	−0.18	−0.25	−0.09	−0.22	0.29	0.44	0.25	−0.02	0.19								
Cd	−0.15	−0.19	−0.16	−0.12	−0.13	0.06	−0.01	−0.11	−0.14	−0.25	0.16	−0.05	−0.03	−0.05	−0.15	−0.12	0.22	0.01	0.22	0.40	0.46	0.08	0.34							
Pb	−0.07	−0.19	−0.22	−0.27	−0.13	0.30	0.002	−0.12	−0.19	−0.18	−0.27	−0.01	−0.01	−0.11	−0.26	−0.05	−0.02	0.14	0.17	0.09	0.05	−0.01	0.40	0.39						
FM	−0.18	0.48	0.41	0.34	0.35	−0.22	0.31	0.26	0.31	0.47	0.46	−0.02	0.25	0.17	0.18	0.09	0.22	−0.06	−0.12	−0.13	0.08	0.003	−0.37	−0.21	−0.33					
FT	0.26	−0.68	−0.54	−0.52	−0.53	0.39	−0.48	−0.53	−0.56	−0.66	−0.56	0.11	−0.50	−0.36	−0.34	−0.05	−0.27	−0.06	0.21	0.08	−0.31	−0.06	0.35	0.14	0.29	−0.79				
ISA	−0.19	0.67	0.47	0.47	0.49	−0.43	0.41	0.54	0.58	0.58	0.55	−0.01	0.55	0.40	0.36	0.01	0.22	0.21	−0.17	0.10	0.36	0.08	−0.24	−0.06	−0.20	0.50	−0.86			
FM-R	−0.33	0.75	0.56	0.54	0.67	−0.35	0.43	0.50	0.48	0.69	0.45	0.02	0.53	0.44	0.45	0.01	0.33	0.16	−0.06	0.03	0.23	0.16	−0.13	−0.03	−0.07	0.42	−0.60	0.48		
FT-R	0.27	−0.86	−0.63	−0.59	−0.72	0.38	−0.51	−0.60	−0.59	−0.79	−0.58	0.06	−0.55	−0.46	−0.47	0.03	−0.35	−0.12	0.18	0.07	−0.24	−0.08	0.32	0.18	0.20	−0.59	0.79	−0.67	−0.86	
IS-R	−0.26	0.72	0.64	0.54	0.48	−0.30	0.46	0.52	0.57	0.67	0.70	0.08	0.53	0.37	0.34	−0.01	0.25	0.19	−0.06	0.17	0.18	0.15	−0.27	−0.10	−0.25	0.62	−0.73	0.71	0.58	−0.78

Bold and italic characteristics represent the significant correlation with $P < 0.001$, respectively.
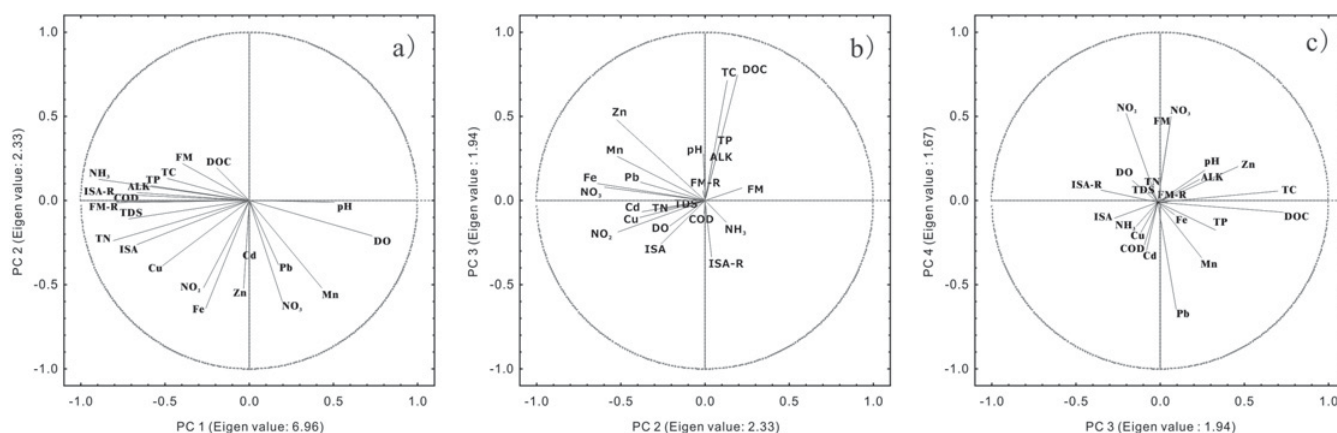
Fig. 2. Principal component analysis (PCA) ordination plots for 22 water quality parameters at the 57 sites sampled in the Taizi River basin. (a) PC 1 × PC 2; (b) PC 2 × PC 3; (c) PC 3 × PC 4.

organic pollution. Nitrogen enrichment was significant in the Taizi River. The average values of total nitrogen were relatively higher than other nutrients according to the Chinese Environmental Quality Standard for Surface Water (Table 1).

The measurement of heavy metals in the sediment showed that iron was the predominant metal in the surface sediment with an average concentration of 3.2×10^5 mg.kg^{-1}. Manganese had an average value of 531.4 mg.kg^{-1}, which was relatively higher than concentrations of the other metals, which were usually lower than 100 mg.kg^{-1}. Compared with the background levels of heavy metals found in regional soils in Liaoning Province, cadmium, copper and zinc were considered the main pollutants in surface sediments of Taizi River (Table 1).

The dominant land use type was forest at both catchment and riparian scales (Table 1). However, land use for several sampling sites was dominated by farmland, considering the maximum value was 78.2% at the watershed scale and 51.2% at the riparian scale. Impervious surface areas were high at some sites, with around 89.8% at the watershed scale. Generally, impervious surface areas were relatively low: around 14.4% at the watershed scale and 3.18% at the riparian scale (Table 1).

Biological indices

The high variation in total abundance of macroinvertebrate, fish and algae showed that samples could represent different environmental and habitat conditions and levels of human disturbance in the research area (Table 1). Considering that land use was dominated by forest, the research area showed relatively low family richness of EPT taxa, with five families on average and a maximum of 13 families. Meanwhile, the taxa richness of fish also showed relatively low values, with six species on average and a maximum of 13 species. According to similar research in the Taizi River, the other biological indices encompassed the range between least and most polluted condition (Table 1).

Disturbance gradients

Spearman rank correlation analysis showed broad correlations among the environmental factors (Table 2). Five water quality factors, including conductivity, TDS, BOD$_5$, permanganate and active phosphate, were eliminated from further analysis according to the high correlation coefficients ($r = 0.75$–0.91). Nickel, chromium and copper showed significant correlations ($r = 0.67$–0.76) for the heavy metal concentrations in the sediment. Both chromium and nickel were eliminated from further analysis. All types of land use showed wide and significant correlations ($P < 0.001$). The same types of land use also showed intra-correlations at both catchment and riparian scales. Considering the land use types and coefficient values, forest was eliminated from analysis at both catchment and riparian scales (Table 2).

Finally, 22 environmental factors were used in the PCA (Fig. 2). The first four axes were constructed to explain the dominant disturbance gradients on the basis of the decline in eigenvalues in the PCA. Four axes accounted for 58.6% of the total variance, within which the first axis accounted for 31.6% (eigenvalue 6.96), the second accounted for 10.6% (eigenvalue 2.33; Fig. 2(a)), the third accounted for 8.8% (eigenvalue 1.94; Fig. 2(b)) and the fourth accounted for 7.6% (eigenvalue 1.67; Fig. 2(c)). The main explanatory parameters for the first PC axis were negatively correlated, including ammonia nitrogen, total nitrogen, TDS, COD, DO, farmland and impervious surface area at the riparian scale. The main explanatory parameter for the second PC axis was iron (Fig. 2(a)). Dissolved organic carbon and total dissolved carbon in the sediment explained the main variance in the positive direction for the third PC axis (Fig. 2(b)). Lead explained most of the variance in the negative direction for the fourth PC axis (Fig. 2(c)).

Core indices

Before selecting core indices, the intra-correlations of biological indices were analyzed using Spearman rank

Table 3. Spearman rank correlation coefficients of biological indicators.

	Ind.-M	TR-M	EPT-M	BP-M	H-M	BMWP	FBI	Ind.-F	SR-F	BP-F	H-F	Ind.-A	TR-A	H-A	IBD	SHE	IPS	DES
TR-M	0.45																	
EPT-M	0.38	*0.95*																
BP-M	0.02	−0.73	−0.70															
H-M	0.08	*0.86*	*0.85*	−0.94														
BMWP	0.39	*0.94*	*0.93*	−0.69	*0.81*													
FBI	0.05	−0.19	−0.23	0.07	−0.11	−0.21												
Ind.-F	0.14	0.26	0.23	−0.16	0.16	0.28	−0.05											
SR-F	0.07	0.09	0.07	0.10	−0.03	0.11	−0.10	0.69										
BP-F	0.13	0.14	0.14	−0.20	0.11	0.14	0.02	0.07	−0.41									
H-F	−0.14	−0.15	−0.14	0.23	−0.15	−0.12	−0.01	0.24	*0.76*	−0.73								
Ind.-A	0.31	0.22	0.20	−0.03	0.06	0.22	−0.34	0.18	0.25	−0.19	0.13							
TR-A	0.16	0.12	0.12	0.00	0.06	0.09	−0.36	0.12	0.36	−0.23	0.30	0.63						
H-A	0.10	0.09	0.12	−0.03	0.08	0.09	−0.23	0.04	0.36	−0.15	0.33	0.08	0.63					
IBD	−0.20	−0.44	−0.38	0.28	−0.38	−0.47	0.08	−0.14	−0.17	−0.08	−0.04	−0.13	−0.22	−0.12				
SHE	−0.04	−0.17	−0.16	0.07	−0.12	−0.12	0.28	−0.15	−0.06	−0.09	0.08	0.11	0.05	−0.07	−0.03			
IPS	−0.17	−0.25	−0.22	0.16	−0.22	−0.23	−0.12	−0.18	−0.09	−0.20	0.06	0.20	0.00	−0.08	0.57	0.38		
DES	0.07	0.21	0.23	−0.18	0.23	0.18	0.39	−0.16	−0.11	−0.13	0.03	−0.13	−0.29	−0.19	0.12	0.31	0.21	
ROTT	0.19	0.24	0.26	−0.20	0.18	0.24	0.04	0.11	0.06	−0.12	0.13	0.08	−0.15	−0.07	0.09	0.25	0.07	0.30

Bold and italic characteristics represent the significant and high correlations ($P < 0.001$ and $|r| > 0.75$).

Table 4. Adjusted coefficients of determination (R^2) from linear regressions used to analyze the response of biological indices to the multiple stressor gradients (PCA axis 1, 2, 3 and 4).

	Equation	Adjusted R^2	*F*-value	*P*-value
PCA axis 1	Ind.-M = −1301 × PCA axis 1 + 2437	0.29	23.69	< 0.001
	TR-M = 3.1 × PCA axis 1 + 19.9	0.48	53.13	< 0.001
	EPT-M = 1.07 × PCA axis 1 + 5.33	0.45	46.43	< 0.001
	BP-M = 0.073 × PCA axis1 + 0.496	0.50	56.10	< 0.001
	H-M = 0.35 × PCA axis 1 + 2.32	0.55	70.80	< 0.001
	BMWP = 9.4 × PCA axis 1 + 54.2	0.47	50.14	< 0.001
	FBI = 0.55 × PCA axis 1 + 3.32	0.37	34.54	< 0.001
	IBD = −0.59 × PCA axis 1 + 12.42	0.15	10.42	0.002
	ROTT = 0.50 × PCA axis 1 + 9.76	0.12	7.88	0.007
PCA axis 2	Ind.-M = 1184 × PCA axis 2 + 2438	0.07	4.93	0.031
	BP-F = 0.039 × PCA axis2 + 0.556	0.10	6.86	0.017
	H-F = −0.12 × PCA axis 2 + 1.63	0.08	5.39	0.032
PCA axis 3	FBI = −0.50 × PCA axis 3 + 3.32	0.07	5.47	0.023
	DES = −0.35 × PCA axis 3 + 15.42	0.08	5.30	0.025
PCA axis 4	EPT-M = −1.01 × PCA axis 4 + 5.33	0.08	5.89	0.018
	TR-A = 3.51 × PCA axis 4 + 33.44	0.07	5.44	0.023
	DES = −0.35 × PCA axis 4 + 15.36	0.09	5.90	0.019

analysis (Table 3). The results showed that most macroinvertebrate indices were highly correlated ($r > 0.75$, $P < 0.001$). The correlation coefficient between taxa richness and family richness of EPT taxa was highest ($r = 0.95$). Taxa richness, family richness of EPT taxa, Shannon–Wiener diversity index and BMWP scores were also correlated ($r = 0.86$–0.94). Shannon–Wiener diversity index was also highly negatively correlated with Berger–Parker index ($r = –0.94$) and positively correlated with BMWP scores ($r = 0.81$). Two fish indices showed significant and high correlation: species richness and Shannon–Wiener diversity index ($r = 0.76$). None of the benthic algae indices showed high correlations in our study (Table 3). Considering the high correlations among BMWP scores, taxa richness and family richness of EPT taxa, one index was recommended as the core index. Between Berger–Parker index and Shannon–Wiener index of macroinvertebrates, and between species richness and Shannon–Wiener index of fish, one index for each group was recommended as the core index.

Four PC axes were used as disturbance gradients to evaluate the sensitivities of biological indices. Overall, 13 biological indices showed significant relationships with the first four PC axes (Table 4). The regression analyses revealed that biological indices of macroinvertebrates could be explained significantly by the first PC axis. The negative direction of the first PC axis represented the main water nutrient enrichment gradient and riparian land cover changes (Fig. 2(a)). Six macroinvertebrate indices showed positive relationships with the first PC axis, and macroinvertebrate abundance showed negative relationships with relatively high explanations (adjusted $R^2 = 0.29$–0.55). Two benthic diatom indices, biological diatom index and Rott saprobic index, also showed

Table 5. Selected references values of core biological indices used for the river health assessment in the Taizi River.

Categories	Core indicators	Regions	Target values	Critical values	Sites number	References
Algae	ROTT	All	20	0	57	Lecointe *et al.* (2003); Qu *et al.* (2014)
	DES	All	20	0	57	Lecointe *et al.* (2003); Qu *et al.* (2014)
Fish	BP-F	All	0.15	0.90	56	Leigh *et al.* (2012); Zhang *et al.* (2013)
	H-F	All	3	0	56	Leigh *et al.* (2012); Wang *et al.* (2013)
Macroinvertebrate	H-M	All	3	0	57	Leigh *et al.* (2012); Qu *et al.* (2012)
	EPT-M	Highland	15	0	23	Park *et al.* (2007); Leigh *et al.* (2012)
		Midland	10	0	20	Kerans and Karr (1994); Leigh *et al.* (2012)
		Lowland	7	0	14	Stribling *et al.* (1999); Leigh *et al.* (2012)

significant explanation with the first PC axis (adjusted $R^2 = 0.12$–0.15). The Descy index showed a significant linear model with the third and fourth PC axes, but at low levels of variance ($R^2 = 0.08$–0.09, $P < 0.05$). Two fish community indices, Berger–Parker index and Shannon–Weiner biodiversity, were negatively correlated with the second PC axes. Taxa richness of benthic algae also showed significant correlation with the fourth PC axis (Table 4).

Based on the linear regression analysis, index representation and intra-correlations among different indices, six indices were used as core indices to construct the integrated river health assessment index. Core indices included two indices each of benthic diatoms, fish and macroinvertebrates (Table 5). The family richness of EPT taxa was finally selected as the core index due to its highly significant relationship with the main disturbance gradient, common application and easy calculation. Correspondingly, two highly correlated indices of BMWP scores and taxa richness of macroinvertebrates were eliminated. The Berger–Parker index of macroinvertebrates was eliminated due to its high correlation with the Shannon–Wiener biodiversity index of macroinvertebrates. Total abundance of macroinvertebrates was also eliminated since it is easily affected by sampling method and operator. Calculation of FBI mainly cited the tolerance values of macroinvertebrates from the USA, so FBI was not included in the core indices. Biological diatom index showed unpredicted correlations (Table 4) and was therefore not selected as a core index. Taxa richness of algae was eliminated due to the relatively low coefficients of determination. With further consideration of the broad applicability and accessibility of the target and critical values of each indicator, six indices were finally selected as core indices, including family richness of EPT taxa and Shannon–Wiener biodiversity index of macroinvertebrates, Descy index and Rott saprobic index of algae and Shannon–Wiener diversity and Berger–Parker index of fish (Table 5).

Target and critical values

The target and critical values of core indices were mainly referred from published papers or cited from similar research reports in the Liao River watershed (Table 5).

For the diatom-based indices, all the indices were standardized systematically from 0 to 20 in OMNIDIA 7.0. Thus, 20 was selected as the target value and 0 selected as the critical value (Table 5). For the Shannon–Wiener biodiversity index, 3 and 0 taxa were, respectively, selected as target and critical values according to locally published research of macroinvertebrate and fish biological integrity. Similarly, the Berger–Parker index was also cited from local research reports, and 15 and 90% were, respectively, used to represent the target and critical values of fish. The target values of EPT related with community composition and sensitive taxa of macroinvertebrates were different in different sampling regions. Target values were 15, 10 and 7 macroinvertebrate families in the highland, midland and lowland regions, respectively (Table 5).

Calculation and evaluation of the integrated biological index

Based on the target and critical values, each index was standardized and averaged to produce a final integrated biological index score at each sampling site. The results showed that 40.4% were in good condition, 45.6% were in fair condition and 10.5% were in poor condition. Only one site was classified either critical or excellent river health condition (Fig. 3). Among those sites, excellent and good river health conditions were mainly located in the highlands. Sampling sites from the midlands showed fair and good river health condition. Except one site from the upstream region, all of the sampling sites with critical and poor river health were restricted to the lowland region (Fig. 3).

Coefficients of determination for multiple linear regression correlations between the final river health score and the 11 environmental parameters that predominantly contributed to the construction of the first four PC axes (Fig. 2) were used to evaluate the sensitivity and stability of the integrated biological index (Fig. 4). As expected, the results showed that the final score was explained significantly by the first PC axis (Fig. 4, $R^2 = 41.1$, $P < 0.001$). The final score was also useful to predict nitrogen enrichment ($R^2 = 16.9$–20.9, $P < 0.001$) and organic pollution ($R^2 = 13.9$, $P < 0.001$). More importantly, the integrated biological index reflected the land use changes of agricultural development and urbanization within a 10-km
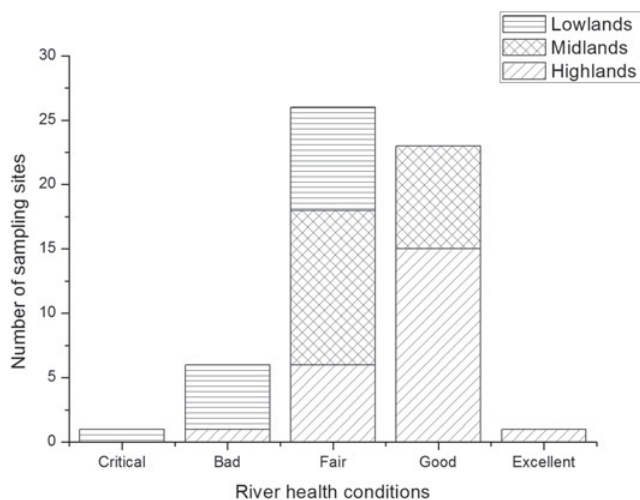
Fig. 3. River health assessment within the three categories of river types in the Taizi River.

Discussion

Usefulness of multiple biological indices

The Taizi River is one example of a river that widely suffers from different kinds of human disturbances in the Liao River basin (Zhang *et al.*, 2009). The PCA results reflected that the first four axes explained the main variation of human disturbances. The negative direction of the first PC axis integrated the dominant disturbances from water quality parameters of organic pollution, nutrient enrichment in water and sediments and land cover changes. The disturbance level increased gradually from highland (upstream reaches) to lowland (downstream reaches).

It is apparent that different categories of aquatic organisms reveal multiple human stressors at different spatial and temporal scales (Boulton, 1999). Our research confirmed that different biological indices showed varied sensitivity to the disturbance types (Table 4). The regression analyses revealed that macroinvertebrate indices showed broadly significant responses to a general environmental gradient in our study. The high sensitivity to different kinds of human disturbances has made macroinvertebrate indices as one of the most widely used indicators of river health (Metcalfe, 1989; Cairns and Pratt, 1993; Hering *et al.*, 2004; Dolédec and Statzner, 2010). Diatom-based indices were mainly developed based on tolerance to organic pollution and eutrophication (Stevenson and Pan, 2004), and have proven to be useful

riparian buffer ($R^2 = 13.1$–18.5, $P < 0.001$). The final scores were not suitable for sediment heavy metal contaminations. Both Fe and Pb did not show significant regression correlations. It may be useful to reflect the organic pollution of sediments, although with relatively low explanation with total dissolved carbon (Fig. 4).

indices to reflect river contamination in the same watershed (Qu *et al.*, 2014). However, these indices did not show similar trends over different sampling periods. It is highly recommended that the suitability and applicability of diatom-based indices be evaluated over different seasons.

Considering the high mobility of fish between impacted and non-impacted areas, the evaluation and selection of fish indices may be difficult. Biodiversity indices of fish showed stronger responses to the second PC axis even with relatively low coefficients of determination (Table 3). Considering that the Taizi River suffers from severe industrial pollution, the concentration of heavy metals in the sediment may reflect the long-term history of heavy metal contamination. Thus, the fish community diversity indices would be more accurate to reflect the relatively long-term disturbances at the watershed scale. The sensitivity of fish indices was relatively low to identify the nutrient enrichment and organic pollution or land use changes in riparian areas in our study. Our study also did not identify correlations between sediments and macroinvertebrates, despite their sensitivity to sedimentation (Prygiel *et al.*, 1999; De Lange *et al.*, 2004; Rabeni *et al.*, 2005) and low levels of heavy metal contamination (Clements *et al.*, 2000; Qu *et al.*, 2010).

The most suitable indices varied according to the levels of contamination or disturbances, across different watersheds, and between different sampling seasons. In our study, we found that macroinvertebrate indices were widely significant with the dominant stressor gradients of habitat disturbances and water contamination, as previously identified by Plafkin *et al.* (1989). In contrast, Hering *et al.* (2006) found that macrophytes and fish communities showed stronger responses to general degradation gradients in European bioassessment programs. Thus, multiple indices may be necessary to reflect multiple human disturbances, although advantages (and disadvantages) overlap among the three groups (Resh, 2008) and different characteristics of each aquatic fauna group can be matched to serve the specific study objectives (Bae *et al.*, 2014). Meanwhile, it is important to select integrated biological indices to evaluate the advantages and disadvantages for restoration programs and also provide for the protection of river biological integrity with better management (Hering *et al.*, 2010).

Target and critical values

It is necessary to define the target and critical values for the purpose of assessing river health condition based on the expected undisturbed or natural condition. However, the difficulty of defining the undisturbed or natural condition has encouraged relevant research and discussions (Karr, 1999; Hawkins *et al.*, 2010). Due to long-term human activities and climate changes, it would be hard to select the totally "undisturbed" natural condition. For the biological integrity assessment, human activities and water quality in the upstream regions of the
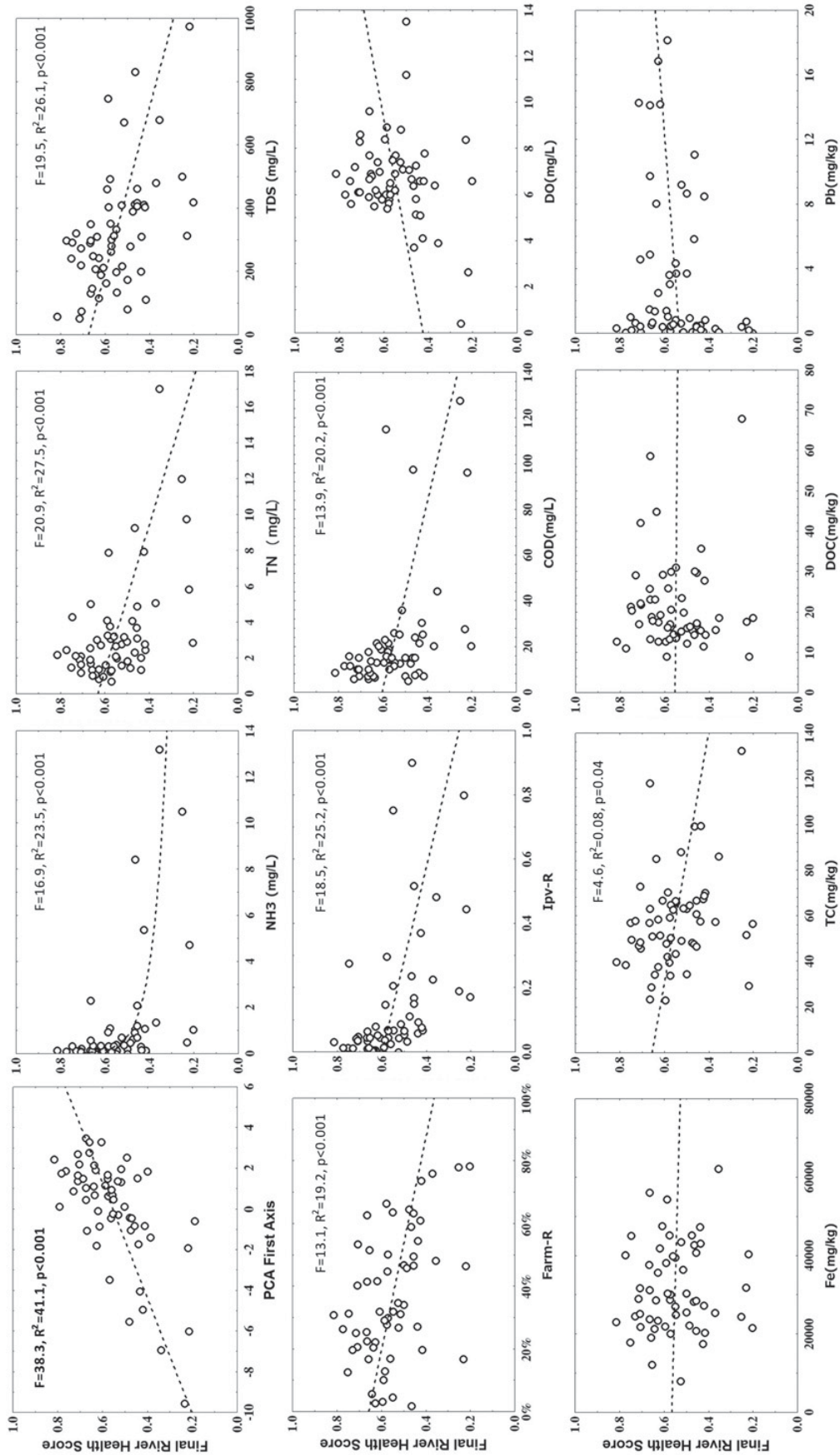
Fig. 4. Scatter plots for the integrated river health assessment results in relation to the four principal component (PC) axes of environmental factors of water quality and sediments, and land use changes. The multiple regression analysis results are presented in the upper corner showing F values, R^2 values and significances ($P < 0.05$).

sampling region were the main standards (Reynoldson *et al.*, 1997; Barbour *et al.*, 1999). According to the EU Water Framework Directive, the reference condition does not necessarily refer to a totally undisturbed condition, although this causes new problems about how to compare the assessment results among different kinds of water bodies (Hering *et al.*, 2010).

Stoddard *et al.* (2006) defined four different types of reference condition for streams: BAC, historical condition, minimally disturbed condition and least disturbed condition. Considering the long-term development history for most Chinese rivers, selecting the BAC as the reference condition would be appropriate and reasonable. Our results also confirmed that selecting sampling sites from the low level of human pressure to serve as the reference condition would be appropriate in the Taizi River. Expert opinions, the national water quality standards, and relevant literature were also useful resources used in our research (Kerans and Karr, 1994; Stribling *et al.*, 1999; Lecointe *et al.*, 2003; Park *et al.*, 2007; Geng *et al.*, 2012; Leigh *et al.*, 2012; Wang *et al.*, 2013; Zhang *et al.*, 2013; Qu *et al.*, 2014).

Importance of stream classification

Stream classification is crucial for river health bioassessment. Firstly, river classification is necessary for setting appropriate reference conditions, and ensures that comparisons are only made between comparable river systems (Bunn *et al.*, 2010). In our study, stream types were classified into highland, midland and lowland based on natural environmental parameters (Kong *et al.*, 2013). Secondly, the composition and function of aquatic organisms usually varies significantly from the headwaters to river mouth, as predicted by the river continuum concept (Vannote *et al.*, 1980). Considering the high spatial patchiness of aquatic organism species composition, it is suitable to establish reference values according to the freshwater ecoregions (Abell *et al.*, 2000). In this study, variations of aquatic organism communities within different river types were also considered (Qu *et al.*, 2013; Wang *et al.*, 2013; Yin *et al.*, 2013). Thirdly, it is also suitable to select river health indicators according to the different stream types. Similar stressors may cause different responses to biological indices among different stream types within the same ecoregion. Leigh *et al.* (2012) already identified that hydrological alteration had different effects on the macroinvertebrate structural and functional attributes between dry lands and tropical streams in Australia. Finally, the river protection and management targets should be adjusted according to the river development and disturbances. Downstream reaches usually have a relatively higher level of development and disturbances than upstream reaches. Similar trends were also identified with relatively good river health condition at highland sampling sites and relatively fair river health condition in the lowland sampling sites (Fig. 3).

Challenges of multiple biological indices

Limited budgets and difficult identification for different categories of aquatic fauna are challenges for the application of multiple biological indices. Selection of indices that could be quickly calculated as rapid bioassessment indicators increases the applicability of multiple indicators in the river health assessment. If these indices could be calculated *in situ*, this may be even more convenient than the traditional methods. The limited number of sampling sites makes it difficult to draw robust conclusions. In our study, only 57 sites were sampled across three different river types. Several diatom-based indices already showed different trends compared with previous research in the same river during different sampling seasons (Qu *et al.*, 2014). Long-term data records and more intensive sampling efforts may be required to provide more stable predictions and confident conclusions (Bae *et al.*, 2011, 2014), especially for fish communities (Kennard *et al.*, 2006). Selecting suitable target and critical values are a particular challenge when implementing a river health project for the first time, especially for those rivers without previous research. Further evaluation using a long-term larger data set and different rivers will contribute to filling this knowledge gap.

Conclusions

It is important to evaluate river health condition using a reliable and stable assessment method for better river management and restoration. In this study, multiple biological indices were developed for the Chinese NRHLP by integrating indices of benthic algae, macroinvertebrates and fish. The sensitive evaluation confirmed its usefulness in the river health assessment, especially for those rivers suffering long-term watershed development. Meanwhile, selection of sensitive indices according to their responses to general disturbance gradients and establishment of target and critical values based on references and expert opinions were realistic for a highly developed watershed. It was also appropriate to establish different target values for indices that were highly correlated with longitudinal community succession of macroinvertebrates from upstream to downstream. For example, different target values of family taxa richness of EPT were used in different regions of Taizi River in this study. Even though the limited sampling sites with single-visit procedures may affect the assessment results, our study provided important strategies for the development of multiple biological indices. Continual evaluation using longer term and larger data sets across different rivers will contribute to filling the knowledge gap.

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