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Research Article: DOC, pH, major ions, and flow velocity were identified as primary determinants for spatial patterns of diatom assemblages. Both diatom indices and indicator revealed the same results, which implies the capacity of diatom indices and indicator analysis in monitoring water quality status.

Spatial Pattern of Benthic Diatoms and Water Quality Assessment using Diatom Indices in a Subtropical River, China

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Research Article

Spatial Pattern of Benthic Diatoms and Water Quality Assessment using Diatom Indices in a Subtropical River, China

Benthic algae including benthic diatoms are one of the most vital primary producers in riverine ecosystems, and their spatial distribution and assemblage structure respond sensitively to various environmental variables. Thus, benthic diatoms have been widely used as indicators of water quality in riverine systems. Here, we present a study on benthic diatoms and water quality assessment using diatom indices in the upper Han River, a subtropical river with a length of 925 km in China. Canonical correspondence analysis indicated that spatial patterns of diatom assemblages could be primarily explained by dissolved organic carbon, pH, major ions, and flow velocity. Two-way indicator species analysis on the diatom assemblages grouped the sampling sites into those from mainstream and tributaries. There were also significant differences in the Shannon–Wiener diversity index, evenness index, species richness, and diatom indices between the mainstream and tributary sites, and diatom indices categorized the mainstream as mesotrophic and the tributaries as oligo-mesotrophic in the upper Han River. Lower water quality in the mainstream led to the dominance of pollution tolerant species such as *Eolimna subminuscule*, while tributaries were characterized by *Achnanthes pyrenaicum* and *A. subatomus*. The research demonstrates the applicability of diatom indices for water quality and ecohealth assessment in subtropical rivers.

Keywords: Bioassessment; Bioindicator; Indicator value; Multivariate statistical analysis; Physical and chemical variables

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1 Introduction

Benthic algae including benthic diatoms are one of the most vital primary producers in riverine ecosystems [1], and their spatial distribution and assemblage structure respond sensitively to a range of environmental variables including temperature, nutrient supply, ionic strength, hydrologic regime, stream substrate, and sediment, and even geology and land use [2–5]. Additionally, benthic algae are commonly found in various habitats throughout the world and are easily collected and preserved, so they have been widely used for water quality assessment on a variety of environmental changes such as deterioration by nutrient enrichment, acidification, and metal contamination [6–9]. Understandably, they are also one of the key groups of organisms recommended by the European water framework directive for surface water quality assessment [10].

In addition to benthic diatom community composition, many diatom indices have been developed for water quality assessment

worldwide. For instance, IPS (specific pollution sensitivity index) and CEE (Commission for Economical Community Metric) are widely used in France [11], and IPS has usually been considered as the reference index to evaluate the applicability of other new diatom indices because it incorporated the largest number of species among all diatom indices [12]. SLAD (Sládeček's index) [13] and CEE have significant relationships with physical and chemical variables indicating trophic level (e.g., soluble reactive phosphorus (SRP), total phosphorus (TP), and total nitrogen (TN)) and organic matter concentration (e.g., dissolved organic carbon (DOC), NH₄-N) [11, 14]. The WAT index (Watanabe's index), developed to predict organic pollution, has been reported to perform well [5, 15]. The IDAP (index diatom Artois-Picardie) was developed in the Artois-Picardie Basin [16], and studies also demonstrate its considerable capacity for water quality assessment in coastal streams [17]. The EPI-D (diatom-based eutrophication/pollution index) has demonstrated its significant correlation with nitrite [17] and dissolved inorganic phosphate in the Vaal and Wilge Rivers, South Africa [14]. However, diatom indices developed in other countries or regions including those widely used in the world are rarely applied to monitor water quality in China. There are also very few studies regarding the development of these indices based on benthic diatoms in Chinese rivers [18], one index based on benthic algae developed in the Xiangxi River [19], and one bio-integrity index of benthic diatom used for evaluating the influence of run-of-river dams on river ecosystems [20].

The upper Han River basin is the water source area of the Middle Route of the South-North Water Transfer Project (MR-SNWTP) in

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Abbreviations: CCA, canonical correspondence analysis; DOC, dissolved organic carbon; MRPP, multi-response permutation procedure; NMS, non-metric multi-dimensional scaling; PCA, principal component analysis; SRP, soluble reactive phosphorus, TP, total phosphorus; TN, total nitrogen

China. The MR-SNWTP will transfer water to the North China Plain for domestic, industrial, and agricultural usages [21–23]. Thus, water quality in the upper Han River will directly affect the daily life of people residing in the water destination area in the near future. Previous studies have been focused on water quality in particular physical and chemical conditions and their relationship with land use and land cover in the Han River basin [21, 24, 25]. The present research summarizes the results of a synoptic study designed to elucidate major environmental factors affecting the distribution of benthic diatoms and their communities in the base flow season in the upper Han River, and further to identify benthic diatom species that could be applied as indicators to assess water quality in the subtropical riverine ecosystems. This research is an important component of the water conservation initiative for the inter-basin water transfer project of the MR-SNWTP [22].

2 Materials and methods

2.1 Study area

The Han River is a tributary of the Yangtze River and it flows west to east and finally falling into the Yangtze in Wuhan City, Hubei Province. Danjiangkou Dam was constructed in 1970s on the upper Han River and formed the Danjiangkou Reservoir. The upper Han River basin, upstream of the Danjiangkou Reservoir, drains an area of 95 200 km² with a length of 925 km (Fig. 1). The basin is in the north subtropical monsoon climate zone with principal vegetation being various forest types including coniferous, deciduous, mixed coniferous, and broad-leaved forests, and shrubs, and herbs. The dominant soil types are yellow brown soil and cinnamon soil [21, 24], and its surface lithology is primarily composed of carbonates [26]. The bottom substrate of rivers is mostly composed of cobble with low proportions of gravel and sand, and a hardpan substrate is found in the head streams. Human settlement consists of small towns or villages in the uplands and larger cities such as Hanzhong, Ankang, and Shiyan located along the upper Han River corridor [22, 23, 25]. Water quality is jointly affected by natural weathering processes [26–28] and human activities such as utilization of pesticides and herbicides for agriculture since there are large areas of crop cultivation such as maize, wheat, rice, cassava, vegetables, and citrus orchards in the basin [21, 24, 25].

Figure 1. The position of sampling sites in the upper Han River Basin and the location of the upper Han River in China.

2.2 Diatom sampling and identification

Epilithic diatoms were collected from 29 sites including 10 sites in the mainstream and 19 sites in tributaries in the riverine network in November 2007 (Fig. 1) [26–28]. Three substrate rocks (with a diameter <25 cm) were collected randomly from the left, centre, and right of transects across the rivers. On deep sites, rocks were collected close to the river bank. The rocks were brushed in a fixed area, i.e., a circular area with a diameter of 7 cm, and periphyton from nine rocks was rinsed several times with distilled water into one container. Samples (100 mL) were preserved in plastic bottles in 4% formaldehyde. In laboratory permanent diatom slides were mounted with NaphraxTM after oxidizing organic materials in samples with sulfuric acid (H₂SO₄), followed by nitric acid (HNO₃), which was modified from Barber and Haworth [29]. Species were identified and a minimum of 400 valves were counted per slide at 1000× magnification using a microscope (Olympus BX51, Olympus, Tokyo, Japan). Diatom identification was carried out according to Prygiel and Coste [30], Krammer and Lange-Bertalot [31–33], and Krammer [34].

2.3 Analysis of physical and chemical variables

Water samples were collected simultaneously with the diatom samplings at the same 29 sites in the river network (Fig. 1). Samples of 500 mL were mixed with 10% v/v sulfuric acid (final pH<2) for analysis of TP, TN, SRP, and chemical oxygen demand (COD_{Mn}). For the analysis of DOC, SRP, and ions, the water sample was first filtered using cellulose nitrate membrane filters (Whatman, aperture 0.45 μm). One small portion of the water sample was mixed with 10% v/v sulfuric acid (final pH<2 in the water sample) to be stored for the DOC and SRP analyses. The second portion of the samples was stored for directly measuring Cl⁻ and SO₄²⁻, while a third portion was added to HNO₃ (final pH<2 in samples) for Na⁺, K⁺, Ca²⁺, Mg²⁺, and Si determination [27]. Water samples were all stored at 4°C in bottles washed by acid in advance for a maximum of 7 days.

Water temperature (T), pH, total dissolved solids (TDS), turbidity (TURB), electrical conductivity (EC), dissolved oxygen (DO), ammonium-nitrogen (NH₄⁺-N), and nitrate-nitrogen (NO₃-N) were measured in situ using YSI 6920 probe (YSI, Yellow Springs, Ohio, USA). Velocity of flowing waters was detected using a global flow probe (FP201, Global Water, Gold River, CA). Major cations (Na⁺, K⁺, Ca²⁺, Mg²⁺, and Si) were measured using inductively coupled plasma atomic emission spectrometry (ICP-AES) (IRIS Intrepid II XSP DUO, Thermo Fisher Scientific, Waltham, MA, USA). Anions (Cl⁻ and SO₄²⁻) were measured using a Dionex ion chromatograph (IC; Dionex, Sunnyvale, CA, USA). TP and SRP were measured by ammonium molybdate spectrophotometric method, TN was determined by alkaline potassium persulfate digestion-UV spectrophotometric method, and COD_{Mn} was determined by potassium permanganate index method [35]. DOC was measured using TOC-V CPH (Shimadzu, Kyoto, Japan).

2.4 Data analysis

Structural attributes such as diversity index, evenness, and species richness have been used as surrogate measurements to characterize benthic algae assemblage relating to physical and chemical variables. We calculated the Shannon–Wiener diversity index [36], Pielou evenness [37], and species richness of the diatom assemblages. In

addition, five diatom indices including the CEE [12], EPI-D [36], IBD [30], IPS [38], and WAT [15] were also calculated. Diatom indices were calculated using the OMNIDIA 7 software V 4.2 [39]. Diatom index scores ranged between 0 and 20, and lower scores implied higher levels of pollution or eutrophication [40]. Class limit values were imposed on diatom index scores to assess levels of pollution [40] (Tab. 1). The relationship between the diatom indices and physical and chemical variables was studied using Spearman rank correlation.

Two-way indicator species analysis (TWINSPAN), a divisive classification method based on correspondence analysis, was performed to define species assemblages and the fidelity of species to an assemblage. We tested the significant difference between groups using a multi-response permutation procedure (MRPP), a non-parametric approach for testing differences of variables among predefined groups. The Sorenson coefficient on abundance data was selected as the measured distance in MRPP and the Monte Carlo randomization procedure was used to test the significance of the null hypothesis [41]. The non-metric multi-dimensional scaling (NMS) was used in conjunction with TWINSPAN classification to determine the major variation in species composition and elucidate patterns in assemblage structure without integrating environmental variables. NMS is appropriate for data that were non-normal, discontinuous distributed [41]. NMS analysis was processed in an autopilot mode [42]. The Sorenson coefficient was used as the distance measure in the analysis. Further, the indicator value method (IndVal) [43] was used to explore the indicator value of the taxa among the groups obtained by TWINSPAN. Normally, the indicator value of a taxon varied between 0 and 100. If one taxon occurred in all sites of a group this taxon will be defined as the maximum value. The significance of the indicator value was tested using the Monte Carlo randomization procedure.

Principal component analysis (PCA) was conducted to derive gradients among physical and chemical variables and the significance was tested by Bartlett's test. Then detrended correspondence analysis (DCA) was carried out based on biotic data to explore the lengths of gradient of four ordination axes. The longest gradient length of four ordination axes was 3.14, so ordination analysis could be processed either by linear model or by unimodal model. Here unimodal model (CCA) was selected. Monte Carlo randomization procedure was used to test the significance of variables at 0.01 level. The canonical correspondence analysis (CCA) was conducted to explore relationships between diatoms and environmental factors. The diatom data were transformed into relative abundance before analysis, and rare species (those occurring in only one sampling site) were excluded from further analysis. To eliminate the influence of extreme values on ordination scores, species data were square root transformed before conducting CCA. Forward selection and Monte Carlo permutations were used to select a minimum set of

variables that had significant and independent effects on epilithic diatom distribution. Species optima and tolerances (the average and standard deviation of the physical and chemical variables) were calculated by the relative abundance of the taxa at each site using the software C² [44]. The criteria in this study referred to see the information from Kilroy et al. [45], (1) occurrence in at least 12 sites, and (2) tolerance to the object variable <0.75.

One way analysis of variance (ANOVA) was used to test the significance of the differences among different groups. If the data were normally distributed the ANOVA was followed by a Student-Newman-Keuls, otherwise, it was processed by a Dunn's test to test the significance between the groups [3]. ANOVA and PCA were completed by SPSS 16.0 for windows, DCA and CCA were run by CANOCO (version 4.5), and NMS, MRPP, and IndVal were carried out using PC-ORD (version 4.0). Among the 29 sampling sites, primary analysis identified sites no. 10 and no. 29 as outliers based on the diatom composition and environmental variables of water [18]. Consequently, both sites were excluded from all analyses.

3 Results

3.1 Relationships among benthic diatoms and physical and chemical variables

There were great spatial variabilities in water physical and chemical variables, and normally mainstream had higher nutrients concentrations than tributaries (Tab. 2). The average of TP in mainstream was 0.07 mg L⁻¹, twice as much as in tributaries, i.e., 0.03 mg L⁻¹. A few sites in tributaries such as Dan River (site 2) had high concentrations of NO₃⁻-N and TN, reaching 6.73 and 6.79 mg L⁻¹, respectively. Generally, nitrogen concentrations showed a higher variability compared to the mainstream (Tab. 2).

Principal component analysis of the physical and chemical variables extracted four components with eigenvalues >1.0, accounting for 69.85% of the total variance (Tab. 3). The first factor, accounting for 36.48% of the total variance, separated SO₄²⁻, NO₃⁻, TN, and NO₃-N with factor loadings >0.80 (Tab. 4). The second component, explaining 14.65% of the total variance, was primarily composed of TP and SRP. The first two components were closely related to major ion concentrations and nutrient level (i.e., nitrogen and phosphorus; $p < 0.001$).

Canonical correspondence analysis indicated that the eigenvalues of the first two axes were 0.26 and 0.18, respectively, accounting for 23.3% of the total variance in the diatom composition, and the species-environment correlations were high for both axis 1 ($r = 0.994$) and axis 2 ($r = 0.996$; $p < 0.01$). Loadings on axis 1 and axis 2 were considerably larger than those on succeeding axes, and they principally expressed variation in DOC concentrations, pH, flow velocity, and major ions. The assemblages of benthic diatoms were mainly affected by DOC, pH, velocity, and major ions such as K⁺, Na⁺, Cl⁻, and F⁻ (Fig. 2), and DO contributed to the distribution of *Achnanthes minutissima*, *A. subatomus*, *A. pyrenaicum* positively. Comparatively, *Navicula capitatoradiata*, *N. reichardtiana*, *Nitzschia dissipata*, *Eolimna minima* were explained mainly by DOC, and the abundance of *Cocconeis placentula* var. *euglypta* was much influenced by velocity (Fig. 2).

Three diatom taxa including *Eolimna subminuscula* (TN 2.64 mg L⁻¹), *Navicula cryptotenella* (TN 2.20 mg L⁻¹), and *Nitzschia palea* (TN 2.12 mg L⁻¹) were considered as potential indicator species for high TN concentration in river water. There were also two indicator

Table 1. Assessment of water trophic status using diatom indices [41]

Index score	Class	Trophy
Class limit values for diatom indices		
>17	High quality	Oligotrophy
15–17	Good quality	Oligomesotrophy
12–15	Moderate quality	Mesotrophy
9–12	Poor quality	Mesoeutrophy
<9	Bad quality	Eutrophy

Table 2. The characteristics of physical and chemical variables in mainstream and tributaries in the upper Han River, China

Parameter	Unit	Range (n = 27)	Mean ± SD (n = 27)	Mainstream		Tributaries	
				Range (n = 9)	Mean ± SD (n = 9)	Range (n = 18)	Mean ± SD (n = 18)
Velocity	m s ⁻¹	8.5–15.0	12.28 ± 1.51	0.10–1.20	0.64 ± 0.29	0.08–1.50	0.44 ± 0.39
t	°C	0.08–1.50	0.51 ± 0.37	12.0–14.0	13.00 ± 0.56	8.50–16.00	12.31 ± 1.90
EC	μS cm ⁻¹	159.90–495.90	282.90 ± 65.65	159.90–346.00	287.13 ± 56.02	181.40–495.90	282.78 ± 71.42
TDS	mg L ⁻¹	103.9–322.30	183.87 ± 42.67	103.9–224.9	186.62 ± 36.42	117.90–322.30	182.49 ± 46.42
TURB	NTU	2.70–45.70	8.78 ± 9.40	3.00–45.70	12.01 ± 13.69	2.70–26.90	7.16 ± 6.21
DO	mg L ⁻¹	8.16–12.11	10.70 ± 0.72	10.13–11.06	10.62 ± 0.31	8.16–12.11	10.74 ± 0.86
pH		6.00–6.88	6.57 ± 0.20	6.42–6.88	6.58 ± 0.15	6.00–6.85	6.56 ± 0.22
NH ₄ -N	mg L ⁻¹	0.12–1.14	0.28 ± 0.22	0.15–0.29	0.23 ± 0.04	0.12–1.14	0.31 ± 0.28
NO ₃ -N	mg L ⁻¹	0.32–6.73	1.51 ± 1.31	0.65–2.30	1.64 ± 0.52	0.32–6.73	1.44 ± 1.57
COD _{Mn}	mg L ⁻¹	0.67–5.58	1.46 ± 0.90	0.75–1.83	1.35 ± 0.44	0.67–5.58	1.52 ± 1.07
TN	mg L ⁻¹	0.62–6.79	1.86 ± 1.18	1.33–2.67	2.02 ± 0.43	0.62–6.79	1.78 ± 1.42
TP	mg L ⁻¹	0.002–0.25	0.04 ± 0.05	0.01–0.25	0.07 ± 0.07	0.00–0.11	0.03 ± 0.02
SRP	mg L ⁻¹	0.00–0.07	0.01 ± 0.01	0.00–0.07	0.02 ± 0.02	0.00–0.02	0.01 ± 0.01
DOC	mg L ⁻¹	0.51–2.06	1.29 ± 0.37	0.75–1.94	1.46 ± 0.39	0.51–2.06	1.21 ± 0.34
Si	mg L ⁻¹	2.65–12.13	5.52 ± 1.74	4.27–12.13	6.17 ± 2.48	2.65–7.66	5.19 ± 1.17
F ⁻	mg L ⁻¹	0.05–0.31	0.14 ± 0.05	0.09–0.37	0.16 ± 0.07	0.05–0.22	0.12 ± 0.04
Cl ⁻	mg L ⁻¹	1.17–19.60	5.54 ± 4.48	3.67–19.60	6.49 ± 4.98	1.17–15.40	5.06 ± 4.28
NO ₃ ⁻	mg L ⁻¹	3.4–49.88	10.24 ± 8.52	7.12–13.33	10.85 ± 2.00	3.40–49.88	9.93 ± 10.43
SO ₄ ²⁻	mg L ⁻¹	12.91–64.43	28.51 ± 12.05	13.60–44.00	27.59 ± 8.14	12.91–64.43	28.97 ± 13.79
K ⁺	mg L ⁻¹	0.41–1.62	1.02 ± 0.31	0.75–1.27	0.99 ± 0.18	0.41–1.62	1.04 ± 0.36
Ca ²⁺	mg L ⁻¹	25.43–68.15	41.12 ± 9.08	25.43–53.08	39.49 ± 8.69	32.12–68.15	41.94 ± 9.41
Na ⁺	mg L ⁻¹	1.63–8.42	3.11 ± 1.42	2.75–4.46	3.14 ± 0.53	1.63–8.42	3.09 ± 1.72
Mg ²⁺	mg L ⁻¹	3.55–20.52	9.08 ± 4.00	4.44–14.23	8.95 ± 3.36	3.55–20.52	9.14 ± 4.38

species for high TP, including *Encyonema minuta* (TP 0.11 mg L⁻¹) and *N. dissipata* (TP 0.12 mg L⁻¹), and only one species *Discostella pseudostelligera* for high DOC concentration (1.74 mg L⁻¹; Tab. 5).

3.2 Site classification using diatom assemblages

Two groups were classified by TWINSpan on the basis of diatom composition, and the classification was validated by MRPP (all $p < 0.001$). Group A included 12 sites including all 9 sites in the mainstream, and group B was composed of the other 15 sites in the tributaries. The sampling sites showed continuous pattern along the NMS axis (Fig. 3), however, sites in group B were generally distributed on the left side of axis 1 and those in group A were on another side. Group A was primarily characterized by *Eolimna subminuscula* with an indicator value of 98. The indicator values of *Achnanthes pyrenaicum* and *A. subatomus* were 89 and 82, respectively, for group B (Tab. 6).

3.3 Diatom indices and biodiversity indices

There was difference in the benthic diatom assemblage between group A and group B. The average Shannon–Wiener diversity index,

Table 3. Total variance explained by principal components analysis (PCA) for physical and chemical variables in the upper Han River basin, China

Component	Extraction sums of squared loadings		
	Total	% of Variance	Cumulative %
1	8.39	36.48	36.48
2	3.37	14.65	51.13
3	2.82	12.28	63.41
4	1.48	6.44	69.85

evenness, and richness were 2.9, 0.8, and 47.6 in group A, and in group B they were 2.1, 0.6, and 33.3, respectively (Fig. 4). Also, the five selected diatom indices had lower values in group A, and their differences among the two groups were significant ($p < 0.01$; Fig. 5). The diatom index scores of CEE, EPI-D, IPS, and WAT in group

Table 4. The first four components extracted by PCA using 22 physical and chemical variables in the upper Han River, China

	Components			
	1	2	3	4
SO ₄ ²⁻	0.878	-0.192	0.140	-0.073
NO ₃ ⁻	0.865	-0.128	-0.055	0.158
TN	0.838	-0.064	0.027	-0.082
NO ₃ -N	0.821	-0.058	0.012	-0.138
Mg ²⁺	0.799	-0.201	0.375	-0.038
NH ₄ ⁺ -N	0.786	0.001	-0.461	0.136
Na ⁺	0.770	0.188	-0.391	-0.080
TDS	0.765	-0.188	0.464	0.092
Ca ²⁺	0.762	0.080	0.020	0.086
K ⁺	0.691	-0.165	-0.197	-0.253
EC	0.689	-0.104	0.448	0.221
Cl ⁻	0.681	-0.068	-0.282	-0.142
pH	-0.605	-0.373	0.175	-0.061
TP	0.131	0.856	0.449	0.016
SRP	0.020	0.845	0.215	0.080
F ⁻	0.527	0.769	-0.087	0.009
TURB	0.116	0.639	0.628	0.021
DOC	0.150	0.590	-0.693	-0.125
velocity	-0.118	-0.014	0.503	0.243
DO	0.226	-0.285	-0.053	0.846
COD _{Mn}	-0.091	0.182	-0.441	0.641
Si	-0.408	0.319	-0.324	0.188

Bold values represent the variable with strong loadings and the strong loadings themselves.

Figure 2. Canonical correspondence analysis (CCA) diagrams showing physical and chemical factors and benthic diatoms in the ordination space of the 1st and 2nd axes. (ADPY, *Achnanthydium pyrenaicum*; ADSU, *Achnanthydium subatomus*; ADCT, *Achnanthydium catenata*; ADMI, *Achnanthydium minutissima*; ADSA, *Achnanthydium saprophilum*; CPEA, *Cocconeis placentula* var. *euglypta*; GMIN, *Gomphonema minutum*; MVAR, *Melosira varians*; NCPR, *Navicula capitatoradiata*; EOMI, *Eolimna minima*; NRCH, *Navicula reichardtiana*; NDPA, *Nitzschia dissipata*; NPAL, *Nitzschia palea*).

Figure 3. Non-metric multidimensional scaling (NMS) ordination of benthic diatom communities. Final stress for one-dimensional solution = 10.04.

A varied from 9.7 to 11.8 and from 14.0 to 16.1 for group B. The Spearman rank coefficient of correlation showed that the diatom indices were significantly correlated with nutrient contents of water, in particular $\text{NO}_3\text{-N}$, TN, TP, DOC, and major ions such as

Table 5. Optimum and tolerance (standard deviation of the optimum) of benthic diatoms for pH, total nitrogen concentration (TN), total phosphorus concentration (TP), and dissolved organic carbon (DOC) calculated by the relative abundance of the taxa in the upper Han River

Taxon	pH		TN		TP		DOC		Number of occurrences
	Optimum	Tolerance	Optimum (mg/L)	Tolerance	Optimum (mg/L)	Tolerance	Optimum (mg/L)	Tolerance	
<i>Achnanthydium atomus</i>	6.7	0.11	1.41	0.26	0.04	0.03	0.85	0.28	12
<i>Achnanthydium catenatum</i>	6.5	0.19	1.71	0.51	0.05	0.05	1.32	0.35	22
<i>Achnanthydium jackii</i>	6.6	0.21	1.14	0.68	0.03	0.05	1.18	0.25	17
<i>Achnanthydium minutissima</i>	6.5	0.25	1.57	0.56	0.03	0.03	1.32	0.25	26
<i>Achnanthydium pyrenaicum</i>	6.7	0.10	1.18	0.61	0.02	0.02	1.09	0.24	27
<i>Achnanthydium saprophilum</i>	6.5	0.30	1.66	0.43	0.04	0.04	1.17	0.35	26
<i>Achnanthydium subatomus</i>	6.6	0.24	1.51	0.51	0.03	0.03	1.11	0.27	27
<i>Achnanthydium subhudsonis</i>	6.7	0.09	1.58	0.40	0.05	0.04	1.05	0.33	12
<i>Amphora inariensis</i>	6.6	0.20	1.99	0.56	0.03	0.03	1.50	0.31	12
<i>Amphora pediculus</i>	6.6	0.22	1.89	0.53	0.05	0.05	1.46	0.37	24
<i>Cocconeis pediculus</i>	6.6	0.10	1.51	0.47	0.02	0.02	1.26	0.35	19
<i>Cocconeis placentula</i>	6.6	0.18	1.67	0.53	0.04	0.04	1.23	0.43	15
<i>Cocconeis placentula</i> var. <i>euglypta</i>	6.6	0.18	1.55	0.45	0.04	0.04	1.18	0.40	27
<i>Cymbella affinis</i>	6.5	0.26	1.63	0.51	0.04	0.05	1.56	0.39	21
<i>Discostella pseudostelligera</i>	6.4	0.30	2.04	0.55	0.05	0.03	1.74	0.35	14
<i>Encyonema minuta</i>	6.3	0.30	1.49	0.57	0.11	0.10	1.54	0.24	12
<i>Encyonopsis leeii</i> var. <i>sinensis</i>	6.5	0.21	1.69	0.63	0.05	0.08	1.33	0.24	19
<i>Eolimna minuta</i>	6.5	0.28	1.77	0.44	0.04	0.03	1.45	0.49	22
<i>Eolimna subminuscula</i>	6.5	0.21	2.64	1.01	0.06	0.07	1.61	0.34	14
<i>Gomphonema minutum</i>	6.5	0.16	1.57	0.56	0.05	0.07	1.31	0.30	25
<i>Melosira varians</i>	6.5	0.29	2.09	0.52	0.05	0.04	1.31	0.30	15
<i>Navicula capitatoradiata</i>	6.5	0.28	1.85	0.56	0.06	0.06	1.31	0.30	23
<i>Navicula cryptotenella</i>	6.5	0.16	2.20	0.70	0.05	0.05	1.31	0.30	14
<i>Navicula novaesiberica</i>	6.6	0.10	1.71	0.61	0.04	0.04	1.49	0.39	15
<i>Navicula radiosafallax</i>	6.6	0.15	1.56	0.50	0.03	0.02	1.40	0.30	14
<i>Navicula reichardtiana</i>	6.5	0.20	2.03	0.59	0.06	0.06	1.54	0.36	25
<i>Nitzschia dissipata</i>	6.5	0.21	1.88	0.39	0.12	0.11	1.42	0.36	22
<i>Nitzschia palea</i>	6.5	0.24	2.12	0.56	0.07	0.07	1.56	0.31	25
<i>Planorhynchium rostratum</i>	6.6	0.22	1.78	0.38	0.04	0.04	1.25	0.45	19
<i>Platessa conspicua</i>	6.6	0.22	1.50	0.52	0.04	0.03	1.17	0.36	21

Taxa listed were present in 12 or more of the total 27 sampling sites (Bold values represent the value of the optimum of high pH or nutrient).

Table 6. Indicator values for the important ($p < 0.01$) taxa in each TWINSpan group

Species	Observed indicator value (%)		p value
	Group A	Group B	
<i>Achnanthes pyrenaicum</i>	11	89	0.0018
<i>Achnanthes subatomus</i>	18	82	0.0002
<i>Amphora inariensis</i>	64	1	0.0060
<i>Amphora pediculus</i>	76	19	0.0038
<i>Cyclotella meneghiniana</i>	51	2	0.0068
<i>Eolimna subminuscula</i>	97	0	0.0002
<i>Fistulifera pelliculosa</i>	59	2	0.0052
<i>Navicula capitatoradiata</i>	80	15	0.0064
<i>Navicula cryptocephala</i>	63	2	0.0028
<i>Navicula reichardtiana</i>	84	14	0.0008
<i>Navicula schroeteri</i> var. <i>symmetrica</i>	58	0	0.0010
<i>Navicula viridula</i> var. <i>germainii</i>	65	1	0.0004
<i>Navicula viridula</i> var. <i>rostellata</i>	62	1	0.0058
<i>Nitzschia dissipata</i>	81	8	0.0034
<i>Nitzschia palea</i>	86	12	0.0028
<i>Surirella angusta</i>	55	1	0.0080

Cl^- , NO_3^- , and Na^+ (Tab. 7). DO had positive relationships with CEE and EPI-D, and SRP only had a negative correlation with CEE. Based on the preset criteria [40], sampling sites in group A were in moderate quality and mesotrophic and those in group B were in good quality and oligomesotrophic in terms of water quality.

4 Discussion

4.1 Environmental factors influencing benthic diatom assemblages

Benthic algal composition has been reported to be determined by the interaction of proximate determinants such as nutrients, intermediate (i.e., longitudinal linkage at reaches), and ultimate factors (i.e., climate, geology, and land use) [46–48]. Major nutrient

concentration (i.e., TP and TN) in ambient aquatic environment was the primary factor explaining variation in benthic assemblages [3, 49, 50]. In this study, CCA showed that proximate determinants such as DOC and major ions were vital in explaining the variation in diatom assemblages. The findings are consistent with the results of Potapova et al. [51].

However, the sum of canonical eigenvalues in the two axes was 0.44 and they only accounted for 23.3% of the total variance in diatom species composition. Meanwhile, PCA of the environmental variables extracted four components with eigenvalues > 1.0 , accounting for 69.85% of the total variance, and the components comprise SO_4^{2-} , NO_3^- , TN, $\text{NO}_3\text{-N}$, TP, and SRP. Thus, it implies that other proximate factors like substratum embeddedness or ultimate factors such as land use/land cover probably also strongly influenced the structure and distribution of benthic diatoms in the upper Han River basin. Previous research indicated that flow velocity was important in influencing the abundance of benthic algae [52], but it did not demonstrate significant effect in this study. It is speculated that biological disturbance such as predator pressure [53] in the small habitat of lotic system determined the spatial pattern of diatom communities structure. In brief, the proximate determinants such as nutrients and biological factors like predator pressure might act at smaller spatial scales [51, 53], and land use probably contributed more to their temporal pattern because ultimate factors operated at catchment scales over a long time [54]. However, the relationship between environmental factors and benthic biota is worthy of future research.

4.2 Trophic status assessed by diatom indices

Diatom indices have been widely applied for assessment of water pollution and trophic status. For instance, IPS has been performed to evaluate water quality in the rivers of southern Poland [55], France [56], and Finland [40]. Some of the diatom indices including IPS, CEE, and EPI-D have been tested to show the feasibility for assessing aquatic pollution in the Vaal and Wilge Rivers, South Africa [57]. In this study, CEE, EPI-D, IBD, IPS, and WAT were significantly correlated to concentrations of nutrient and major ions, and they were significantly different among the two groups (i.e., sites in the mainstream and tributaries). Based on the diatom indices, water quality in group A (includes all sites in the mainstream) was mesotrophic and that in group B (i.e., sampling sites in tributaries) was oligomesotrophic, which is compatible with the physical and chemical variables (Tab. 2). The significant difference between the water quality in the mainstream and tributaries could result from the followings: (1) nutrients from agricultural pollution in upper tributaries converge in the mainstream, and (2) large cities with dense human activities are generally located along the mainstream of the upper Han River.

4.3 Approaches of river water assessment

While diatom indices have been approved to be applicable for water quality assessment, indicator analysis could provide measurement of the biological integrity and trophic status of water. Autecology metrics could be calculated based on species optima for primary environmental variables which affect the algal community structure and weighted by relative abundances of algae species [5, 58]. Despite cosmopolitan distribution of diatoms, their variations in autecologies were small among different rivers. For instance, the

Figure 4. Comparison of biodiversity indices between TWINSpan subgroup A (9 sampling sites in the mainstream and 3 polluted sampling sites in tributaries) and subgroup B (15 sampling sites in tributaries).

Figure 5. Average values and standard deviations of diatom indices (CEE, EPI-D, IBD, IPS, and WAT) between TWINSpan subgroup A (9 sampling sites in the mainstream and 3 polluted sampling sites in tributaries) and subgroup B (15 sampling sites in tributaries).

Table 7. Spearman rank correlation coefficients between physical and chemical variables and diatom indices

	DO	NO ₃ N	TN	TP	SRP	DOC	F ⁻	Cl ⁻	NO ₃ ⁻	Na ⁺
CEE	0.494^{a)}	-0.467 ^{a)}	-0.463 ^{a)}	-0.632 ^{b)}	-0.432 ^{a)}	-0.554 ^{b)}	-0.592 ^{b)}	-0.529 ^{b)}	-0.382 ^{a)}	-0.554 ^{b)}
EPI-D	0.514^{b)}	-0.455 ^{a)}	-0.420 ^{a)}	-0.504 ^{b)}	-0.293	-0.449 ^{a)}	-0.511 ^{b)}	-0.609 ^{b)}	-0.364	-0.606 ^{b)}
IBD	0.210	-0.512 ^{b)}	-0.445 ^{a)}	-0.489 ^{b)}	-0.240	-0.556 ^{b)}	-0.546 ^{b)}	-0.758 ^{b)}	-0.404 ^{a)}	-0.695 ^{b)}
IPS	0.200	-0.478 ^{a)}	-0.459 ^{a)}	-0.543 ^{b)}	-0.303	-0.434 ^{a)}	-0.490 ^{b)}	-0.658 ^{b)}	-0.363	-0.592 ^{b)}
WAT	0.323	-0.623 ^{b)}	-0.619 ^{b)}	-0.459 ^{a)}	-0.186	-0.484 ^{a)}	-0.481 ^{a)}	-0.672 ^{b)}	-0.592 ^{b)}	-0.617 ^{b)}

Bold characters mean the significant correlation.

Only variables with significant correlations were presented here.

^{a)} Correlation is significant at the 0.05 level (two-tailed).

^{b)} Correlation is significant at the 0.01 level (two-tailed).

optimum of *Nitzschia dissipata* for TN was 1.88 mg L⁻¹, and its optimal value for inorganic nitrogen was 1.35 mg L⁻¹ in Columbia Plateau (US) [58]. The similarity in species such as *Cocconeis pediculus*, *C. placentula*, *Encyonema minuta*, and *Nitzschia palea* between this study and others could also be found [58]. Among diatoms, nitrogen-heterotrophic species (e.g., *Melosira varians*, *Eolimna subminuscule*, and *Nitzschia palea*) had greater nitrogen optima compared to nitrogen-autotrophs [59]. In this study, *Eolimna subminuscule* and *Nitzschia dissipata* had higher optimal values of TN (2.64 and 1.88 mg L⁻¹, respectively) indicating the high concentration nitrogen in the mainstream. It was inferred that trophic status in the mainstream of the upper Han River was a mesotrophic level with distinct indicator species because these two species were indicators of eutrophic and mesotrophic status, respectively [59], compatible with the assessment using diatom based indices.

5 Conclusions

There were large variabilities in physical and chemical variables in the mainstream and tributaries of upper Han River, China. DOC, pH,

major ions, and flow velocity were identified as the primary determinants for the spatial patterns of diatom assemblages. The inference models (optima environmental variable for species), indicator analysis, and diatom indices have shown that benthic diatoms respond sensitively to ambient environmental factors. Although there were slight differences, both diatom indices and indicator analysis indicated that water in the mainstream was of poor quality or mesotrophic status and that in tributaries were of good quality or oligomesotrophic status. This implies the capacity of diatom indices and indicator analysis in monitoring water quality status in subtropical rivers.

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