A bioindicator assessment framework of river ecosystem health and the detection of factors influencing the health of the Huai River Basin, China

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Abstract

River ecosystem health assessments are a hot topic in global water resources management. The Huai River Basin is a representative basin faced with serious environmental problems due to high water pollution and numerous water projects. Comprehensive investigation of the factors affecting river ecosystem health is an urgent precursor to the successful management of the Huai River Basin. Based on a series of aquatic ecosystem indicators, monitoring and standards, such as phytoplankton, zooplankton and benthic macroinvertebrate data, an assessment framework of river ecosystem health is proposed using a multi-step gray correlation evaluation method. Environmental impact factors including climate, land cover, edaphic factors, hydrology, water projects and water quality were identified using the [Kruskal-Wallis test](#) and distance-based redundancy analysis method. The ecosystem was found to be sub-healthy with 44% (31/71) of sites in a healthy condition, 51% (36/71) sub-healthy and 5% (4/71) unhealthy. Climate and hydrology were the dominant factors that together contributed 37.5%, 24.1% and 6.9% of the variation in phytoplankton, zooplankton and benthic macroinvertebrates, respectively. The main variables affecting variation were minimum temperature, relative humidity, solar radiation, and the magnitude, frequency, duration and timing of monthly runoff. Together, these variables

1 explained 24.5% of the variation in the ecosystem health of the whole river. Thus, to
2 improve river ecosystem health in the basin, the regulation of dams and sluices should
3 give priority to ecological outcomes and the threat of climate change should not be
4 underestimated. This study provides a blueprint for ongoing ecological monitoring and
5 restoration programs in China and is expected to be a successful case and reference to
6 transition from water quantity management to river health management in the Huai
7 River Basin.

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9 *Key words:* River ecosystem health; multi-step gray correlation method; multivariable
10 analysis; Huai River Basin

12 **Introduction**

13 River ecosystem health assessments are one of the hot topics in global water resources
14 management (Karr 1999; Arthington *et al.* 2010). Furthermore, it is an urgent task in a
15 transition from water quantity management to water quality management, and even river
16 health management in China (Meng *et al.* 2009; Liu and Liu 2009). According to the
17 report “Rivers at Risk: Dams and the future of freshwater ecosystems” from 2004, there
18 were 21 river basins in the world with dramatically degraded ecosystems, and the
19 Yangtze River in China was one of the top three (WWF and WRI 2004). In China, the
20 ecosystems of 8 major river basins have been destroyed by pollution, and the percentage
21 of sub-healthy and unhealthy rivers in 2010 was 62.9% in the Hai River Basin, 59.5% in
22 the Liao River Basin, 58.1% in the Huai River Basin, 52.4% in the Songhua River Basin,
23 31.8% in the Yellow River Basin, 11.4% in the Yangtze River Basin and 15.1% in the
24 Pearl River Basin, based on the assessment of traditional water quality indices (EPAC
25 2002, 2010). Preserving and repairing river ecosystem health will promote economic
26 and social sustainability and have consequently been identified internationally as
27 research priorities (Rapport *et al.* 1998; Norris and Thoms 1999). River ecosystem
28 health assessments and the detection of environmental impact factors will provide a
29 foundation for river restoration and will improve basin sustainability (Meng *et al.*
30 2009).

31 An ecologically healthy river is a river that retains the major ecological features
32 and functions of a river (Norris and Thoms 1999; Liu and Liu 2009). Currently,

1 biological methods have risen to prominence in the assessment of river condition,
2 having gradually replaced traditional water quality assessment techniques (Norris and
3 Thoms 1999; Wright *et al.* 2000). Examples of biological methods include the River
4 Invertebrate Prediction and Classification System (RIVPACS) (Wright *et al.* 2000), the
5 Australian River Assessment System (AUSRIVAS) (Simpson and Norris 2000) and the
6 Benthic Invertebrate Community Structure (BEAST) (Reynoldson *et al.* 1997). The
7 biological approach compares the actual river species with the natural species in an
8 undisturbed river. However, application of this method is restricted by high data
9 requirements and the existence of an undisturbed river for comparison, which is
10 increasingly rare, especially in China (Norris and Thoms 1999). Alternatively, indicator
11 assessment approaches identify the social benefits of riverine development in addition
12 to the benefits of preserving an ecosystem's natural structure and functions (Rapport *et*
13 *al.* 1998; Fairweather 1999). The approach involves the selection and assessment of
14 some characteristic indicators, followed by comparisons with established standards to
15 quantify the health status of an ecosystem. Indicators should represent all aspects of the
16 ecosystem, including aquatic species, chemistry and physical structure. This method
17 better considers the integrity and diversity of an ecosystem and comprehensively
18 reflects ecosystem health, and it has been used to assess river ecosystems globally.
19 Examples include the index of biological integrity (IBI) (Karr 1981); the riparian,
20 channel and environmental (RCE) inventory (Petersen 1992); and the index of stream
21 condition (ISC) (Ladson *et al.* 1999). The widespread application of this method is
22 attributable to its low data requirements.

23 River ecosystem health assessment is an urgent issue in China that faces many
24 challenges, such as high spatial heterogeneity in large river systems, rapid development
25 of the regional economy and population growth, the high density of water engineering
26 projects and increasingly serious water pollution. The Huai River Basin is representative
27 of Chinese rivers, with conflicts and tradeoffs between high population densities,
28 economic development and environmental sustainability. A comprehensive assessment
29 of river ecosystem health in the Huai River Basin would expedite the successful
30 implementation of an integrated river basin management strategy and [transition from](#)
31 [water quantity management to river health management](#). Previous investigations were
32 focused on water quality assessments based on physical and chemical characteristics,

and it is a new challenge to assess river ecosystem health based on biological indicators. This study investigated the spatial distribution and abundance of phytoplankton, zooplankton and benthic macroinvertebrates at 71 sites in the Huai River Basin. The objectives of this study are (1) to propose an integrated river ecosystem health assessment framework for the Huai River Basin based on multiple bioindicators using the multi-step gray correlation method; (2) to analyze the species composition and assess the spatial distribution of ecosystem health status of the Huai River Basin; and (3) to identify the environmental factors that influence the variation of river ecosystem health using nonparametric multivariate analysis. This study is expected to provide the basis for river monitoring and restoration programs that incorporate bioindicators and support water resource planning and sustainable development in the Huai River Basin.

Material and methodology

Study area

The Huai River Basin (30°55' -36°36'N, 111°55' -121°25'E) is located in eastern China between the Yangtze River Basin and the Yellow River Basin (Figure 1). There are two main sub-basins, the 190,000 km² Huai River Drainage area and the 80,000 km² Yishusi River Drainage area. The Huai River Basin lies in the south-north climate transition zone with approximately 888 mm mean annual precipitation and 8.35×10^{11} m³ mean annual runoff.

The Huai River Basin is the sixth largest river basin in China, with an average population density of 623 person/km², which is approximately 4.5 times the national average. More than 5,700 dams and 5,000 floodgates have been constructed for flood control and water supply (Zhang *et al.* 2010). The industry boom in the basin, including paper mills, chemical plants and breweries, further threatens river health due to poor sewage disposal practices. Many ecological and environmental problems are emerging; for example, river fragmentation, lake and wetland shrinkage, loss of biodiversity and reduced water quality are all associated with declining ecological function. Previous studies in the Huai River Basin paid more attention to hydrology (Xia *et al.* 2011), environment and the impact of human activities, and ecological monitoring and assessment studies has been insufficient. A comprehensive assessment of river

ecological condition and an understanding of the main influential factors determining river health will be important precursors to environmental improvement in the basin.

Data selection

Ecological sample sites and metrics. Seventy-one sample sites were chosen based on the monitoring stations for routine hydrology and water quality assessments (Water Resources Bulletin of the Huai River Basin, 1997–2008), which are distributed in the Hongru River, Shaying River, Guo River, Huai River, Southern mountain rivers, Beijing-Hangzhou Grand Canal and Yishusi River (Figure 1). Sampling was conducted over 10 days beginning on July 10, 2008, prior to flooding. Due to high regulation in this basin, the intra-annual flow has been extremely stable since the 1970s, and the runoff hydrograph during the sampling period was close to the average hydrograph in the post-dams periods (Zhang *et al.* 2010) (Figure 2). Plankton, including phytoplankton and zooplankton, and macroinvertebrates were selected as the main assessment indicators because both are widely available and convenient to sample. Replicate samples were collected at each site according to relevant published guidelines (EPAC, 1993; MWR 1998). Although fish are often employed as a bioindicator in river ecosystem health studies, the fish communities of the Huai River Basin are already under severe stress, and many have already become extinct or extremely rare due to serious pollution (Zhao *et al.*, 2012). To avoid further diminishing fish stocks and difficulties with sampling from such a sparse community, more prevalent biota were chosen as biological indicators.

Phytoplankton were collected at approximately 10 cm below water surface using plastic polymethyl methacrylate (PMMA) water collection apparatus. The 1-L samples were stored in pre-cleaned bottles and preserved with 1.5% Lugol's iodine solution. After 24 hours, sufficient precipitation had occurred to decant the supernatant, leaving 30 ml of concentrated phytoplankton. Numerical phytoplankton analysis was carried out using Utermohl's inverted plankton microscope according to literature (Sicko-Goad *et al.* 1977; Rott 1981). The phytoplankton abundance of each site (number of individuals/L) were estimated by averaging the sample abundance of subsamples.

Zooplankton, including protozoa and rotifer, were collected within 5 cm of the surface in the middle of the river using PMMA bottles. Larger taxa, including cladocera

1 and copepods, were collected using a No. 25 phytoplankton net (aperture size: 0.065
2 mm). All zooplankton samples were stored in pre-cleaned bottles with 1.5% Lugol's
3 solution. In the laboratory, each sample was deposited for 24 hours and concentrated,
4 then split in a Folsom plankton splitter to subdivide to two subsamples. Cladocera and
5 copepods were identified using the method described by Evans *et al.* (1982). The
6 zooplankton abundance of each site (number of individuals/L) were estimated by
7 averaging the sample abundance of subsamples.

8 Most of these sites were distributed in the middle and lower reaches where the
9 substratum predominantly consists of mud and sand. Benthic macroinvertebrate samples
10 were collected from the riverbed by the improved Peterson grab sampler (outlet: 29
11 cm×19 cm), washed through a 1 mm sieve and stored in plastic bags with 2 ml of 75%
12 ethyl alcohol. In the laboratory, macroinvertebrates were identified to genus level
13 (number of individuals per m²).

14 *Environmental data* There are many physical and chemical factors that potentially
15 influence river ecosystem health such as the watershed area, population density,
16 distances down from sluices and dams, water quality, climate, land cover, edaphic
17 factors, flow regimes and so on. For the Huai River Basin, forty-four environmental
18 variables from six major independent categories (climate, land cover, edaphic factors,
19 flow regimes, water projects and water quality) were chosen for statistical detection and
20 population density, as an integrated and indirect factor, was ignored (Table 1). Climate
21 factors likely provide energy and water for ecosystem growth and reproduction
22 (Stenseth *et al.*, 2002; Moisan *et al.*, 2002). Land cover and edaphic factors may provide
23 biogenic materials and substrate for organisms and form habitat (Mackay *et al.* 2003).
24 Flow regime (including magnitude, frequency, duration and timing of particular flows
25 and the variability of the overall flow regime), is a major physical determinant, and its
26 variation could affect the energy and material transmission process and disturb the
27 process of organism growth (Bunn and Arthington, 2002). Water quality includes
28 nutrients and oxygen for organisms (Turak *et al.* 1999); a high concentration of
29 nutrients could cause eutrophication and aquatic hypoxia, which inhibit organism
30 growth. Furthermore, a high density of dams and sluices fragmented most rivers,
31 directly disturbed natural flow regime characteristics and consequently affected river
32 ecosystem health in the Huai River Basin. Thus, the influence of dams and sluices

cannot be ignored (Zhang *et al.*, 2010; 2012), and their impact is a long-term cumulative process. For this reason, long-term series of regulated and unregulated runoff were needed to analyze the impact of water projects on flow regimes and their potential impact on river ecosystem health.

Climatic data were obtained at 10 weather stations from the China Meteorological Administration (<http://cdc.cma.gov.cn/index.jsp>). The values of climate variables were calculated by the area weighted average method. Land use and soil types were obtained from the Data Center for Resources and Environmental Sciences (<http://www.resdc.cn/>). Areas of land use and soil types were calculated by ArcGIS 10.0 software at each sub-basin that was controlled by the sample site. Hydrological data included monthly regulated discharges between 1961 and 2000, which were obtained from the Huai River Commission. Corresponding unregulated discharges were obtained from the output of the Zhang *et al.* (2010) hydrological model. The change of flow regimes between unregulated and regulated discharges was calculated for flow regime factors. The selected variables were magnitude, frequency, duration and timing of change in flow events, which influenced the ecological integrity of rivers both directly and indirectly (Poff *et al.* 1997). The values of hydrologic variables were calculated by programming using Compaq Visual Fortran 6.0 software according to Zhang *et al.* (2012). Seven water quality variables were selected according to national guidelines for surface water quality (EPAC 2002). Direct measurements or samples for lab analysis were taken at each site for each of these variables. However, it was very difficult to collect data on these six categories, especially flow regimes data, for all 71 sites. In addition, it was unfeasible to simulate runoff processes using hydrologic models in the [reticular river networks](#) downstream of the Huai River Basin; therefore, only the flow regimes at the upper and middle streams were modeled. Thus, 33 sites in the upper and middle regions of the Huai River Basin were selected to test the environmental factors. Their geographic features are shown in Table 2.

River ecosystem health assessments

Assessment bioindicators. Data on the ecosystem's biodiversity and macroinvertebrate tolerance to pollution were obtained from the literature and from the primary data collected in this study to assess ecosystem health status, including species

diversity, richness, dominance and evenness. The Shannon-Weaver diversity index (H), which reflects species diversity in the river ecosystem, has been a popular and well-developed index in the ecological literature (Shannon and Weaver 1949). The maximum value occurs when different species are uniform in abundance. Margalef's species richness index (D) reflects the number of different species in the river ecosystem (Margalef 1958). Species richness is related to species diversity, but they are not the same thing; richness does not take proportional abundances into account and is therefore the actual count of species (Tuomisto 2010). If a large number of species are identified, the D value will also be high. Simpson's dominance index (S) reflects the dominance degree of species in the river ecosystem, which varies between zero and one (Simpson 1949). The smaller the value is, the more prevalent the dominant species are. Pielou's evenness index (J) is a measurement of how evenly distributed the abundance is among extant species (Pielou 1959). The larger the value, the more uniformly the species are distributed. The Hilsenhoff biotic index (HB) reflects the tolerance of macroinvertebrate species to pollution, and also indirectly reflects the degree of river pollution. The larger the value, the more heavily the river was polluted. All of these calculations were performed using Microsoft Office Excel 2003 software.

- Shannon-Weaver diversity index (H)

$$H = -\sum_{i=1}^s \left[\left(\frac{n_i}{N} \right) \ln \left(\frac{n_i}{N} \right) \right] \quad (1)$$

- Margalef's species richness index (D)

$$d = \frac{s-1}{\ln N} \quad (2)$$

- Simpson's dominance index (S)

$$S = 1 - \sum_{i=1}^s \frac{n_i(n_i-1)}{N(N-1)} \quad (3)$$

- Pielou's evenness index (J)

$$J = \frac{H}{\ln s} \quad (4)$$

- Hilsenhoff biotic index (HB)

$$PTI = \frac{\sum_{i=1}^s (n_i \cdot t_i)}{N} \quad (5)$$

where n_i is the individual number of the i th species in the samples per liter (ind/L), N is the total number of individuals in the samples ind/L, s is the number of species in the sample, and t_i is the tolerance value of the i th macroinvertebrate species. The value of each species was gathered from the literature (Hilsenhoff 1987; Wang *et al.* 2004).

River ecosystem health standards. Currently, river ecosystem health standards are understudied in China (Liu and Liu 2009). In this study, the main hypothesis of “healthy ecosystems” was that species were abundant and diverse, but not dominant, and the macroinvertebrate species were less tolerant to water pollution. Based on past research experiences, long-term runoff data and environmental monitoring data in the Huai River Basin, river ecosystem health (IndH) was separated into five categories to maintain consistency with the five standard categories in the national environmental quality for surface water (EPAC 2002), according to the objective of this study (Table 3), as follows:

Morbid ($IndH=1$): Natural characteristics have been severely disturbed by human activities, and the number of biological organisms is low. Key ecosystem processes are not functioning, and the ecosystem is extremely unstable.

Unhealthy ($IndH=2$): Natural characteristics have been disturbed by human activities, and species composition and ecosystem structure have been drastically altered. Dominant species are prevalent. Ecosystem services are in decline. The stress of human activities has overwhelmed the ecosystem’s capacity, resulting in instability.

Sub-healthy ($IndH=3$): River characteristics are somewhat disturbed, and biodiversity and ecosystem structure have been changed to some extent. The pressure of human activities on the ecosystem has exceeded the ecosystem’s capacity. However, the ecosystem still demonstrates the ability to recover.

Healthy ($IndH=4$): River characteristics are normal and biodiversity and ecosystem structure remain stable. The main ecosystem services are functioning, and the pressure of human activities is within the ecosystem’s capacity.

Very healthy ($IndH=5$): Human activities have had a negligible impact on river condition. Biological species are abundant and uniformly distributed. The ecosystem structure is stable, and its services are diverse.

Integrated Assessment Model. River ecosystem health (IndH) was quantified in two steps. First, the four ecological indexes (H, D, S, and J) at each site were calculated by integrating each corresponding index of phytoplankton, zooplankton and benthic macroinvertebrates, and the values of PTI of benthic macroinvertebrates at each site were also calculated. A weighting algorithm was used to integrate the different indexes, thus,

$$BI(i) = w_1 I_1 + w_2 I_2 + w_3 I_3 \quad (6)$$

where $BI(i)$ is the integrated value of H, D, S, J and HB indexes at site i . I_x are the ecological index values of phytoplankton, zooplankton and benthic macroinvertebrates, and w_x is the weight applied to each index. The weights of these three aquatic organisms were determined based on their habits, representativeness, previous studies and sensitivity analysis. Benthic macroinvertebrates often assemble in certain reaches and were less affected by the variation of flow regimes. The community thus provides more information for chronically monitoring river ecosystem health (Dai and Ni 2008; Masese *et al.* 2009; Fellows *et al.* 2006). Zooplankton and phytoplankton have a short life cycle and will quickly adapt to short-term impacts (Zhao *et al.* 2008). Species diversity and biomass of zooplankton and phytoplankton are widely used as ecological indicators for river ecosystem health assessment (Xu *et al.* 1999). Based on the Delphi method (Altschuld, 2003), the weights of these three aquatic organisms were determined by expert knowledge gained from long-term experience with river ecosystem health. Those experts included professional and technical personnel in the Huai River Water Resources Protection Bureau and water environment and ecology researchers in the Chinese Academy of Sciences. After comprehensive analysis of the opinions of these experts on indicator weights, the weights of three indicators, i.e., benthic macroinvertebrate (w_1), zooplankton (w_2) and phytoplankton (w_3), were determined to be 0.50, 0.25, and 0.25, respectively. When one biota component was missing in the data, the weighting values of the two remaining biota components were set as 0.50. A sensitivity analysis of the effect of weights was also conducted preliminary by setting three groups of weights: 0.50, 0.25, 0.25; 0.33, 0.34, 0.33; and 0.50, 0.30, 0.20, and contrasting the health status of these different weights groups.

Secondly, the state of the ecosystem health of each site was determined by comparing $BI(i)$ values with the standard values of these five categories. The multi-step

grey correlation evaluation method was used in this step, which measured the geometric similarity between samples and a certain grade standard matrix according to the correlation degree. The weight value of each index was based on the samples, which overcame the limits of subjective weighting (Xia 1999). Thus, the five values of correlation degree were calculated. The higher the correlation degree, the closer the sample was to the corresponding standard category, and the ecosystem health category was confirmed. All of these steps were performed by the multi-step grey correlation evaluation system software (register number: 2006SRBJ2140) developed by Xia *et al.* (2006) issued by National Copyright Administration of China.

Multivariate analyses

There were 71 sites distributed in the whole Huai River Basin and 31 sites in the upper and middle regions. Thus, to overcome the lack of long-term monitoring, we analyzed the spatial variation of ecosystem health to detect influential factors. The surrounding environment and catchment of each site was quite distinct, with diverse climatic elements, flow regime characteristics, water quality, land cover, soil, etc. The nonparametric multivariate analysis was employed to examine multi-species responses in the river ecosystem. Firstly, the nonparametric Kruskal-Wallis test was applied to qualitatively detect the effects of watershed area, distances down from sluices and dams, water quality degree on the ecosystem health. If the p-value was less than or equal to the significance level, their effect on ecosystem would be significant. Secondly, distance-based redundancy analysis (db-RDA), which is a new multivariate technique for testing the significance of individual terms in a multifactorial analysis-of-variance model for multispecies response variables, was used to partition the variability in the data (Legendre and Anderson 1999; McArdle and Anderson 2001). All the environmental data and biological data were standardized by logarithm ($x+1$) transformation because logarithmic data generally showed strong matrix correlations at the biological species, genus and family levels in the environmental data (Heino J. 2008). The resemblance between the samples and environmental factors were calculated by the semi-metric Bray-Curtis distance measure, and significant influential factors were selected step by step based on Akaike's Information Criterion (AIC) selection criterion. The significance level was set at 0.05, and the relationship was considered to

be statistically significant if the p-value was less than or equal to 0.05. All of these statistical analyses were carried out in PRIMER software.

Results and Discussions

Species composition and distribution

Phytoplankton A total of 58 phytoplankton taxa consisting of 39 genera were identified at 71 sites (Figure 3a). The most dominant divisions, in descending order, were Chlorophyta (17 genera), Cyanobacteria (12 genera), Bacillariophyta (six genera), Dinoflagellate (two genera) and Euglena (two genera). Cyanobacteria and Chlorophyta constituted 74.4% of all taxa and 99.8% of the total counted individuals (Figure 3a). The highest diversity was observed at Dawangmiao (14 taxa) in the Huiji River, which accounted for 24.1% of all taxa. The lowest diversity (2 taxa) was observed at Huaian and Nanzhao in the Huai River and Sanhezha in Hongze Lake, which together comprised only 3.4% of all taxa.

Zooplankton A total of 104 taxa consisting of 74 genera were identified, including Rotifer (24 genera), Cladocera (11 genera), Copepoda (17 genera), Protozoans (11 genera), Nauplius (two genera) and others (nine nauplii) (Figure 3b). The dominant genera were Rotifer and Cladocera, which accounted for 65.1% of all genera and 88.1% of total individuals (Figure 3b). The highest number of taxa was observed at Yingshang in the Shaying River, which had 21 taxa and constituted 20.2% of all taxa, whereas the lowest number of taxa (only one) was observed at Hengpaitou in the Pi River, which only constituted one percent of all taxa. The highest abundance was 14528.33 ind/L observed at the Bozhou site in the Guo River, and the lowest abundance was at the Hengpaitou site in the Pi River, which had less than 1 ind/L.

Benthic macroinvertebrate Benthic macroinvertebrates were found at 59 investigated sites. Only 24 taxa were identified, including Gastropoda (10 genera), Lamellibranchiate (seven genera), Oligochaeta (three genera), Crustacea (two genera), Hirudinea (one genus) and Insecta (one genus) (Figure 3c). The predominant genera were Gastropoda and Oligochaeta, which constituted 54.2% of all genera and 86.4% of the total counted individuals (Figure 3c). The highest number of taxa (10) was observed at the Suqian site in the East Canal, which constituted 41.7% of all taxa, whereas the lowest number of taxa were observed at Huaibin, Nanzhao and Huainan in the Huai

1 River, Jishu in the Dongyu River, Liangshan in the Zhuzhao River, Yanzhou and Linsu
2 in the Yishusi River and Sanhezha in Hongze Lake (only one taxon was identified at
3 each site). The highest abundance, 2976 ind/m², was observed at the Sixian site in the
4 Hui River, whereas the lowest abundance, 18 ind/m², was observed at Huaibin, Nanzhao
5 and Huainan in the Huai River; Jishu in the Dongyu River; Yanzhou in the Si River;
6 Linshu in the Shu River; and Laozishan and Sanhezha in Hongze Lake.

8 *River ecosystem health conditions in the Huai River Basin*

9 At all 71 sites, predominant species were evident. There were only 39 genera of
10 Phytoplankton, and Cyanobacteria and Chlorophyta were predominant, with relative
11 densities of 94.4% and 5.4%, respectively. Among the 63 genera of Zooplankton,
12 Rotifer (44.0%) and Cladocera (44.1%) were the predominant genera. Moreover, there
13 were only 24 genera of benthic macroinvertebrates, and Gastropoda (62.3%) and
14 Oligochaeta (24.1%) were the most predominant. Thus, the whole ecosystem in the
15 Huai River Basin was in a sub-healthy condition (Figure 4 and Table 4).

16 There were 31 sites in healthy condition, accounting for 44% of all sites. The
17 healthy ecosystem sites were distributed in the lower reaches and the southern mountain
18 streams. Possible explanations for their healthy conditions are large distances from
19 dams and sluices, the maintenance of natural habitat niches and the low level of
20 anthropogenic disturbance in the mountainous sites (See Figure 1). There were 21 sites
21 over 20.0 km downstream from dams and sluices, and these sites accounted for 77% of
22 the healthy sites. There were 36 sites in sub-healthy condition with fragile ecosystems,
23 accounting for 51% of the total sites. All of these sites were in the upper reaches and in
24 lakes. Thirty-nine percent (14/36) of the sites in the upper rivers were disturbed by river
25 regulation, and 42% (15/36) of the sites were seriously polluted which were worse than
26 Class V.

27 The other four sites were in unhealthy conditions and accounted for 5% of all sites.
28 All of these sites were disturbed by intensive human activities. Pizhou and Liangshan
29 are located in the Beijing-Hangzhou Grand Canal, which is one of busiest waterways in
30 China (Figure 1). Thus, the incessant vehicular traffic and artificial channels have
31 destroyed the river habitat. Moreover, water quality degrees at all of these four sites
32 were worse than Class V, which means the rivers were polluted heavily. The water

quality variables that exceeded the standards were total nitrogen (average values of 4.69 mg/L and the standard of Class V: 2.00 mg/L) and ammonia nitrogen (average values of 2.81 mg/L and the standard of Class V: 2.00 mg/L) (Figure 4).

The sensitivity analysis of weights allocated to the phytoplankton, zooplankton and benthic macroinvertebrates was conducted, and the results revealed that 76% (54/71) and 73% (52/71) of the sites were in the same health category when the weights of the three organisms were changed from 0.25-0.25-0.50 to 0.33-0.34-0.33 and 0.20-0.30-0.50, respectively. Thus, changing the weights did not significantly alter the proportion of sites allocated to healthy, sub-healthy or unhealthy categories (Table 4). The weights of 0.25, 0.25 and 0.50 were chosen as a compromise between simplicity and representation of the theoretical contributions of each group to river ecosystem health.

Factors influencing aquatic ecosystem health

The proceeding analysis suggests that the factors that influence river ecosystem health are watershed area, distance downstream from dams or sluices, climate, hydrological regulation, pollution, soil properties, land use and human activities such as sand mining and commercial fishing.

The watershed area ranged from 580 km² to 119,986 km², and the watershed area of 30 of the 33 sites (91%) was not greater than 40,000 km². The ecosystems of most sites (26/33) were sub-healthy, with two sites categorized as healthy and four sites categorized as unhealthy, and the water quality degree was Class III at 10 sites, Class IV at seven sites and Class V or worse at 16 sites. Most unhealthy and sub-healthy sites (18/33) were located less than 50 km downstream of dams or sluices. Moreover, most unhealthy and sub-healthy sites (18/33) were located less than 50 km downstream of dams or sluices and the same as the polluted sites (12/33) with water quality degrees of Class IV or worse. By Kruskal-Wallis test, the effect of watershed size on both water quality degree and ecosystem health was not significant, but the effect of the distance down from dams or sluices was significant on ecosystem health, but not on water quality degree. Also the water quality affected on the ecosystem health significantly (Table 5).

Using multivariate analyses, similarity with the variation of phytoplankton,

1 zooplankton, benthic macroinvertebrate samples as well as the whole ecosystem and the
2 factors passed the significant test are shown in Table 6 and the affected proportion was
3 quantified.

4 *Phytoplankton* The solar radiation (SRD), the number of zero-flow months
5 (ZERON), minimum temperature (MINT) and relative humidity (RHUM) have
6 statistically significant relationships with phytoplankton. Together these factors
7 accounted for 37.5% of the spatial variation of phytoplankton, and climate factors (SRD,
8 MINT and RHUM) were the factors with the strongest influence. These climatic factors
9 accounted for 29.3% of spatial variation, suggesting strong climatic control of the
10 growth rate and condition of phytoplankton. Climatic factors were followed in
11 importance by hydrologic factors (ZERON), which accounted for 8.2% of the spatial
12 variation. These factors were often controlled by the regulation of dams and sluices and
13 they controlled habitat form and the dynamics of nutrient exchange.

14 *Zooplankton* The SRD, area of paddy and grassland (PADDY and GRASS),
15 predictability and seasonality of mean monthly runoff (PMMF and SMMF) and
16 ammonia nitrogen ($\text{NH}_3\text{-N}$) all have statistically significant relationships with
17 zooplankton abundance. Together, these factors can explain 42.3% of the spatial
18 variation in zooplankton levels. Like phytoplankton, climatic factors (such as SRD)
19 were the factors with the most influence, accounting for 13.5% of variation. Changes in
20 solar radiation likely drive the exchanges of heat, momentum and water that ultimately
21 determine zooplankton growth, recruitment and migration patterns (Stenseth *et al.*,
22 2002; Moisan *et al.*, 2002). The second most influential factors, PADDY and GRASS,
23 which accounted for 12.9% of variation, were land use variables, which provide
24 substrate for the zooplankton. The third most influential group was hydrologic factors,
25 PMMF and SMMF, which accounted for 10.6% of variation and affect the breeding,
26 spawning and maturation processes of zooplankton. The final variable was water
27 quality, $\text{NH}_3\text{-N}$, which accounted for 5.3% of variation and could affect aquatic nutrient
28 levels and the growth rates of zooplankton.

29 *Benthic macroinvertebrates* Both the $\text{NH}_3\text{-N}$ and the 10-year average recurrence
30 interval of flood (ARI10) variables have a statistically significant relationship with the
31 spatial variation of benthic macroinvertebrates. These factors together explained 19.7%
32 of the variation. The notable absence of climate as a significant factor is likely due to

the insulating properties of the water column above the bottom-dwelling macroinvertebrates. The amount of variation explained by water quality and hydrologic factors was 12.8% and 6.9%, respectively.

Ecosystem. Results showed that SRD, predictability of mean monthly runoff (PMMF), baseflow index (BI) and paddy soil (PADS) have statistically significant relationships with river ecosystem health. SRD, a climatic factor, was the variable with the most influence and accounted for 13.1% of the variation in river ecosystem health. SRD was followed by hydrologic factors (PMMF and BI together explained 11.4% of variation), which influence the breeding, spawning and maturation processes and control the provision of biologically useful habitats. Climate and hydrological factors were followed by soil (PADS), which explained 5% of variation. Soil determines the availability of nutrients for ecosystem growth and decay processes.

Conclusions

River ecosystem health assessment is both an urgent and difficult requirement for integrated river basin management in China. Assessing ecosystem health is difficult primarily because of the paucity of suitable monitoring programs. This attempt at assessing river ecosystem health in the Huai River Basin showed two main results:

(1) The Huai River ecosystem is in a sub-healthy and fragile condition at present. Of the 71 sites investigated, 43.7% were categorized as healthy, mostly in the lower reaches of the Huai River and the East Canal, the southern mountain streams and the Shaying River. Just over half (50.7%) of the sites were categorized as sub-healthy, most of which were in the upper reaches of the Hongru and Yishusi Rivers, Nansi Lake and Hongze Lake. The other sites (5.6% of all sites) were categorized as unhealthy, and these were Yingshang in the Shaying River, Dawangmiao in the Guo River and Pizhou and Liangshan in the East Canal. The assessment results were reasonable, as they were close to the results of Zhao *et al.* (2008) and the national environment state bulletin in 2010 (EPAC 2010). The bioindicator assessment framework would promote the use of biological indicators in conventional water monitoring plans in China, and accelerate the transition of water management from water quantity management to water quality and even river health management.

(2) The key factors influencing river ecosystem health were detected by

1 multivariate analysis. Climatic factors were identified as the factors with the most
2 influence on ecosystem health. The mechanism by which climate drives exchanges of
3 heat, momentum and water that ultimately determine ecological growth, recruitment and
4 migration patterns is well understood (Stenseth *et al.* 2002; Moisan *et al.* 2002). The
5 second most influential group was hydrologic factors, which are known to directly
6 affect the formation of habitat and nutrient exchange, which are the lifecycle processes
7 of freshwater organism biodiversity (Poff *et al.* 1997; Webb *et al.* 2010). The third most
8 influential groups were land use, water quality and edaphic factors, which determine the
9 provision of organic matter and nutrients for the formation, reproduction and growth of
10 riverine organisms and affect the biodiversity of aquatic biological communities
11 (Timchenko *et al.* 2000). Also, the effect of the distance down from dams or sluices was
12 significant on ecosystem health. To improve river ecosystem health in the Huai River
13 Basin, the simple economic-oriented regulation of dams and sluices, as well as water
14 quality control, should be modified with ecological outcomes as a priority, such as
15 integrated “water quantity - quality - ecology” regulation. Additionally, the threat of
16 climate change on river ecosystem should be necessarily taken into account in
17 integrated river basin management.

18 As an initial exploration on the Huai River Basin ecosystem instead of the
19 traditional water quality assessment techniques, we set out to analyze the macroscopic
20 distribution of river ecosystem health in the different flow regime periods and the main
21 influencing factors. Using only one time sample is insufficient and contains many
22 uncertainties affected by sampling methods, climate, flow regimes and the regulation of
23 dams or sluices. Therefore, it was difficult to interpret all variations of the ecosystem.
24 However, we were able to compare the spatial variation of the ecosystem health of all
25 71 sites, detect all of the potential influential factors based on their surrounding
26 environment and interpret 19.7% - 42.3% of variation in the ecosystem. The results
27 were still helpful and significant considering that our capacity is inadequate for
28 predicting the effects of human activity on freshwater ecosystems and given the
29 complex mechanisms of human activity on ecosystems all over the world, not just in
30 China. Further research should include additional biological indicators (such as fish and
31 periphyton), assessment standards, weighting determination, sample site selection, the
32 effect of dams and sluices, climate and long-term monitoring.

Acknowledgements

This study was supported by Huai River Project for Major Science and Technology Program for Water Pollution Control and Treatment (No.2014ZX07204-006) and Natural Science Foundation of China (No. 41271005). Thanks to Cheng Xushui, Zhou Jiebing, Zhang Yanzai from Huai River Water Resources Protection Bureau, Dr. Zhang Liang, Dr. Liu Xin from Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences and Prof. Liu Min, Dr. Lin Xiao from East China Normal University for their contribution for samples collection and Dr. Rob Rolls from Griffith University, Australia for data analysis. Thanks to Elsevier languages editors, Stephen Fujiwara in University of Technology Sydney, Kass Hingee in the CSIRO Mathematical and Information Sciences, Australia and Dr Xiaoyan Zhai in Wuhan University, China for their help to revise the paper. Thanks also to the anonymous referees for their valuable comments and suggestions, which significantly improved the quality of the paper.

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1 Table1 The selected environmental variables to test the responses in river ecosystem

Categories	Variables	Unit	Abbreviation
Climate	Precipitation	mm	PCP
	Potential evapotranspiration	mm	PET
	Maximum temperature	°C	MAXT
	Minimum temperature	°C	MINT
	Relative humidity	%	RHUM
	Solar radiation	J/m ²	SRD
	Wind speed	m/s	WSP
Hydrology	Mean monthly runoff	m ³ /s/km ²	MMF
	CV monthly flow	none	CVMMF
	Baseflow index	none	BI
	Low flow discharge (90 th percentile)	none	LOW90
	High flow discharge (>10 th percentile)	none	HIG10
	10-year Average Recurrence Interval of flood	none	ARI10
	Low flow spell count (<90 th percentile)	none	LOWC90
	High flow spell count (>10 th percentile)	none	HIGC10
	Low flow spell duration (<90 th percentile)	none	LOWS90
	High flow spell duration (>10 th percentile)	none	HIGS10
	Number of zero-flow month	none	ZERON
	Predictability of MMF	none	PMMF
	Constancy of MMF	none	CMMF
	Seasonality of MMF	none	SMMF
Water quality	Potential of Hydrogen	none	PH
	Ammonia nitrogen	mg/L	NH ₃ -N
	Permanganate index	mg/L	COD _{Mn}
	Total phosphorus	mg/L	TP
	Total nitrogen	mg/L	TN
Land use	Dry farmland	km ²	DFL
	Urban	km ²	URBAN
	Paddy	km ²	PADDY
	Forest	km ²	FOREST
	Grassland	km ²	GRASS
	Water	km ²	WATER
Soil	Cinnamon soil	km ²	CINNS
	Alluvial soil	km ²	ALLUS
	Urban soil	km ²	URBAS
	Aeolian sandy soil	km ²	AEOS
	Back soil	km ²	BLACKS
	Brown soil	km ²	BROWNS
	Loessal soil	km ²	LOES
	Skeleton soil	km ²	SKELS
	Paddy soil	km ²	PADS
	Lake soil	km ²	LAKES
	Chisley soil	km ²	CHISLS
	Skeletol soil	km ²	SKELTS

1 Table 2 The geographic features of selected sites and their controlled catchments

Site name	Elevation (m)	Distance down from dams or sluices (km)	Water Temperature (°C)	Watershed area (km ²)	Main land use and its proportion
Minggang	60	53.76	30.70	3007	Dry farm(47%) and Forest (52%)
Xixian	59	68.07	30.60	9651	Dry farm(40%) and Forest (41%)
Huaibing	55	>200	28.70	16005	Dry farm(40%) and Forest (41%)
Nanzhao	31	>200	28.20	30136	Dry farm(65%) and Forest (18%)
Lutaizi	28	>200	28.70	89762	Dry farm(66%) and Paddy (15%)
Huainan	45	39.83	28.70	89762	Dry farm(66%) and Paddy (16%)
Bengbu	47	>200	29.80	119986	Dry farm(70%) and Paddy (16%)
Wugouying	69	18.60	30.00	786	Dry farm(47%) and Forest (59%)
Yuhuangmiao	93	15.86	31.00	751	Dry farm(76%) and Forest (24%)
Runan	67	28.48	30.30	4405	Dry farm(80%)
Bantai	58	118.11	29.90	11116	Dry farm(90%)
Pingdingshan	84	25.43	33.00	2680	Dry farm(57%) and Forest (43%)
Luohe	71	40.17	30.00	12057	Dry farm(74%) and Forest (25%)
Huaxing	90	46.37	29.60	5200	Dry farm(71%) and Forest (29%)
Huangqiao	69	99.06	32.30	7022	Dry farm(96%)
Zhoukou	66	2.04	31.00	19443	Dry farm(83%)
Jieshou	66	34.13	29.80	28756	Dry farm(87%)
Fuyang	16	101.70	30.00	38294	Dry farm(90%)
Yingshang	16	75.14	30.20	39965	Dry farm(91%)
Fugou	71	0.00	29.80	5745	Dry farm(96%)
Zhoukou	43	0.54	28.40	7022	Dry farm(96%)
Taikang	31	19.78	29.90	580	Dry farm(100%)
Bozhou	37	25.75	28.90	10457	Dry farm(100%)
Mengcheng	49	50.25	29.60	13641	Dry farm(100%)
Huaiyuan	28	85.35	30.60	14153	Dry farm(100%)
Dawangmiao	60	161.30	28.90	3089	Dry farm(100%)
Zhangdian	37	65.49	28.90	1314	Dry farm(100%)
Xinyang	101	5.47	29.20	1062	Forest (100%)
Hengpaitou	66	41.79	26.70	4234	Forest (68%) and Grassland(28%)
Matou	52	48.88	30.00	5405	Forest (59%) and Grassland(25%)
Zhengyang	22	101.13	31.50	6736	Forest (42%) and Paddy(34%)
Yeji	68	30.10	29.80	1913	Forest (46%) and Grassland(33%)
Shangsi Bridge	68	30.75	30.30	803	Forest (80%)

1 Table 3 The standard values of different indices corresponding to the different river
2 ecosystem healthy degrees

Healthy Degree Index	Morbid	Unhealthy	Sub-healthy	Healthy	High-Healthy	References
H(river)	0	0-1	1-2	2-3	>3	Kong et al. 2000
H(lake)	0	0-0.5	0.5-1.5	1.5-2.5	>2.5	
D	0-1	1-2	2-3	3-6	>6	Cai et al. 2002; Chen et al. 2007
S	0.0-0.2	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	Cai et al. 2002; Ji et al. 2007.
J	0.0-0.2	0.2-0.4	0.4-0.6	0.6-0.8	0.8-1.0	Cai et al. 2002 ; Zhao et al. 2008
HB	>8.8	7.7-8.8	6.6-7.7	5.5-6.6	<5.5	Hilsenhoff 1987;Wang et al.,2004
IndH	1	2	3	4	5	Ye et al. 2007

3

1 Table 4 The values of five indices of the 71 monitoring sites in the Huai River Basin

ID	River Name	Site name	H	D	S	J	HB	IndH	IndH(1)	IndH(2)
1	Huai River	Minggang	0.83	0.71	0.71	0.52	-	3	3	3
2		Xixian	0.69	0.62	0.35	0.51	6.35	4	4	4
3		Huaibin	0.95	1.18	0.52	0.75	5.96	3	4	4
4		Nanzhao	0.75	0.73	0.38	0.55	5.96	3	4	4
5		Lutaizi	0.8	0.64	0.56	0.66	5.96	4	4	4
6		Huainan	0.83	0.92	0.44	0.54	5.96	3	4	4
7		Bengbu	0.88	1.45	0.51	0.53	5.96	4	4	4
8		Linhuaiquan	0.69	0.55	0.45	0.74	5.98	4	4	4
9		Wuhe	0.71	0.68	0.41	0.69	6	4	4	4
10		Xuyi	0.92	0.8	0.74	0.71	-	3	3	3
11		Jinhu	0.5	0.36	0.64	0.49	8.59	3	3	3
12	Hongru River	Wugouying	0.43	0.12	0.5	0.56	7.96	3	3	3
13		Yuhuangmiao	0.63	-1.21	0.57	0.91	7.48	3	3	4
14		Runan	1.43	0.96	0.83	0.92	8.1	4	3	4
15		Bantai	1	0.54	0.59	0.74	6.1	4	4	4
16	Shaying River	Pingdingshan	0.87	0.68	0.43	0.54	5.96	4	4	4
17		Luohe	0.45	0.49	0.24	0.41	6.21	3	4	4
18		Huaxing	0.61	0.62	0.33	0.59	5.98	4	4	4
19		Huangqiao	0.74	0.39	0.42	0.64	6.54	4	4	4
20		Zhoukou	0.68	0.43	0.4	0.58	6.53	4	4	4
21		Jieshou	0.6	0.32	0.4	0.71	6.03	4	4	4
22		Fuyang	0.41	0.3	0.24	0.35	5.96	4	4	4
23		Yingshang	0.41	0.28	0.61	0.27	-	2	3	3
24	Jialu River	Fugou	0.36	0.17	0.49	0.53	9.09	3	3	3
25		Zhoukou	0.66	0.44	0.75	0.63	9	3	3	3
26	Guo River	Taikang	0.19	0.36	0.09	0.17	5.96	3	4	4
27		Bozhou	0.28	0.19	0.32	0.31	6.14	3	3	3
28		Mengcheng	0.89	0.61	0.43	0.54	5.96	4	4	4
29		Huaiyuan	0.22	0.31	0.1	0.24	5.97	4	4	4
30		Dawangmiao	0.73	0.5	0.7	0.49	-	2	3	3
31		Zhangdian	0.66	0.57	0.31	0.39	5.96	4	4	4
32	Hui River	Guzheng	0.52	0.25	0.3	0.55	5.98	4	4	4
33		Yongcheng	0.38	0.34	0.17	0.25	5.96	4	4	4
34		Sixian	0.33	0.23	0.17	0.24	5.98	4	4	4
35	Pi River	Xinyang	0.62	0.24	0.84	0.71	-	3	3	3
36		Hengpaitou	0.67	0.38	0.17	0.61	-	3	3	3
37		Matou	0.88	3.01	0.49	0.68	5.96	3	4	4
38		Zhengyang	0.93	1.09	0.49	0.61	5.96	3	4	4
39		Yeji	0.38	0.37	0.21	0.47	6.06	4	4	4
40		Shangsi Bridge	0.53	0.14	0.35	0.65	6.06	4	4	4
41	Dongyu River	Jishu	0.52	0.71	0.29	0.43	5.96	3	4	4
42	Wanfu River	Sunzhuang	0.76	0.76	0.74	0.56	8.95	3	3	3
43	Zhuzhao River	Liangshan	0.44	0.28	0.25	0.4	5.96	3	4	4
44	Yishusi River	Yanzhou	0.49	0.5	0.29	0.45	5.96	3	4	4
45		Yishui	0.6	0.26	0.35	0.67	5.99	4	4	4
46		Yinan	0.52	0.63	0.66	0.45	-	3	3	3
47		Linyi	0.59	0.4	0.68	0.64	-	3	3	3
48		Gangshang	0.7	0.39	0.44	0.76	6.01	4	4	4
49		Juxian	0.8	0.9	0.42	0.64	7.92	3	3	3
50		Linsu	0.71	2.53	0.4	0.55	5.96	3	4	4
51		Chentang Bridge	0.88	1.62	0.75	0.7	-	3	4	4

52	South-North Water Transfer East Canal	Jiangdu	0.38	0.36	0.2	0.28	5.96	3	3	3
53		Gaoyou	0.77	0.53	0.52	0.85	6.04	4	4	4
54		Baoying	0.85	0.48	0.63	0.82	7.52	4	4	4
55		Huaian	1.05	1.92	0.77	0.78	-	3	3	3
56		Siyang	0.48	0.37	0.26	0.45	6.19	3	4	4
57		Suqian	0.59	0.46	0.34	0.59	5.99	4	4	4
58		Pizhou	0.48	0.29	0.74	0.39	-	2	2	2
59		Taierzhuang	0.77	0.53	0.44	0.76	5.98	4	4	4
60		Jining	0.09	0.32	0.52	0.1	-	3	3	3
61		Liangshan	0.34	0.26	0.18	0.32	8.82	2	3	3
62	Hongze Lake	Laozishan	0.29	0.33	0.17	0.27	6.1	3	3	3
63		Lihewa	0.53	0.28	0.29	0.49	5.98	4	4	4
64		Chengzihu	0.8	0.46	0.52	0.58	7.13	4	3	4
65		Erhezha	0.57	0.27	0.49	0.66	6.04	4	3	4
66		Sanhezha	0.52	0.2	0.28	0.38	5.96	3	3	3
67	Nansi Lake	Weishandao	0.38	0.56	0.2	0.25	5.96	4	4	4
68		Erjibashang	0.45	0.22	0.5	0.59	7.87	3	3	3
69		Erjibaxia	0.41	0.4	0.26	0.3	5.96	3	3	3
70		Nanyang	0.38	0.23	0.26	0.53	7.32	3	3	3
71		Dushandao	0.03	0.05	0	0.51	-	3	3	3

1 Note: IndH is the ecosystem health degree when the weights of three organisms were 0.50, 0.25,
2 0.25, respectively; IndH(1) is the ecosystem health degree when the weights of three organisms were
3 0.33, 0.34, 0.33, respectively; IndH(2) is the ecosystem health degree when the weights of three
4 organisms were 0.50, 0.30, 0.20, respectively. The overstriking number in IndH(1) and IndH(2)
5 columns means the different health degree comparing to InH column.

6

Table 5 The affected of watershed size, dams or sluices and water quality on river ecosystem in the Huai River Basin

	Watershed size	Distance	Water quality	Grouping Variable
p-value	0.491	0.601	-	Water quality
p- value	0.427	0.003*	0.034*	Eco-health

Note: 'p-value' is a measure of probability and the criterion of statistical significance is 0.05; '*' is the significant factors.

Table 6 The influential factors on river ecosystem in the Huai River Basin

Bio-indictors	Variables	Category	p-value	Prop.	Cumul. Prop.
Phytoplankton	SRD	climate	0.001	0.164	0.164
	ZERON	hydrology	0.044	0.082	0.246
	MINT	climate	0.005	0.078	0.324
	RHUM	climate	0.041	0.051	0.375
Zooplankton	SRD	climate	0.001	0.135	0.135
	PADDY	landuse	0.005	0.073	0.208
	PMMF	hydrology	0.015	0.066	0.274
	GRASS	landuse	0.024	0.056	0.33
	NH ₃ -N	water quality	0.016	0.053	0.383
	SMMF	hydrology	0.04	0.04	0.423
Benthic macroinvertebrate	NH ₃ -N	water quality	0.015	0.128	0.128
	ARI10	hydrology	0.05	0.069	0.197
The whole ecosystem	SRD	climate	0.001	0.131	0.131
	PMMF	hydrology	0.023	0.061	0.192
	BI	hydrology	0.039	0.053	0.245
	PADS	soil	0.048	0.05	0.295

Note: ‘p-value’ is a measure of probability, the tail probability of the distribution of a test statistic in statistical significance testing; ‘Prop.’ is the proportion of ecology variation affected by the predictor; ‘Cumul. Prop.’ is the cumulative proportion of ecology variation affected by all the predictors.

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Figure 2

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Figure 3
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Figure 4
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- 1 Fig.1 Location of the Huai River Basin and sampling sites distribution
- 2 Fig.2 The comparison of average daily runoff at Fuyang and Bengbu stations in
- 3 per-dams period (1950-196), post-dams period (1970-2008) and the sample year
- 4 (2008).
- 5 Fig.3 The phytoplankton (a), zooplankton (b) and zoobenthos(c) composition of all the
- 6 71 investigated sites in the Huai River Basin
- 7 Fig.4 The ecosystem health degree and water quality degree of all the 71 investigated
- 8 sites in the Huai River Basin