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Multi-assemblage biotic integrity index customized for different hydrological phases: Spatiotemporal aquatic health monitoring of a subtropical coastal urban river

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Abstract

Urban rivers in subtropical coastal regions suffer from intensive anthropogenic disturbances and strong seasonal hydrological fluctuations. However, most biological integrity assessments rely on single biotic groups and overlook hydrological seasonality, leading to unstable and one-sided evaluations. In this study, we developed hydrological period-specific Indices of Biotic Integrity (IBI) based on phytoplankton (P-IBI), zooplankton (Z-IBI), periphytic algae (A-IBI), benthic macroinvertebrates (B-IBI) and fish (F-IBI). A comprehensive multi-taxa assemblage IBI (m-IBI) was further integrated from the five single-group indices. Coupled with the Water Quality Index (WQI), we evaluated the the spatial and seasonal variation of aquatic ecosystem health in the Dongxixi River, Xiamen. Results revealed that the overall ecological status of the whole river basin remained sub-healthy throughout all three hydrological periods. Single-taxon IBIs exhibited obvious spatiotemporal heterogeneity, whereas m-IBI showed higher stability and consistent correlation with WQI. Core biological indicators driving ecosystem status shifted with hydrological conditions, reflecting seasonal succession and functional complementarity among multi-trophic aquatic communities. Correlations between individual IBIs and physicochemical factors differed markedly across seasons. As a synthetic metric integrating multi-level biological responses, m-IBI eliminates evaluation bias originating from single-taxon limitations and presents robust performance across all hydrological periods. This study emphasizes the importance of integrating hydrological seasonality and multi-assemblage communities into river health evaluation, and provides a practical framework for assessment and ecological restoration of subtropical coastal urban rivers.

Introduction

Urban rivers are vital water resources for production, domestic use, agricultural irrigation and urban landscaping, and provide critical habitats for aquatic organisms in cities(Cui et al., 2024). Rapid urbanization and intensive farming generate continuous pollutant loads, including nitrogen, phosphorus and organic contaminants, transported into river ecosystems via surface runoff and domestic wastewater discharge(X. Xu et al., 2023). This has resulted in widespread ecological degradation, the loss of native species habitats, and elevated ecological risks to both aquatic organisms and human residents(Su et al., 2025). Aquatic ecosystem health monitoring aims to quantify community structural shifts, track functional degradation and identify emerging environmental stressors timely, which is the core foundation of sustainable watershed management(Z. Zhao et al., 2025).

Traditional approaches for aquatic ecosystem health assessment are primarily based on water quality parameters or single biological indicators, including the Water Quality Index (WQI), Index of Biotic Integrity (IBI), multimetric indices (MMIs) and species diversity indices(Biney, Gyamfi, Karikari, & Darko, 2025; Vadas et al., 2022). Intensified human activities generate massive pollutant inputs, which largely deteriorate the water environment of urban rivers. Merely reducing pollutant loads back to pre-disturbance levels cannot restore stable river ecosystems, as full functional recovery requires the reconstruction of intact aquatic biotic assemblages(Cui et al., 2024). Against this background, biotic integrity assessment has become a primary monitoring technique for freshwater ecosystems, with the

IBI regarded as a highly representative, dependable multimetric evaluation framework(C. Zhao et al., 2019).

The IBI framework integrates multiple metrics reflecting community composition, functional traits, and ecological responses, delivering stronger discrimination capacity than single indicators(Beck & Hatch, 2009; Zheng et al., 2023). Initially developed for fish assemblages, the IBI has been extended to benthic macroinvertebrates, periphytic algae, phytoplankton, zooplankton, microbes and aquatic macrophytes(Beck & Hatch, 2009; Q. Liu, Yin, & Yi, 2024; Z. Xu et al., 2025). Aquatic biota across different trophic niches exhibit divergent responses and sensitivities to water depth, sediment properties and eutrophic stress(Cui et al., 2024). These interspecific differences yield mutually complementary monitoring indicators capable of reflecting ecosystem disturbances(Lu et al., 2024). Fish, as top predators with long life cycles and strong habitat dependence, reflect cumulative long-term river environmental conditions but respond slowly to short-term environmental fluctuations(Wu et al., 2020). Benthic macroinvertebrates attach to fixed riverbed substrates and are sensitive to low-concentration toxic pollutants, making them effective early-warning indicators for sediment degradation(Wu et al., 2020). As primary producers, phytoplankton and periphytic algae react rapidly to surrounding environmental variations but cannot reliably represent long-term cumulative disturbance(Tang, Xu, Yang, & Zhang, 2026; G. Zhang, Duan, & Li, 2024). Furthermore, periphytic algae respond rapidly to local environmental conditions (e.g., flow, depth, water quality) and are more representative than phytoplankton for site-specific assessment(Lu et al., 2024; G. Zhang et al., 2024). Zooplankton plays a crucial role in food-web energy transfer, and their abundance and diversity directly reflect river trophic levels(Mutethya, Yongo, Qi, et al., 2024). Microbes exhibit short life cycles and high metabolic rates, and their community structure and functional genes can rapidly indicate the self-recovery capacity of aquatic ecosystems under pollutant stress(Wang, Zhang, Zhang, & Chang, 2023; M. Zhang et al., 2019). Overall, various taxonomic organisms have unique life history characteristics, leading to varied performance in indicating environmental shifts. IBIs built upon a single taxon thus fail to reflect the complete ecological conditions of aquatic ecosystems(Huang, Xu, Liu, Guan, & Li, 2022). Previous studies have shown that assessments based on one biological group often produce inconsistent or contradictory results, especially in watersheds under mixed human disturbances(Carlisle, Hawkins, Meador, Potapova, & Falcone, 2008; Lu et al., 2024; Z. Zhao et al., 2025; Zhu et al., 2023). To improve accuracy and representativeness, multi-taxon integrated indices are increasingly recommended, yet few studies have considered hydrological seasonality when constructing multi-assemblage IBIs for subtropical urban rivers.

The Dongxixi River, Xiamen’s largest coastal urban watershed, presents prominent seasonal flow variation and persistent urban-agricultural compound interference. In this study, we established season-specific single-taxon IBIs and a multi-assemblage IBI (m-IBI) for five biotic groups. This study aimed to: (1) evaluate the spatiotemporal gradients of aquatic biotic integrity across three hydrological period; (2) compare the temporal stability and discrimination performance of single-taxon and multi-assemblage IBIs; (3) identify dominant physicochemical drivers of biological integrity under distinct runoff regimes;

(4) establish a standardized multi-assemblage IBI workflow adaptable to subtropical coastal urban river monitoring and ecological restoration.

Materials and methods

Study area and sampling site distribution

The Dongxixi River is located in a subtropical monsoon climate zone in Xiamen, China, with a catchment area of 491.48 km². Upstream reaches feature mountainous terrain with steep slopes and rapid flow, while midstream and downstream sections pass through flat plains subjected to intensive urban construction and crop cultivation. This river acts as the core ecological barrier of northeastern Xiamen and supplies domestic and agricultural water for Tong'an and Xiang'an Districts.

Eight sampling sites were set along the main channel and primary tributaries (Fig. 1), covering one national-controlled section (D5), five provincial-controlled sections (D1-D4, D8) and two city-controlled sections (D6-D7). Samples were collected in three representative hydrological periods: September 2023 (wet season, high runoff induced by typhoon rainfall), December 2023 (dry season, low base flow and pollutant accumulation) and April 2024 (normal season,stable moderate discharge).

Sample collection and processing

Water temperature (WT), pH, dissolved oxygen (DO), and electrical conductivity (EC) were measured in situ using a Hach® HQ4300 portable multi-meter. In addition, 2 L of water was collected at each site and transported to laboratory to analyze the permanganate index (COD_{Mn}), total nitrogen (TN), total phosphorus (TP), ammonia nitrogen (NH_3-N), and chemical oxygen demand (COD) within 48 h. All water quality detection procedures strictly followed national standard analytical methods for freshwater monitoring.

Periphytic algae

Three to five cobbles (64–256 mm) or ten pebbles (16–64 mm) were collected within a 50 m river reach at each site. Periphytic algae were scrubbed from a defined area of approximately 100 cm² using a stiff-bristled brush and rinsed into pre-cleaned bottles with purified water. Samples were fixed with Lugol's solution at a final concentration of 10–15% (v/v). Algal identification and enumeration were performed under a microscope (CX31RTSF, Olympus, Japan) using a plankton counting chamber.

Phytoplankton

A 2.5 L water sample was collected at a depth of 0.5 m and preserved with 1.5% Lugol’s iodine solution. All samples were settled in darkness for 48 h and subsequently concentrated to 50 ml for microscopic analysis. Phytoplankton enumeration and species identification were performed at ×400 magnification using a 0.1 mL plankton counting chamber.

Zooplankton

30 L of surface water (0.5 m depth) was filtered through a 25# plankton net, and concentrated organisms were transferred to a 100 mL sample bottle. Samples were fixed with 1.0% Lugol's solution, settled in darkness for 48 h in the laboratory, and further concentrated before enumeration. Zooplankton were identified and counted to the lowest possible taxonomy under 400× magnification.

Benthic macroinvertebrate

A D-frame net (30×30 cm, 425 μm mesh) was applied to sample a total area of 1 m^2across mixed habitats (riffles, pools, riparian vegetation, depositional areas). All samples from a single site were combined as one composite sample, rinsed to remove large particles and preserved in sealed containers with 7% formaldehyde solution. Prior to identification, samples were rinsed with deionized water or phosphate buffer to eliminate residual fixative. All macroinvertebrates were identified to genus or family level using standard taxonomic keys.

Fish

Environmental DNA (eDNA) metabarcoding was adopted to characterize fish community structures. 1L water samples were collected from both surface and bottom layers, fully homogenized and stored in sterile polyethylene bottles. For each homogenized sample, 350–500 mL water was filtered through 0.45 μm mixed cellulose filter membranes (Merck Millipore, Germany). Milli-Q blank water was processed synchronously as negative control to detect exogenous DNA contamination risk. Filter membranes were stored at − 20°C before delivery to One Health Biotechnology (Suzhou) Co., Ltd for DNA extraction and high-throughput sequencing. PCR amplification was performed using the primers MetafishF1(5'-TCGTGCCAGCCACCGCGGTTA-3') and MetafishR1(5'-ATAGTGGGGTATCTAATCCCAG-3')(Xiong, MacIsaac, & Zhan, 2024). The 50 μL PCR reaction mixture contained 25 μL of Taq polymerase, 50 ng of template DNA, 2 μL of each primer (10 μM), and ddH_2O to the final volume. The thermal cycling protocol comprised an initial denaturation at 98°C for 30 s; 35 cycles of 98°C for 10 s, 58°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 5 min. The amplified products were gel-purified and sequenced on an Illumina NovaSeq 6000 system.

Development of IBIs

Selection of reference sites

To develop the IBIs, sampling sites were classified into reference and disturbed sites. Reference sites serve as benchmarks and were selected based on criteria including minimal discernible anthropogenic disturbance, good water quality, and high habitat quality(Xiaoyi Hu et al., 2022; Y. Zhang, Ban, Li, Wang, & Xiao, 2020). Three upstream sites (D1, D2, D3) satisfying all thresholds were defined as reference sites, while the remaining five sites were categorized as disturbed sites.

Selection of metrics

A total of 19 phytoplankton(Huang et al., 2022; Lee et al., 2023; Y. Li & Li, 2023; Z. Zhao et al., 2025), 19 zooplankton(Lee et al., 2023; Lu et al., 2024; Mutethya, Yongo, Zhang, Guo, & Changqing, 2024; Z. Zhao et al., 2025), 24 periphytic algae(Lu et al., 2024; Z. Xu et al., 2025; Z. Zhao et al., 2025), 35 benthic macroinvertebrate(Huang et al., 2022; Lu et al., 2024; C. Zhao et al., 2019; Z. Zhao et al., 2025), and 32 fish(Huang et al., 2022; Meng et al., 2023; Yu et al., 2024; C. Zhao et al., 2019) candidate metrics were preselected, covering species composition, abundance, diversity and functional traits with proven sensitivity to anthropogenic stressors (Table 1).

Candidate metrics were screened through three sequential steps: distribution range analysis, discriminant capacity analysis, and Pearson correlation analysis to obtain the core metrics. First, eliminate indicators with extremely narrow value ranges, excessive fluctuations, over half zero detection rates or non-monotonic response trends along disturbance gradients. Second, boxplots were used to compare interquartile ranges (IQRs) of metrics across reference and disturbed sites, and only parameters with a difference in IQRs ≥ 2 were retained. Finally, pearson correlation analysis was performed in SPSS 19.0, and highly redundant metrics ($|r| \geq 0.75$, $p < 0.05$) were removed.

Metric standardization and IBI classification

Retained core metrics were standardized via ratio transformation, with all scores constrained from 0 to 1 (scores > 1 were recorded as 1). The final IBI score of each site equals the average standardized value of all core metrics within the corresponding taxon system(Huang et al., 2022; Zhu et al., 2021). For metrics that decrease with increasing disturbance:

Standardized score = Site value / 95th percentile value of all sites).

For metrics that increase with increasing disturbance:

Standardized score = (Maximum value − Site value) / (Maximum value − 5th percentile value of all sites).

The 25th percentile value of reference-site IBI scores served as the ecological health threshold. Sites with IBI > IBIE were classified as "Healthy", indicating minimal anthropogenic disturbance. All scores below IBIE were divided into four equal intervals, corresponding to "Sub-healthy", "Moderate", " Poor", and "Extremely poor"(Huang et al., 2022).

Water quality index (WQI) calculations

The WQI integrates multiple discrete physicochemical parameters into a single dimensionless score to reflect comprehensive water quality status(Xue et al., 2025). In this study, eleven water quality parameters were selected for WQI calculation, and the formula is as follows:

$$WQI = \sum_{i=1}^{n} C_i P_i / \sum_{i=1}^{n} P_i$$

Where *n* represents the number of selected water quality parameters; *Ci* is the normalized value of parameter *i*; *Pi* is the weight of parameter *i* (*Pi* ∈ [1,4]). Parameter weights were adopted from published literature and are listed in Table S1(J. Gao et al., 2023; Tian et al., 2019; Xue et al., 2025; K. Zhao et al., 2025).

Data analysis

Microsoft Excel 2020 supported raw data preprocessing, basic IBI calculation and boxplot plotting. SPSS 19.0 was used for Pearson correlation analysis of candidate biological metrics. OriginPro 8.5 constructed linear regression models to quantify fitting performance between IBIs and WQI. Correlation matrices and visualization were generated using the “ggplot2” and “corrplot” packages in R.

Table 1
The aquatic biological integrity evaluation index system.

Candidate Metrics and Groups				
Z-IBI	**A-IBI**	**B-IBI**	**F-IBI**	**P-IBI**
Species Composition	**Species Composition**	**Species Composition**	**Species Composition**	**Species Composition**
Z1 Zooplankton taxa	A1 Periphytic algae taxa	B1 Benthos taxa	F1 Fish taxa	P1 Phytoplankton taxa
Z2 Rotifer taxa	A2 Bacillariophyta taxa	B2 EPT taxa	F2 Cypriniformes taxa %	P2 Bacillariophyta taxa
Z3 Cladoceran+Copepods taxa	A3 Chlorophyta taxa	B3 Ephemeroptera taxa	F3 Cyprinidae taxa %	P3 Chlorophyta taxa
Z4 Protozoa taxa	A4 Cyanobacteria taxa	B4 Trichoptera taxa	F4 Cobitidae taxa %	P4 Cyanobacteria taxa
Z5 Rotifer taxa %	A5 Bacillariophyta taxa %	B5 Aquatic insect taxa	F5 Siluriformes taxa %	P5 Bacillariophyta taxa %
Z6 Protozoa taxa %	A6 Chlorophyta taxa %	B6 Crustacea + Molluscs taxa	F6 Perciformes taxa %	P6 Chlorophyta taxa %
Z7 Top 2 dominant taxa %	A7 Cyanobacteria taxa %	B7 Annelida taxa	F7 Gobiidae taxa %	P7 Cyanobacteria taxa %
Species Biomass	A8 Sensitive species taxa %	B8 Arthropoda taxa	**Biodiversity Index**	**Relative Abundance**
Z8 Zooplankton biomass mg/L	**Relative Abundance**	B9 Mollusca taxa	F8 Shannon-Wiener index	P8 Phytoplankton
Z9 Protozoa biomass mg/L	A9 Bacillariophyta %	B10 Dominant taxon %	F9 Margalef index	P9 Bacillariophyta
Z10 Rotifer biomass mg/L	A10 Chlorophyta %	B11 Top 3 dominant taxon %	F10 Peilou index	P10 Chlorophyta
Z8 Cladoceran+Copepods biomass mg/L	A11 Cyanobacteria %	**Relative Abundance**	**Relative Abundance**	P11 Cyanobacteria

Note: Filamentous green algae: Cladophorales, Ulotrichales, Zygnematales; Mobile diatom: Navicula, Nitzschia, Surirella; Stipitate diatom: Gomphonema Ehrenberg, Licmophora, Achnanthes; EPT: Ephemeroptera, Trichoptera, Plecoptera; Diatom Entropy = Total individuals of Centrales / Total individuals of Pennales.

Candidate Metrics and Groups				
Z9 Cladoceran+Copepods biomass %	A12 Achnanthes %	B12 Benthos abundance	F11 Fish abundance	P12 Bacillariophyta %
Relative Abundance	A13 Cymbella %	B13 EPT %	F12 Cypriniformes fishes %	P13 Chlorophyta %
Z10 Zooplankton abundance	A14 Nitzschia %	B14 Ephemeroptera %	F13 Cyprinidae fishes %	P14 Cyanobacteria %
Z11 Rotifer abundance	A15 Navicula %	B15 Trichoptera %	F14 Cobitidae fishes %	P15 Top 3 dominant abundance %
Z12 Cladoceran+Copepods abundance	A16 Filamentous green algae %	B16 Chironomidae %	F15 Siluriformes fishes %	**Biodiversity Index**
Z13 Protozoa abundance	A17 Oscillatoria %	B17 Gastropoda %	F16 Perciformes fishes %	P16 Shannon-Wiener index
Biodiversity Index	**Ecological Types**	B18 Oligochaeta %	F17 Gobiidae fishes %	P17 Peilou index
Z17 Shannon-Wiener index	A18 Mobile diatom %	B19 Lamellibranchia	F18 Alien species %	P18 Margalef index
Z18 Peilou index	A19 Stipitate diatom %	B20 Tubificidae %	**Nutrition Structure**	P19 Diatom entropy
Z19 Margalef index	**Density and Biomass**	B21 Crustacea + Molluscs %	F19 Omnivorous fishes %	
	A20 periphytic algae density	**Pollution Tolerance**	F20 Herbivorous fishes %	
	A21 periphytic algae biomass mg/L	B22 Intolerant %	F21 Predatory fishes %	
		B23 Tolerant %	**Pollution Tolerance**	
	Biodiversity Index	B24 BMWP index	F22 Sensitive %	

Note: Filamentous green algae: Cladophorales, Ulotrichales, Zygnematales; Mobile diatom: Navicula, Nitzschia, Surirella; Stipitate diatom: Gomphonema Ehrenberg, Licmophora, Achnanthes; EPT: Ephemeroptera, Trichoptera, Plecoptera; Diatom Entropy = Total individuals of Centrales / Total individuals of Pennales.

Candidate Metrics and Groups			
	A22 Shannon-Wiener index	B25 ASPT index	F23 Tolerant %
	A23 Peilou index	B26 FBI index	F24 Moderate %
	A24 Margalef index	**Nutritional Structure**	**Reproductive Co-location group**
		B27 Filterers %	F25 Fish species spawning floating eggs %
		B28 Scrapers %	F26 Fish species spawning demersal eggs %
		B29 Predators %	F27 Fish species spawning adhesive eggs %
		B30 Collect-Gatherers %	F28 Fish species spawning drifting eggs %
		B31 Shredders %	F29 Fish species with special spawning types %
		Biodiversity Index	**Habitat Environment**
		B32 Shannon-Wiener index	F30 Middle and upper layers fish %
		B33 Margalef index	F31 Middle and lower layers fish %
		B34 Pielou index	F32 Bottom fish %

Note: Filamentous green algae: Cladophorales, Ulotrichales, Zygnematales; Mobile diatom: Navicula, Nitzschia, Surirella; Stipitate diatom: Gomphonema Ehrenberg, Licmophora, Achnanthes; EPT: Ephemeroptera, Trichoptera, Plecoptera; Diatom Entropy = Total individuals of Centrales / Total individuals of Pennales.

Candidate Metrics and Groups
Habitat Quality
B35 Clingers %
Note: Filamentous green algae: Cladophorales, Ulotrichales, Zygnematales; Mobile diatom: Navicula, Nitzschia, Surirella; Stipitate diatom: Gomphonema Ehrenberg, Licmophora, Achnanthes; EPT: Ephemeroptera, Trichoptera, Plecoptera; Diatom Entropy = Total individuals of Centrales / Total individuals of Pennales.

Results

Establishment of the IBIs and river ecosystem health assessment

Following distribution range analysis, discriminatory capacity analysis, and correlation analysis for all candidate indicators, core indicators were selected and subsequently integrated to develop hydrological period-specific IBI evaluation systems. The final indicators and calculation formulas for each IBI across different hydrological periods are detailed in Table S2 to Table S4, and the grading criteria for "Healthy, Sub-healthy, Moderate, Poor and Extremely Poor" are list in Table S5.

WQI assessment revealed a consistent declining trend in water quality from upstream to downstream across all hydrological periods (Fig. 2). Notably, 100% of sampling sites were classified as sub-healthy in the dry seasons, whereas 75% were sub-healthy and 25% were moderate in both the wet and normal seasons (Fig. 2). Based on average WQI scores, the overall ecological status of the Dongxixi River was “sub-healthy”. Spatial analysis revealed no significant spatial heterogeneity in WQI across hydrological periods (Fig. S1).

Based on calculated IBIs values, the aquatic ecosystem health status of the eight sampling sites was classified for each hydrological period (Fig. 3). Detailed assessment results for each index are as follows:

P-IBI: 2 healthy, 3 sub-healthy and 3 moderate in the wet season; 2 healthy, 4 sub-healthy and 1 poor in the dry season; 2 healthy, 5 sub-healthy and 1 moderate in the normal season. Mean scores: 2.295, 2.136 and 4.806, all corresponding to the sub-healthy category.

Z-IBI: 3 healthy, 2 sub-healthy and 3 moderate in the wet season; 2 healthy, 4 sub-healthy and 2 moderate in the dry season; 4 healthy, 2 sub-healthy and 2 moderate/poor in the normal season. Mean scores: 2.864, 3.205 and 2.106, all fell into the sub-healthy level.

A-IBI: 2 healthy, 3 sub-healthy and 3 moderate/poor in the wet season; 3 healthy, 1 sub-healthy and 4 moderate/poor in the dry season; 5 healthy, 2 sub-healthy and 1 poor in the normal season. Mean scores: 2.836 (wet season, sub-healthy), 2.436 (dry season, sub-healthy), and 2.288 (normal season, healthy).

F-IBI: 2 healthy, 2 sub-healthy and 4 moderate in both wet and dry seasons; 2 healthy, 3 sub-healthy and 3 moderate/poor in the normal season. Mean scores: 3.640, 3.932 and 2.552, all indicating sub-healthy.

B-IBI: 2 healthy, 1 sub-healthy and 5 moderate/poor in the wet season; 2 healthy, 1 sub-healthy and 5 moderate/poor in the dry season; 2 healthy, 3 sub-healthy and 3 moderate/poor in the normal season. Mean scores: 1.868 (wet season, moderate), 3.690 (dry season, moderate), and 2.924 (normal season, sub-healthy).

m-IBI: 2 healthy, 2 sub-healthy and 4 moderate in the wet season; 2 healthy, 2 sub-healthy and 4 moderate/poor in the dry season; 2 healthy, 4 sub-healthy and 2 moderate in the normal season. Mean scores: 9.098, 10.357 and 9.923, all corresponded to sub-healthy.

In general, the entire Dongxixi watershed maintained a sub-healthy baseline ecological status under all hydrological periods. A-IBI and B-IBI displayed unique seasonal grading patterns, driven by divergent biological traits and phase-sensitive core metric combinations.

Relationships among the IBIs and between IBIs and WQI

Linear regression analysis was employed to assess the performance of single-taxon IBIs by quantifying their fitting patterns with the WQI (Fig. 4). In the wet season, WQI was significantly and positively correlated with Z-IBI (r^2=0.778, p<0.05), F-IBI (r^2=0.534, p<0.05) and m-IBI (r^2=0.525, p<0.05). In the dry season, only Z-IBI maintained a significant positive correlation with WQI (r^2=0.509, p<0.05). In the normal season, significant positive correlations were detected between WQI and P-IBI (r^2=0.819, p<0.05), B-IBI (r^2=0.514, p<0.05) and m-IBI (r^2=0.602, p<0.05).

Pearson correlation analysis further quantified the associations between m-IBI and other IBIs across hydrological periods (Fig. 5). In the wet season, m-IBI exhibited significant correlations with Z-IBI (*P*<0.05), A-IBI (*P*<0.01), F-IBI (*P*<0.01), and B-IBI (*P*<0.001). In the dry season, m-IBI retained significant correlations with Z-IBI (*P*<0.05), A-IBI (*P*<0.01) and F-IBI (*P*<0.001), and developed a new correlation with P-IBI (*P*<0.05). In the normal season, m-IBI showed an extremely strong correlation with F-IBI (*P*<0.001) and highly significant correlations with P-IBI and B-IBI (*P*<0.01). This pattern reflects seasonal taxon turnover and functional complementarity across multi-trophic food webs.

Correlation between IBIs and physicochemical factors

Pearson correlation analysis was performed to analyze the response characteristics of six IBIs to water quality parameters in three hydrological periods (Fig. 6). The results revealed strong hydrological differentiation in the correlation patterns and distinct specificity in the responses of single-taxon IBIs to water quality parameters. Specifically, in the wet season (Fig. 6a), P-IBI was mainly positively correlated with organic and nutrient indicators including COD□□, COD, NH_3-N, TP. Z-IBI responded to the widest range of variables, including WT, DO, COD□□, NH_3-N, TP, EC and Tur. F-IBI and m-IBI had consistent response rules, both significantly correlated with WT, EC and Tur. B-IBI only showed significant

correlation with WT and COD. Notably, no significant correlations were detected between any IBIs and TN, DO, or pH in the wet season, implying weak effects of these parameters on aquatic biotic integrity under high-flow conditions. In the dry season (Fig. 6b), P-IBI responded mainly to nutrients and physicochemical variables (NH_3-N, TN, TP, EC). Z-IBI was only significantly positively correlated with NH_3-N, TN, and EC. A-IBI and F-IBI exhibited identical response patterns to WT, pH, COD, and NH_3-N. B-IBI expanded its sensitivity to WT, DO, NH_3-N, TN, and EC, while m-IBI correlated only with WT. In the normal season (Fig. 6c), the correlation of all single-taxon IBIs exhibited consistent response patterns, mainly concentrated on EC, Tur, and COD_{Mn}. Specifically, Z-IBI and F-IBI showed identical significant positively correlations with EC and Tur. Beyond this shared pattern, P-IBI was additionally correlated with TN, B-IBI and m-IBI with COD_{Mn}.

Discussion

This study established a hydrological period-specific IBI assessment system for the Dongxixi River, and further quantified the spatiotemporal characteristics of river ecological health by coupling IBI assessments with WQI analysis. We also elucidated the correlation patterns among six IBIs, between IBIs and WQI, and between IBIs and physicochemical variables across different hydrological periods. Although numerous studies have constructed IBI systems for urban river health assessment in subtropical China(J. Li et al., 2017; Tan, Ma, Bunn, & Zhang, 2015; Zhu et al., 2023), most have overlooked the seasonality of hydrological fluctuations and their regulatory effects on biotic-abiotic coupling relationships, which limits the applicability of IBIs for long-term regional monitoring. In this study, the six IBIs showed divergent evaluation results, and their correlations with WQI and physicochemical parameters exhibited strong hydrological dependence. These findings improve the mechanistic understanding of linkages between aquatic biotic communities and environmental factors in subtropical coastal rivers and provide a scientific basis for selecting regionally adapted IBIs for long-term health monitoring in southeastern China.

Seasonal variation of river ecological health driven by runoff differences

WQI scores declined moderately in the wet season, primarily driven by rainfall surface runoff carrying massive non-point source agricultural and urban pollutants into the river, overwhelming dilution effects of increased discharge(Ajloon, Dong, Ayejoto, Ayeni, & Sabo, 2024; A. Zhao et al., 2025).

The six IBIs exhibited different responses to hydrological changes, reflecting the interspecific differences in life history characteristics of various aquatic organisms. A-IBI had the most dramatic seasonal changes. In the normal season, suitable water temperature, stable water volume, appropriate flow velocity, and low pollutant disturbance create ideal substrate environments for periphytic algae(Feng, Wang, & Xie, 2011; Zębek, 2013), which explains why the majority of sampling sites attained a healthy grades in this period. In contrast, vigorous runoff in the wet seasons and sustained pollutant sedimentation in the dry season both damage algal attachment substrates and reduce community integrity, thereby lowering A-IBI scores to the sub-healthy category. Benthic macroinvertebrates are

sensitive to cumulative habitat degradation and multiple co-occurring environmental stressors(Xiaohong Hu et al., 2022; Su et al., 2025; Q. Zhang, Yang, Wan, Wang, & Wang, 2021). Rainwash input of pollutants in wet seasons and persistent pollutant sedimentation in dry seasons both damage benthic habitats, resulting in moderate degradation evaluated by B-IBI in these two periods. Sediment pollutant stress is relieved during normal flow seasons, and B-IBI recovers to sub-healthy grades accordingly. P-IBI, Z-IBI, F-IBI, and m-IBI maintained stable sub-healthy status across all hydrological periods, with weak seasonal fluctuations. Previous studies reported obvious seasonal variation of plankton IBIs(Huang et al., 2022; Z. Li, Ma, Sun, Lu, & Fan, 2022; Lin et al., 2021; Mutethya, Yongo, Zhang, et al., 2024), which differs from the results of this study. This contradiction was associated with the dominant environmental stressor characteristics of different watersheds. In this study, the Dongxixi River was in a mesotrophic state across all hydrological periods. Stable background nutrient concentrations prevented drastic fluctuations in the plankton community structure(Goh et al., 2026; Z. Liu et al., 2023). As top predators, fish have long life cycles, high stress tolerance, and extensive activity range, allowing them to withstand moderate environmental disturbances and thus exhibit stable long-term responses(Wu et al., 2020; Zhu et al., 2023). Tolerant and non-native fish species occupy the dominant position in the Dongxixi River, which also stabilizes the evaluation results of F-IBI in different hydrological periods. Thus, P-IBI, Z-IBI, F-IBI and m-IBI with weak seasonal fluctuation can represent annual ecosystem health based on one-off sampling surveys. By comparison, A-IBI and B-IBI exhibit strong seasonal variability and are thus more applicable to real-time and continuous monitoring schemes.

Longitudinal spatial degradation gradient induced by human interference

WQI evaluation demonstrated a universal degradation trend from upstream to downstream transects, a typical pattern for subtropical small-medium rivers under urban-agricultural compound interference(Ajloon et al., 2024; Mo, Xu, Liu, Wu, & Chen, 2024). In particular, sites D7 and D8 exhibited moderate water quality, driven by persistent pollutant loads originating from dense residential zones, intensive agricultural cultivation, and clustered urban built-up areas.

Assessments based on IBIs showed significant spatial heterogeneity across all three hydrological periods, with ecosystem health in the upper reaches of the Dongxixi River consistently superior to that in the middle and lower reaches. This longitudinal gradient reflects the well-documented spatial pattern of river ecosystem degradation from upstream to downstream under urbanization pressure(Jargal, Atique, Kim, Mamun, & An, 2022). The upper reaches of Dongxixi have lower population density and minimal human disturbance, whereas the middle and lower reaches are predominantly urban and agricultural zones, which profoundly alter habitat conditions and disrupt the life histories of aquatic organisms.

Comparison between WQI and IBI results showed that both approaches effectively distinguish the ecosystem health status between reference and disturbed sites. However, notable discrepancies exist in the evaluation results at identical sampling locations. Taking national controlled section D5 as an example, WQI consistently indicated sub-healthy conditions with relatively favorable water quality across all hydrological periods. However, biological evaluation scores derived from IBIs exhibited large

variations, ranging from poor to healthy grades. This discrepancy arises from divergent responses of distinct biological communities to the type and intensity of environmental stressors(Huang et al., 2022; Z. Zhao et al., 2025). It also proves that simply relying on water quality physicochemical monitoring cannot fully reflect actual river ecological degradation conditions. In addition, biological community recovery always lags behind water quality improvement after pollution interception, which reminds river managers to combine biological monitoring with conventional water quality testing in daily supervision work.

Advantages of m-IBI for long-term watershed monitoring

In this study, correlations between single-taxon IBIs and WQI varied across hydrological periods, indicating that reliance on any single-taxon IBI is insufficient to reliably represent water quality conditions across all hydrological stages. Notably, the m-IBI exhibited the most stable and consistent positive correlations with WQI across all hydrological periods, indicating its superior stability in reflecting comprehensive water quality dynamics. This is because m-IBI integrates responses from multiple biological groups, thereby reducing uncertainty caused by seasonal variability inherent in single-taxon indices.

Pearson correlation analysis further revealed stable and strong correlations between the m-IBI and multiple aquatic biotic indices across all hydrological periods. Therefore, this integrated index can reflect both structural and functional traits of different biological groups as a whole. Additionally, the core biotic indicators correlated with the m-IBI differed greatly among hydrological seasons. Accordingly, these composite indices can reflect dynamic shifts in species succession and functional complementarity among aquatic communities.This targeted optimization greatly improves the stability and comprehensiveness of ecological evaluation under variable flow conditions, confirming the practical value of m-IBI for long-term monitoring of subtropical coastal urban rivers.

Hydrological periods mediate abiotic-biotic coupling relationships

Previous IBI studies mostly restricted correlation analysis to one single sampling period, ignoring the regulatory effects of seasonal flow variations on biota-environment coupling relationships(Cui et al., 2024; Lunde & Resh, 2012; Rocha et al., 2020; Z. Zhao et al., 2025). Our findings reveal that correlation patterns between IBIs and physicochemical factors display strong temporal variability across hydrological seasons. During the wet season, most IBIs were positively correlated with organic matter (COD□□, COD) and nutrients (NH_3-N, TP), reflecting the dominant pressure of non-point source pollution inputs driven by rainfall runoff. In the dry season, the response parameters of IBIs shifted to habitat-related indicators (WT, pH, DO, EC) and nutrients (TN, NH_3-N). During the normal season, stable hydrological conditions reduced spatial heterogeneity in water quality, resulting in all IBIs being primarily correlated with EC, Tur, and COD□□. These results demonstrate that river health assessment must account for hydrological seasonality to avoid biased conclusions.Targeted pollution control priorities can be formulated seasonally: non-point source interception for agricultural and urban runoff in the wet

seasons, minimum ecological flow maintenance and sediment remediation in the dry seasons, and regular organic pollutant monitoring during stable normal flow periods.

Practical monitoring and restoration suggestions for subtropical coastal urban rivers

Urban rivers bear unique compound anthropogenic stressors that cannot be fully characterized via separate physicochemical monitoring or single-taxon biological surveys. The inconsistent IBI evaluation results we obtained at downstream urban sections fully prove that single aquatic taxon cannot objectively reflect overall river ecological degradation, highlighting the necessity of multi-assemblage integrated evaluation frameworks(Z. Gao et al., 2026).

This study puts forward two practical recommendations for ecological monitoring and restoration of the Dongxixi Watershed:

First, optimize long-term monitoring strategies. The m-IBI is recommended for annual routine ecological baseline surveys, while monthly supplementary sampling of A-IBI and B-IBI should be arranged during wet and dry seasons to capture short-term habitat disturbances. Second, expand restoration objectives beyond merely reducing pollutant loads. Nutrient control should be coupled with physical habitat rehabilitation, such as reconstructing alternating riffle-pool sequences and deploying natural cobble substrates to rebuild suitable habitat conditions for periphytic algae and benthic macroinvertebrates.

The m-IBI framework established in this study can be extended to other subtropical coastal urban watersheds in southeast China. For regional adaptation, researchers only need to optimize local biological indicator assemblages based on the unique riverine environmental features of the study area.

Conclusion

This study developed independent hydrological phase-specific single-taxon IBIs and an integrated multi-assemblage m-IBI system for a subtropical urban river. The whole river basin maintained a general sub-healthy ecological state in wet, dry and normal season. All single-group IBIs displayed obvious seasonal fluctuation and upstream-downstream degradation spatial gradients. On the contrary, the m-IBI had stable and significant correlations with WQI under all three hydrological backgrounds. The core biological indicators that determine river ecological health change with seasons, which reflects seasonal species succession and complementary ecological functions of multi-trophic aquatic communities. The coupling relationships between each single IBI and water physicochemical indicators also show strong seasonal mediation effects, with distinct dominant pollution stressors corresponding to wet, dry and normal season respectively. This integrated m-IBI framework overcomes the evaluation biases of single-taxon IBIs and maintains stable performance under fluctuating seasonal flows. Combining hydrological seasonal characteristics with multi-trophic biotic assemblages greatly enhances the reliability of urban river health assessment. The assessment framework developed in this study offers a replicable protocol for routine aquatic ecological monitoring and targeted ecological restoration of subtropical coastal urban rivers worldwide.

Declarations

Ethical approval

No formal ethical approval is required.

Consent to participate

All authors participated and contrib uted to the study.

Consent for publication

The authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare no competing interests.

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Author Contribution

H.Z: Conceptualization, methodology design, field investigation, data collection, sample measurement, data analysis, and manuscript writing. Z.Z: Conceptualization, methodology design, field investigation, data collection, sample measurement, data analysis, visualization, and manuscript writing. Y.L: Methodology design, data analysis, and manuscript revision. F.Y: Data analysis and manuscript revision. C.Y: Data analysis and manuscript revision. All authors reviewed the manuscript.

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Figures

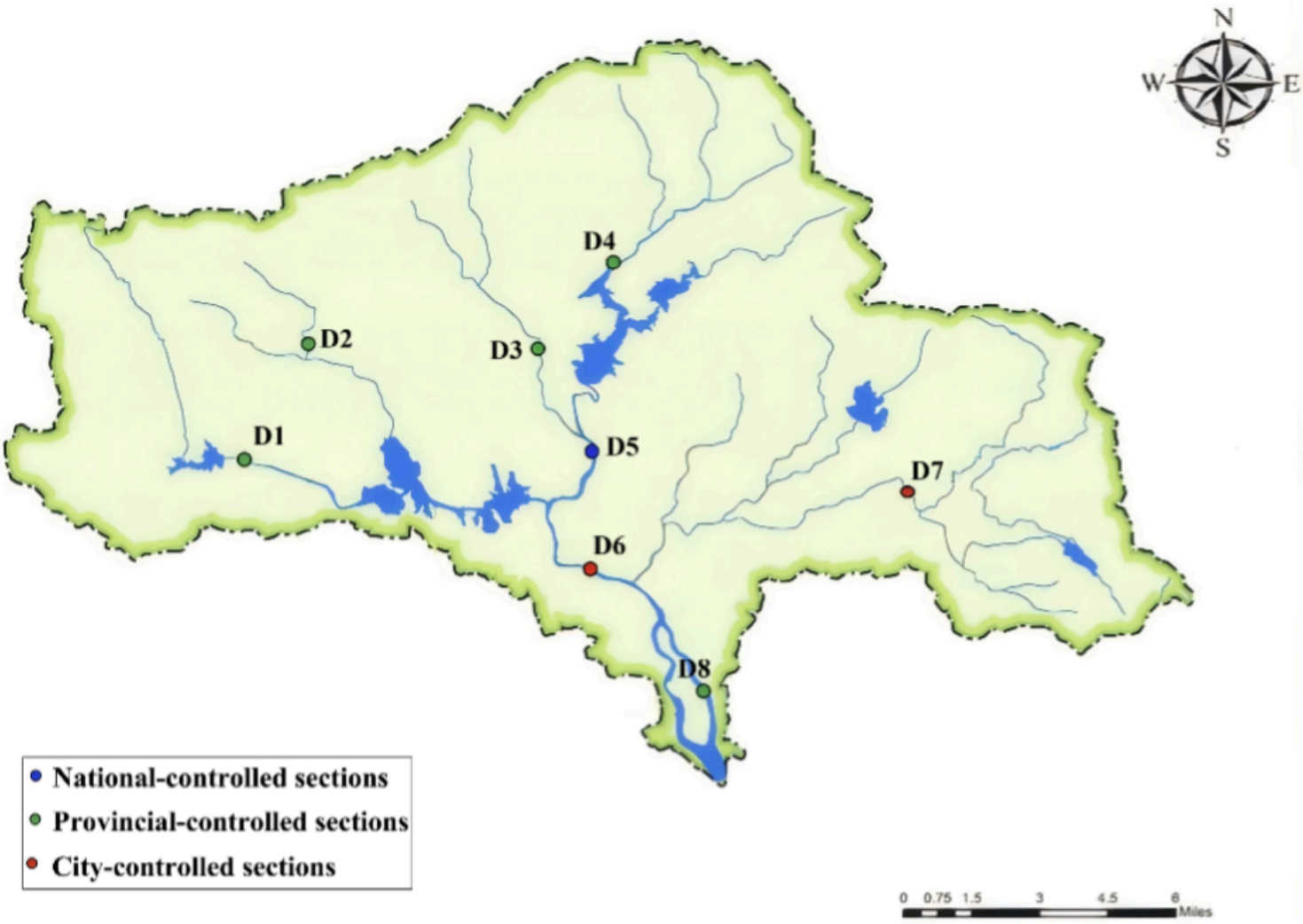


Figure 1

Spatial distribution of eight sampling sites along the Dongxixi River. Symbols distinguish national, provincial and municipal monitoring sections.

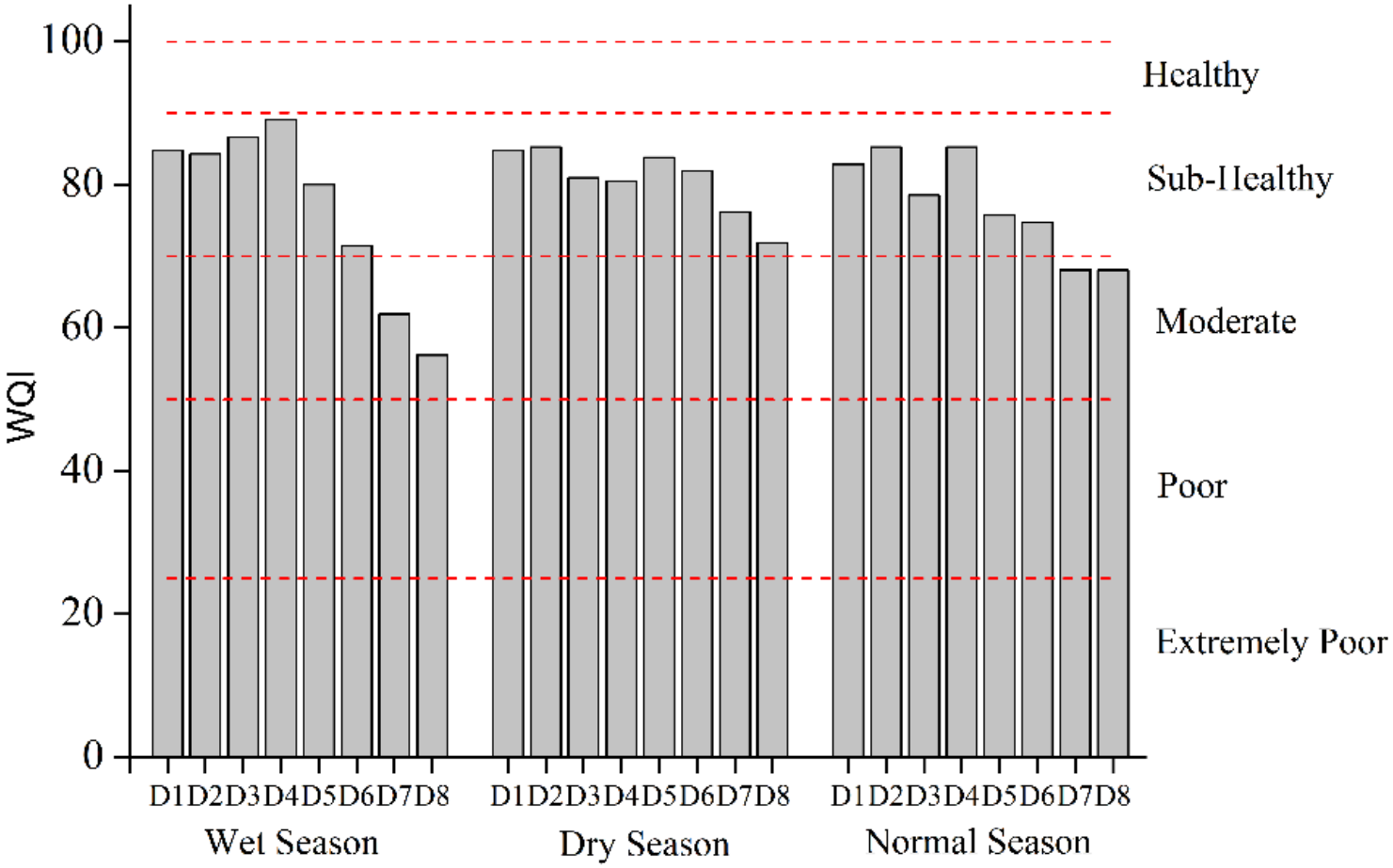


Figure 2

Spatiotemporal proportions of ecological health grades derived from WQI evaluation across three hydrological period of the Dongxixi River.

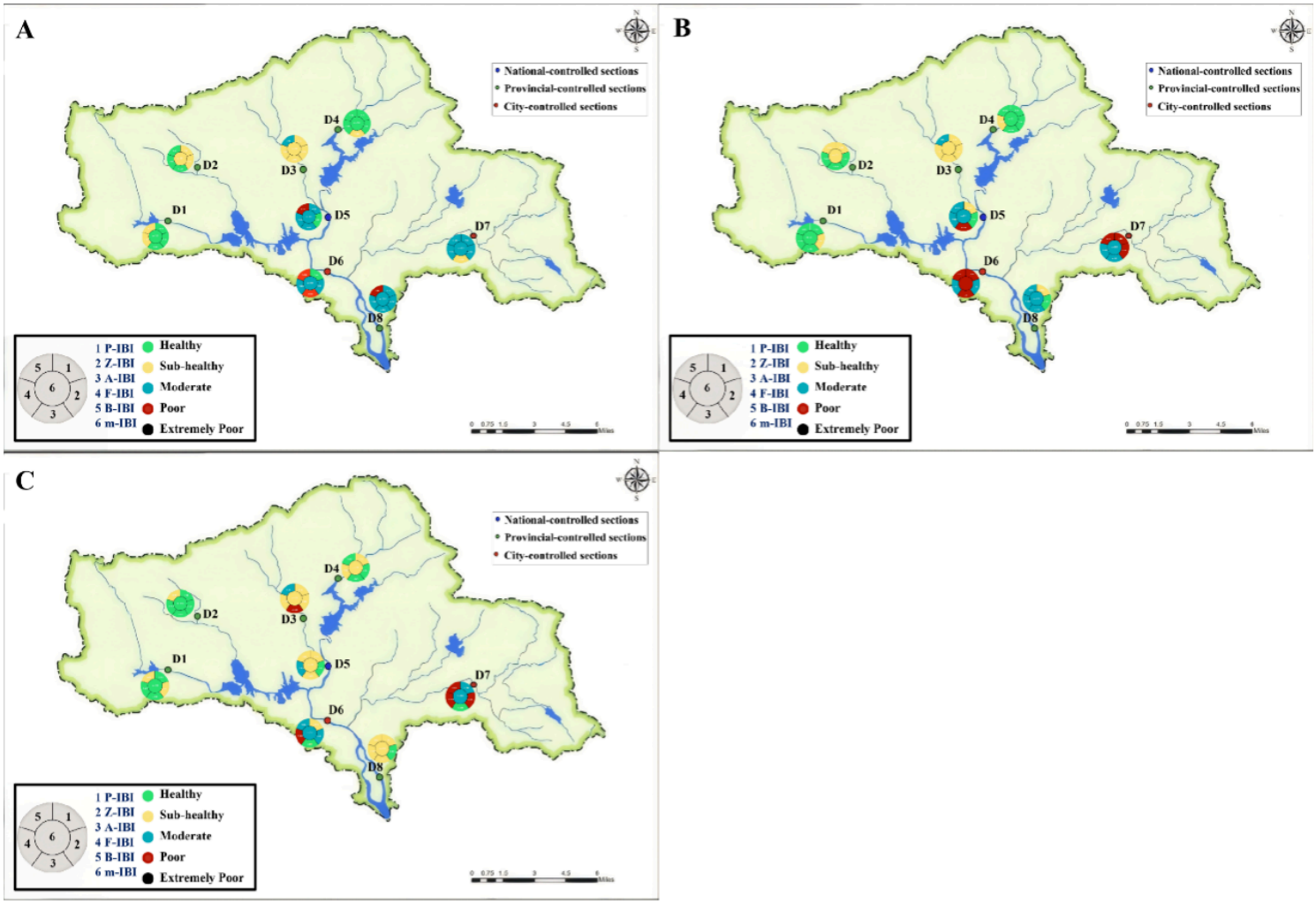


Figure 3

Temporal and spatial distribution of aquatic ecosystem health levels in Dongxixi River based on 6 IBIs. Colors of zones 1-6 represent health grades for P-IBI, Z-IBI, A-IBI, F-IBI, B-IBI, and m-IBI, respectively. (a) Wet season; (b) Dry season; (c) Normal season.

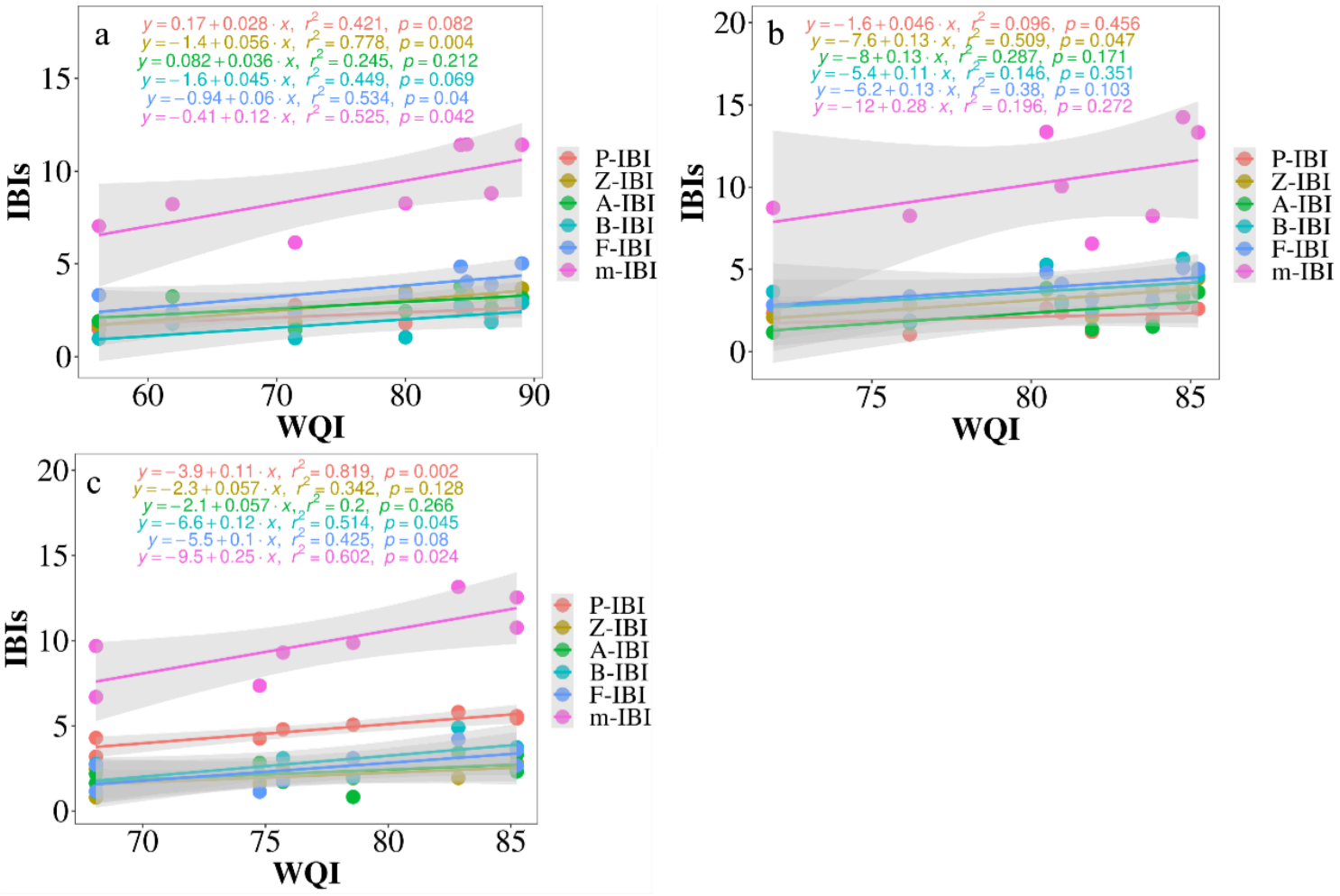


Figure 4

Linear relationships between 6 IBIs and WQI. (a) Wet season; (b) Dry season; (c) Normal season.

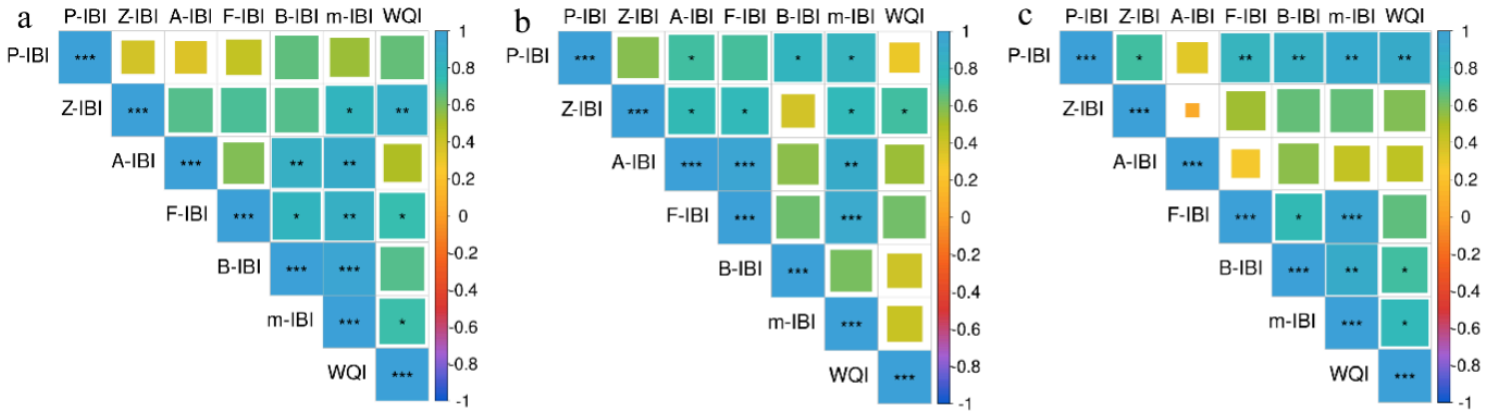


Figure 5

Pearson correlation coefficients between 6 IBIs and WQI across three hydrological period. (a) Wet season; (b) Dry season; (c) Normal season. (□ P<0.05, □□ P <0.01, □□□ P <0.001)

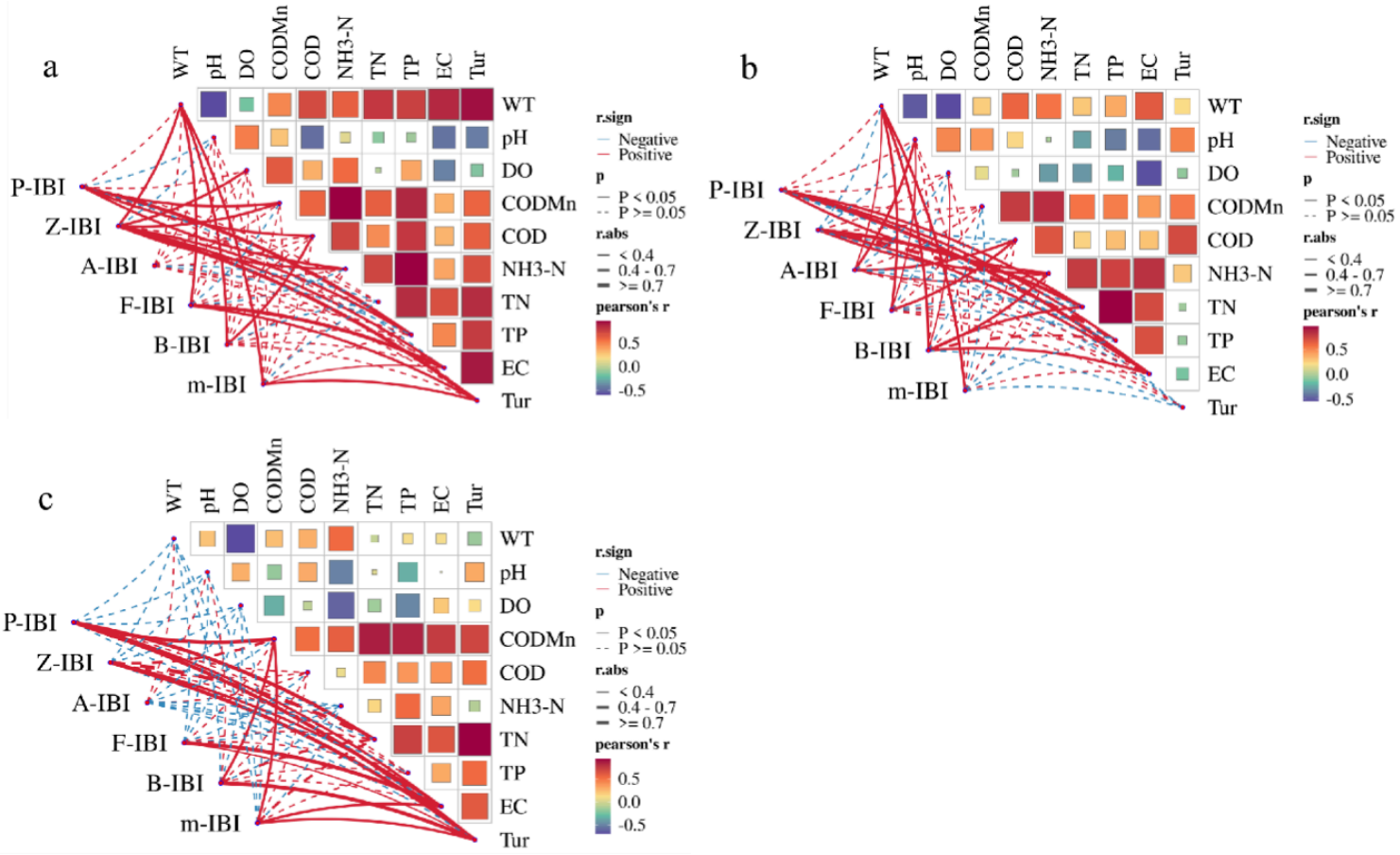


Figure 6

Correlations between physicochemical indexes and IBIs. (a) Wet season; (b) Dry season; (c) Normal season. Red and blue lines represent positive and negative correlations, respectively. Solid lines indicate significant correlations (P<0.05), and dashed lines indicate non-significant correlations (P>0.05). Line thickness is proportional to correlation strength. *P<0.05, **P<0.01, ***P<0.001.

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