Using fish-based biological index to indicate eco-environmental status along the longitudinal gradient of a subtropical river

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Abstract

River ecosystems are facing a deepening biodiversity crisis. Developing robust biotic indicators to assess ecological status across large spatial scales are important. In the subtropical Liuxi River of southern China, 34 fish indicators, including 4 genera and 30 species, were selected from 108 fish species by linear discriminant analysis. These indicators were combined into 18 groups and assigned scores according to their species-specific requirements for food resources and habitat patterns. The ecological and trophic functioning of optimized indicators can reflect not only the community diversity and food web properties but also the environmental quality of the ecosystem. Three formulas for calculating the index of fish indicators (IFI) were developed based on the scoring of each indicator and weighted by relative abundance (individual number, i.e., IFI_N) and relative biomass (wet weight, i.e., IFI_B). Spearman correlation analysis showed that IFI_B exhibited a more powerful explanation of biodiversity and environmental factors than IFI_N and unweighted IFI. Therefore, we conclude that IFI_B has absolute advantages in constructing an indicator-based environmental evaluation system since it contains comprehensive information on biology and ecology. In the future, the application of indicator scoring methods can contribute greatly to the conservation and development of aquatic ecosystems.

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Introduction

Rivers maintain unique biotic resources and provide critical water supplies to people, yet river systems are directly threatened by human activities and stand to be further affected by anthropogenic climate change (Dudgeon, 2000). Multiple environmental stressors, such as channelization, agricultural runoff, and alien species invasion, threaten river ecosystems (Wiens, 2002). These stressors endanger the biodiversity of 65% of the world's river habitats putting thousands of aquatic wildlife species at risk (Vörösmarty et al., 2010). Aquatic organisms, such as fish, phytoplankton, benthic flora (e.g., diatoms and macrophytes), and macroinvertebrates are commonly used as indicators to reflect the biological integrity and river health. Fish, with more complex life history than invertebrates and plants, exhibited specific requirements on food

resources, refuge/nursing ground, and migration pathway during their growth stages (Borja et al., 2008). They are sensitive indicators of water pollution, habitat degradation, migration barriers, and overall ecosystem productivity (Wang et al., 2018b; Winemiller, 1990).Therefore, fish are favored by researchers and monitors to reflect comprehensive information on river ecology and environment (Mujiyanto et al., 2021).

From the perspective of river food web, fish are top predators and exert both top-down and bottom-up control effects on other communities (Southerland et al., 2007; Souza and Vianna, 2020). Fish with high food selectivity (e.g., piscivores, insectivores, molluscivores, algivores, and herbivores) depended greatly on local available resources (e.g., aquatic insect larvae, decapods, molluscs, diatoms, and macrophytes) (Wang et al., 2019; Wang et al., 2018b). In this context, fishes with specific feeding habits could be regarded as the core nodes of predator-prey links, their species richness and population number are of great importance to the diversity and structure of local food webs (Wang et al., 2020a) and even the health and functioning of aquatic ecosystems (Wang et al., 2018a; Wang et al., 2021). Additionally, the temporal variation and spatial distribution of these fish indicators were closely associated with the changing river environment, especially for the sections under human interference (Wang et al., 2020a; Wang et al., 2020b). However, the indicative function of fish indicators as predator-prey linkages has been less explored, and few studies have applied fish indicators in monitoring and evaluating eco-environmental status from a perspective of the whole river food web.

Despite the development of different fish indices (e.g., fish-based integrity/health index, see Jordan and Vaas 2000; Karr 1981), doubts concerning their suitability and sensitivity are still noted, and the need for further improvements to link pressures with index response has been claimed (Pérez-Domínguez et al., 2012). Some efforts have been employed through the European Union Water Framework Directive (EUWFD), which attributes ecological status to an aquatic ecosystem, but there is still a need for more studies (Souza and Vianna, 2020). Considering that these approaches have been developed primarily based on the biotic and abiotic features of temperate regions, the adequacy or adaptability of many indices in subtropical and tropical environments is still not clear (Boyero et al., 2009). This scenario is justified by the scarcity of studies assessing the efficacy of the many existent indices to the tropical reality (Pasquaud et al., 2013). There is, therefore, a need for robust monitoring tools, especially in developing countries in subtropical and tropical regions, as these areas are more susceptible to human-driven changes in the middle and lower river environments (Kennish, 2002; Silva Júnior et al., 2016).

The assessment of biological indexes has been an integral part of water quality monitoring and management programs for many years. However, most worldwide surveys were conducted according to the EUWFD, with less evaluation system constructed based on regional river characteristics (Souza and Vianna, 2020). In particular, although using biological indicators to assess aquatic diversity and identify potential threats is important for the rivers under human disturbance, there was no independent evaluation system for the rivers in fast-developing Asia, especially in densely populated southeast Asia (Dudgeon, 2000). Therefore, developing robust fish indicators with a complete evaluation process, which includes selecting markable species, giving ecological scores, assessing current status, and diagnosing potential problems, is important for Asian rivers located in the tropics and subtropics (Butchart et al., 2011). Such tools are essential to support the establishment of proper management strategies for the preservation of these ecosystems.

Compared with other biomonitoring objects, fish are more favored mainly due to their easy collection, handling, and identification, as well as wide social attention (Souza and Vianna, 2020). In this context, our idea is that fish indicators with specific ecological requirements, especially on feeding, nursing, and migrating, could not only reflect the ecological status, but also the environmental conditions. Moreover, an ecosystem assessment should be carried out as simply and effectively as possible, and thus, we tried to use a semi-quantitative method based on fish indicators to reflect river ecological status from a perspective of biological integrity and food web structure (e.g., food chain length and food web diversity). The objectives of this study are to 1) select key freshwater fish species with indicative function along a subtropical river, 2) construct a scoring system that was effective to evaluate the eco-environmental status, especially for the disturbed river sections, and 3) explore the associations between fish indicator-based assessment and practical status of local

river environment.

2 Materials and Methods

2.1 Study region and sampling sites

The Liuxi River is regarded as the mother river (of high cultural significance) of Guangzhou, the capital city of Guangdong Province in southern China. The study area has a typical subtropical monsoon climate. The mean annual precipitation of the watershed is 1800 mm, mostly occurring in April–September. The Liuxi River watershed (with an area of 2300 km^2) is situated in the northeastern corner of the Pearl River Delta, which has experienced rapid development in the last two decades. The watershed spans four county-level districts (Huadu, Luogang, Baiyun, and Conghua), which occupy 70% of the watershed area (Fig. 1). The water of the Liuxi River is used for a wide range of purposes, such as drinking water, agriculture, industry, and recreation. However, over the past several decades, the Liuxi River (especially the lower reaches) has experienced rapid agricultural, industrial, and urban development. The water quality and habitat integrity of river ecosystems are disturbed by rapid population growth and associated anthropogenic activities, leading to drastic extraneous interference from humans.

Fourteen sampling sites (half mainstream and half tributary) were chosen from headwaters to the estuary along the Liuxi River (Fig. 1). Environmental data, including habitat characteristics and physicochemical parameters of water quality (see Tables S1 in Supplementary Material), were provided by a nationally accredited (China Metrology Accreditation) third-party testing agency. Forests are the dominant land use type in the watershed, accounting for 55% of the total area. Approximately 33% of the watershed is used for agriculture, including orchards (20%), paddy rice fields (9%) and vegetable growing areas (4%). The rest of the watershed is occupied by built-up areas (9%) and water (3%). The development in the watershed is spatially unbalanced. The upstream areas (sites 1–4) are covered by dense forests, while agricultural activities are concentrated in the midstream areas (sites 5–10). The area close to the watershed outlet (sites 11–14) is highly urbanized.

2.2 Fish sampling

Fish samples were collected in 2022 from the headwaters to the estuary (sites #1–#14 in Fig. 1) along the subtropical Liuxi River during the rainy (June to July) and dry (December) seasons. Each site was sampled two times over a season following the basic guidelines of Barbour et al. (1999) and Hauer and Lamberti (2007). Electrofishing equipment was used to effectively stun and collect fish (individual weight < 10 kg) in a 2 m wide × 2 m long × 3.5 m deep water column. Due to varying water levels, two electrofishing operations were conducted as follows: 1) At wadeable sites, single-pass backpack electrofishing was performed simultaneously by two operators moving in a zig-zag fashion. Electrofishing equipment was adjusted at low voltage and mixed frequency, and the walking speed was controlled to ensure a sampling effort of approximately 8 m^2 min^{-1}over 30 min. 2) At nonwadeable sites, a 6-m-long welded hull boat was used for boat-electrofishing, and a bamboo quant was used to propel the boat to eliminate noise disturbance to fish. Electrofishing equipment was adjusted at a high voltage and main frequency, and the paddling speed was controlled to ensure a sampling effort of approximately 6 m^2 min^{-1}. Boat-electrofishing was conducted over a distance of 500 m, spanning both riverbanks at a depth of 1-3 m (Flotemersch et al., 2006). With the help of local fishermen, gill nets (mesh sizes of 10, 20, 30, 40 mm between adjacent nodes, high × long areas of 1.0 m× 60 m, 1.5 m × 100 m, 2.5 m × 150 m, 3.5 m × 200 m) and hoop nets (mesh sizes of 5, 10, 15 mm, volume of 0.25 m high × 0.35 m wide × 10 m long) were used as passive methods to supplement the requisite specimen in cases where the latter was precluded by high depth and large fish sizes.

2.3 Data analysis

All statistical analyses were conducted in R 4.0.5 (R Core Team, 2021) with the primary packages *cluster* , *factoextra* ,*phyloseq* , *microeco* , *PerformanceAnalytics* , and*ggplot2* . Cluster analysis for establishing grouping zones relied on the Bray-Curtis dissimilarities of fish abundance (individual number). Statistically significant cluster groupings were identified using a bootstrap randomization technique in which the nonzero

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values were resampled and used to generate pseudovalues of Bray-Curtis dissimilarities under the null hypothesis. A frequency distribution of pseudovalues was generated from 1000 randomizations of the data matrix, and the 95th percentile was used as the critical value to determine between-group significance. A stepwise forward selection was performed to reduce the linearly correlated environmental factors, and the variables showed significant differences in their values.

Linear discriminant analysis effect size (LEfSe) is an algorithm for high-dimensional indicator discovery that identifies taxa by characterizing the differences between two or more biological conditions (Segata et al., 2011). LEfSe emphasizes both statistical significance and biological relevance, allowing researchers to identify discriminative features that are statistically different among biological classes. The nonparametric factorial Kruskal-Wallis sum-rank test is first used to detect features with significant differential abundance with respect to the class of interest. Second, LEfSe uses linear discriminant analysis to estimate the effect size of each differentially abundant feature and rank the feature accordingly (Liu et al., 2021). Spearman's correlation analysis was used to measure the strength and direction of monotonic association between fish indicator scores and traditional biodiversity indices as well as environmental variables.

3 Results

Spatial patterns of fish fauna in the Liuxi River

One hundred thirteen species belonging to 13 orders, 342 families, and 88 genera were found along the Liuxi River, including 103 native species and 10 exotic species (see details in Table S1 of Supplementary Material). Seven spatial zones (i.e., zones I to VII shown in Fig. 2a) were grouped by clustering analysis based on the individual number of fish assemblages. Sites 1-2 in zone I were located in the headwaters (Fig. 2b), where fish assemblages were dominated by Cyprinidae 62.7% (mainly composed of Danioninae 30.2% and Barbinae 24.8%), Gastromyzontidae (18.0%), and Gobiidae (11.6%). Sites 3-4 in zone II were located in the upper mainstream, where fish assemblages were dominated by Cyprinidae 68.6% (mainly composed of Cyprininae 14.2%, Rhodeinae 13.9%, Gobioninae 12.8%, and Culterinae 12.4%), Cichlidae 15.7%, and Cobitidae 9.1%. Sites 6-7 in zone III were located in the midstream tributaries, where fish assemblages were dominated by Cyprinidae 75.2% (mainly composed of Gobioninae 32.7% and Danioninae 31.4%), Cobitidae (8.1%), and Gobiidae (8.0%). Zone IV included mainstream site 5 and tributary sites 9-10, where fish assemblages were dominated by Cyprinidae 68.1% (mainly composed of Danioninae 28.7%, Gobioninae 11.0%, and Rhodeinae 8.9%), Gobiidae (13.7%), and Cobitidae (7.0%). Sites 8 and 11 in Zone V were located in the middle-lower main stream, where fish assemblages were dominated by Cyprinidae 61.9% (mainly composed of Culterinae 23.0%, Labeoninae 8.2%, Cyprininae 6.5%, and Xenocyprininae 6.4%) and Cichlidae 18.4%. Sites 12 and 14 in Zone VI were located near the river mouth, where brackish fishes appeared and fish assemblages were dominated by Cyprinidae 54.5% (mainly composed of Culterinae 25.3% and Labeoninae 22.1%), Cichlidae 17.2%, Loricariidae 6.7%, and Engraulidae 5.8%. Site 13 in Zone VII was located in the downstream rural tributary, where fish assemblages were dominated by Cichlidae 52.6%, Loricariidae 26.3%, and Clariidae 10.5%.

Fish indicators in the subtropical Liuxi River system

The differences in spatial distribution of fishes in the Liuxi River are mainly reflected at the genus and species levels (Fig. 3), suggesting that genus-/species-level indicators were effective to distinguish fish communities in each zone. The difference at the family level was lower than that at the genus level because Cyprinidae contained more species than other families. LEfSe analysis results showed that the fish assemblages in Zone I were marked by Balitoridae, those in Zone III were marked by Cobitidae, those in Zone IV were marked by Cyprinidae, those in Zone V were marked by Mastacembelidae, those in Zone VI were marked by Engraulidae, and those in Zone VII were marked by Loricariidae. There were no family-level fish indicators in Zone II. At the genus and species levels, a total of 4 genera and 30 species were selected by LEfSe, including *Pseudogastromyzon changtingensis*, *Vanmanenia* (*V. pingchowensis* and *V. gymnetrus*), *Acrossocheilus parallens*, and *Zacco platypus* in Zone I; *Rhodeus sinensis*, *Rasbora steineri*, *Hemiculter leucisculus*, *Acheilognathus barbatulus*, *Cobitis* (*C. sinensis* and *C. arenae*), *Channa argus*, and *Pelteobagrus* (*P. fulvidraco*

, *P.intermedius* , and *P . vachelli*) in Zone II;*Pseudogobio vaillanti* , *Microphysogobio* (*M. kiatingensis* and *M. fukiensi*), *Sarcocheilichthys nigripinnis* , *Opsariichthys bidens* , *Abbottina rivularis* ,*Botia pulchra* , *Rhinogobius giurinus* , and*Glyptothorax fokiensis* in Zone III; *Cirrhinus molitorella* ,*Squalidus argentatus* , and *Hemibagrus guttatus* in Zone IV;*Erythroculter ilisaeformis* , *Parabramis pekinensis* ,*Hemibarbus medius* , *Xenocypris davidi* , *Schistura fasciolatus* , *Mastacembelus armatus* , and *Silurus asotus* in Zone V; *Hypophthalmichthys molitrix* , *Ctenopharyngodon idellus* , and *Coilia grayi* in Zone VI; and *Tilapia zilli*and *Hypostomus plecostomus* in Zone VII.

Grouping of fish indicators based on their ecological properties

Based on the LEfSe screening results, 34 indicator species with similar ecological characteristics were grouped into the same item according to their requirements for spawning, nursing, feeding, and migrating (Table 1). Given that species belonging to the same genus or subfamily commonly exhibit similar environmental indications, it is necessary to provide alternative species based on the target species, which not only enriches the evaluation system, but also facilitates routine monitoring. During the process of optimizing the indicator items, four principles were considered: (1) keep as many different genera or subfamilies as possible from a taxonomic aspect, (2) select species with significant environmental and trophic functioning; (3) prioritize national protected species, and (4) exclude alien invasive species with strong tolerance (e.g., Cichlidae and Loricariidae in zone VII). Eighteen indicator items were defined, including both indicator species and their congeners (belonging to the same genus or subfamily) with similar ecological niches. These indicator items can reflect not only the specific living environment but also the ecological properties of local ecosystems, such as community diversity, food chain length, and food web integrity.

The principle of the ten-point scoring system is to define which indicators should be given a score of 5. Fish indicators with a score of 5 have two characteristics: 1) they are widely distributed and able to swim across lotic and lentic water bodies (e.g., from river to reservoir), such as *Erythroculter pseudobrevicauda* and*Zacco platypus* , and 2) they have a certain tolerance to the degradation of water and habitat, such as *Rhodeus sinensis* and*Rhinogobius giurinus* . Given that the appearance of local top predators could indicate a long food chain and integrated food web, fish indicators with scores of 8-10 should be benthic consumers at high trophic levels (e.g., *Silurus asotus* and *Channa maculate*). In particular, the native protected species such as *Hemibagrus guttatus* and endangered species on the red list such as *Anguilla japonica* are not only species protected by the state but also important migratory species that indicate "stream – river - lake" connectivity; thus, they received the highest score of 10. Except for Gastromyzontidae, which lives in mountain rapids (a narrow habitat niche), fish indicators with low scores were those at low trophic levels and indicated short food chains, such as filter-feeding*Hypophthalmichthys molitrix* and scrape-feeding *Cirrhinus molitorella* . Given that our scoring system is constructed based on accumulative positive feedback of each fish indicator, exotic species were excluded because they have no positive contribution to local environment.

Using fish indicators to evaluate the simple properties of food web

According to the principle of 'simple, quick, precise, and practical', a formula was developed to evaluate the integrity of the local food web based on the significance of fish indicators.

$$IFI_Q = \sum_{i\,=\,1}^{m} E_i$$

$$IFI_N = \sum_{i\,=\,1}^{m} E_i \times \frac{n_i}{N}$$

$$IFI_B = \sum_{i\,=\,1}^{m} E_i \times \frac{b_i}{B}$$

IFI (index of fish indicator) is the environmental evaluation score of the local river ecosystem based on the ecological significance of fish indicators. At a sampling site, E_i and n_i are the evaluation score (see Table 1)

and individual number of a single fish indicator species i ($i = 1 \sim m$), respectively. N is the total individual number of local fish indicators. IFI_Q is a qualitative index that equals the sum of the E_i value of each fish indicator. IFI_N is the semiquantitative index weighted by the relative contribution of individual number (i.e., $\frac{n_i}{N}$), and IFI_B is weighted by the relative contribution of biomass (i.e., $\frac{b_i}{B}$). For example, if the number of indicator species appearing at the sampling site is m, $IFI_N = E_1 \times \frac{n_1}{N} + E_2 \times \frac{n_2}{N} + \dots + E_m \times \frac{n_m}{N}$. After calculating the IFI, the criteria for judging food chain length, food web integrity, and the diversity of the local ecosystem were explored (see Table 2).

Evaluation effects of IFI associated with conventional ecological indices

Spearman correlation coefficients between IFI and traditional ecological indices (e.g., Shannon-Wiener diversity of fish community and benthic index of biotic integrity) as well as environmental factors were calculated to evaluate the practical application effect of IFI (Fig. 4). The results showed that IFI, IFI_N and IFI_B exhibited significantly ($P < 0.05$) positive correlations with Shannon-Wiener diversity, B-IBI, evaluation, DO (mg/L), and flow velocity (cm/s) and significantly negative correlations with total nitrogen (mg/L), NH_3-N (mg/L), and electronic conductivity (μS/cm). Generally, IFI calculated by a concise scoring formula could comprehensively reflect the relationships between biological properties and environmental conditions. Among the three IFI designed, IFI_B exhibited the best performance – significantly correlated with most biotic and environmental factors. Compared with IFI_Q and IFI_N, the greatest advantage of IFI_B is its reasonable accounts for the composition of fish communities, especially for carnivorous and herbivorous fishes with large body sizes.

4 Discussion

4.1 Fish indicators and their spatial distribution patterns

The 34 fish indicators selected in the Liuxi River reflected the site-specific water environment quality and habitat distribution patterns. In the longitudinal gradient along the river, they reflected not only the spatial differences in fish flora along the upper, middle and lower reaches but also the differences between the mainstream and tributaries (Petry and Schulz, 2006). For example, fish in zones I and III indicated wadable mountain headwaters which had higher elevations and lower water temperatures than zones located in downstream plains (Wang et al., 2018b). In a subtropical monsoon climate, perennial rainfall, fast flowing velocity, high dissolved oxygen, and scattered riffle and pebble substrates provide favorable conditions for benthic algae and invertebrates (Dudgeon, 2008). These food resources are the main energy supply for periphytivorous and insectivorous food chains, which could be indicated by rheophilic *Vanmanenia*, *Opsariichthys bidens*, *Zacco platypus*, and *Micronemacheilus pulcher*.

In tributaries, although the water quality is better than that in the mainstream, the original gravel and coarse sand substrates are illegally extracted or buried during agricultural land expansions (Lasne et al., 2007). The populations of Nemacheilidae (*Micronemacheilusi* and *Schistura*) and Botiidae feeding on aquatic insects and Gobioninae (*Hemibarbus* and *Pseudogobio*) hidden in coarse sand substrate declined due to the loss of their feeding and living habitats (Wang et al., 2019). During field sampling, we found that several tributaries were seriously disturbed by humans. The subfluvial pebbles were removed or buried due to channel reconstruction, and some were piled up on the bank for the manufactured landscape. The reduction in pebble coverage area led to the homogenization of the substrate type, leading to a decrease in the diversity of fish and other aquatic assemblages. In addition, tributaries in the lower urban reach have been subjected to severe anthropogenic modification (Wang et al., 2021). The reinforced bank led to a separation between the riparian zone and river channel, which destroyed the aquatic vegetation that was necessary for fish reproduction. In particular, due to the discharge of domestic sewage, extremely high COD_{Mn} and low DO were recorded at tributary site #13 in zone VII, where only invasive species (e.g., *T. zillii*, *H. plecostomus*, and *Pangasius sutchi*) resistant to pollution survived, with no fish indicators observed.

Wang et al. (2018b) reported that the longitudinal decrease in insect consumption and increase in detritus consumption by fish constitute two opposing vectors governing the energy pathways, with midstream transitions indicated by the increased consumption of hydrophytes, mollusks, and crustaceans. Along the

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Liuxi River, the successive presence of upstream fish indicators feeding on aquatic insects, periphyton, and gastropods; midstream indicators feeding on submerged plants, bivalves, odonate larvae, shrimps, and fish; and downstream indicators feeding on plankton, annelids, and sedimentary organic matter are three key processes accounting for local fish community diversity and the trophic network of the food web (Wang et al., 2018a). It could be expected that the cumulative addition of indicators that had already appeared upstream would lead to peak indicator richness in the middle or lower reaches (Eick and Thiel, 2014). As expected, the greatest richness of fish indicators and the highest *IFI* scores were observed in the mid-upper reaches (Welcomme et al., 2006).

4.2 Scoring and evaluation system based on fish indicators

As top consumers in river ecosystems, fish play more important roles than other assemblages (e.g., benthic diatoms or invertebrates) not only because of their species-specific requirements for water and habitat but also because of the trophic interactive roles they play in the food web (Wang et al., 2018b). For example, if littoral zones disappeared due to artificial reforming, the survival rates of *Opsariichthys bidens* and *Zacco platypus* would sharply decreased. Due to the influence of agricultural development, the original pebble substrate was buried; as a result, *Vanmanenia* and *Rhinogobius* , which crawl on pebble surfaces, are threatened. Moreover, because *Rhinogobius* are important food resources for carnivorous fish, their population decrease had negative impacts on top predators, thus affecting the whole food web through trophic cascading (Wang et al., 2018a).

The spawning process of *Hemibagrus guttatus* and *Anguilla japonica* requires a certain migration distance, which is totally dependent on hydrologic and hydrodynamic connectivity (Petry and Schulz, 2006). However, water conservancy projects destroyed the original fluvial morphology and obstructed the migratory route, leading to a decline in migratory fish stocks (Southerland et al., 2007). In addition, the fish indicators selected in this paper occupied important trophic nodes of the predator-prey links and thus can indicate which food chains only existed in specific environments, such as periphytivorous, insectivorous, and molluscivorous. The integrity of these food chains is important for maintaining the health of aquatic ecosystems (Wang et al., 2023).

The purpose of constructing this scoring system is to overcome some deficiencies of traditional ecological indices, such as Shannon-Wiener diversity and the fish index of biotic integrity (Souza and Vianna, 2020). In terms of calculation, traditional indices often underestimate the environmental conditions in upstream tributaries, especially headwaters, where the diversity and integrity of the fish community is low but water and habitat quality is high (Carignan and Villard, 2002). For example, although the fish species richness and community diversity in headwater zone I were low, there was little external interference, and the local environment remained pristine. Thus, the evaluation of the environment in zone I should be high, which used to be underestimated by traditional ecological indices (Bal et al., 2018). In this regard, our scoring system intended to give a realistic environmental assessment by using *IFI* , which was developed based on environmental and trophic functioning of fish indicators (Sosa-López et al., 2005). The Pearson's correlation results further demonstrated that the IFI_B score weighted by relative biomass performed well in reflecting the practical conditions of the local river environment, which provides evidence for managers to make effective decisions (Mujiyanto et al., 2021).

4.3 Practical use of fish indicators in ecological evaluation

Fish with specific feeding and reproductive characteristics not only carry information about their population dynamics but also reflect the comprehensive needs of water quality (e.g., flow velocity, dissolved oxygen, and water depth), substrate types (e.g., gravels, rocks, and pebbles), and habitat patterns (e.g., riffle, pool, and riparian zones), which are critical environmental factors determining the properties (e.g., diversity and integrity) of local ecosystems. The fish indicator species identified in this paper occupied different trophic levels in river food webs and represented important trophic nodes within predator-prey relationships; thus, they played important roles in the energy transfer pathways of ecosystems (Wang et al., 2018b). Therefore, the emergence of these fish indicators has important impacts on the structure and functioning of the food

web. They can indicate not only the environmental quality of river segments but also the specific food chains occurring in heterogeneous habitats (Bal et al., 2018). The integrity of these food chains is important for maintaining aquatic biodiversity and ecosystem health.

It is worth mentioning that although national protected species and endangered species can be indicators of multiple functions in river environments, it is difficult for them to play a key role in field monitoring due to their small number and legal protection (Southerland et al., 2007). Therefore, we recommend that the monitoring of protected species be achieved in two ways: (1) substitution by using other species with similar ecological indicative functions, e.g., a large fin w to indicate habitat areas where a spotting w is likely to occur, and (2) using environmental DNA technology to conduct long-term monitoring in areas where protected species may occur. The fish indicators selected and the scoring system constructed in this study are suitable not only for traditional ecological data (e.g., individual number and biomass) but also for operational taxonomic unit abundance obtained by high-throughput sequencing.

Conclusion

At present, most studies focus on quantitative field monitoring, and the evaluation effects rely heavily on ecological indices, with the results underestimating environmental conditions. The fish indicators selected and scoring system constructed in this study can be easily used by environmental technicians and is conducive to improving the efficiency of field monitoring and the rationality of environmental evaluation. With the functioning of fish indicators as the basis and the scoring system as a guiding principle, researchers could evaluate the integrity and health of local aquatic ecosystems.

Acknowledgements

Data Availability Statement

All data supporting this study are provided as supplementary information accompanying this manuscript.

References

Bal P, Tulloch AI, Addison PF, McDonald-Madden E, Rhodes JR. Selecting indicator species for biodiversity management. Frontiers in Ecology and the Environment 2018; 16: 589-598.

Barbour MT, Gerritsen J, Snyder B, Stribling J. Rapid bioassessment protocols for use in streams and wadeable rivers. Washington, USA: USEPA, 1999.

Boyero L, Ramirez A, Dudgeon D, Pearson RG. Are tropical streams really different? Journal of the North American Benthological Society 2009; 28: 397-403.

Butchart SHM, Walpole M, Collen B, Van Strien A, Scharlemann JPW, Almond REA, et al. Global biodiversity: Indicators of recent declines. Science 2011; 328: 1164-1168.

Carignan V, Villard M-A. Selecting indicator species to monitor ecological integrity: a review. Environmental monitoring and assessment 2002; 78: 45-61.

Dudgeon D. The ecology of tropical Asian rivers and streams in relation to biodiversity conservation. Annual Review of Ecology and Systematics 2000; 31: 239-263.

Dudgeon D. Tropical Stream Ecology. USA: Elsevier, 2008.

Eick D, Thiel R. Fish assemblage patterns in the Elbe estuary: guild composition, spatial and temporal structure, and influence of environmental factors. Marine Biodiversity 2014; 44: 559-580.

Flotemersch JE, Stribling JB, Paul MJ. Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers. Cincinnati, Ohio, USA: Office of Research and Development, USEPA, 2006.

Hauer FR, Lamberti GA. Methods in Stream Ecology. London, UK: Academic Press, 2007.

Jordan S, Vaas P. An index of ecosystem integrity for Northern Chesapeake Bay. Environmental Science & Policy 2000; 3: 59-88.

Karr JR. Assessment of biotic integrity using fish communities. Fisheries 1981; 6: 21-27.

Lasne E, Bergerot B, Lek S, Laffaille P. Fish zonation and indicator species for the evaluation of the ecological status of rivers: example of the Loire basin (France). River Research and Applications 2007; 23: 877-890.

Liu C, Cui Y, Li X, Yao M. microeco: an R package for data mining in microbial community ecology. FEMS Microbiology Ecology 2021; 97: fiaa255.

Mujiyanto, Syam AR, Nastiti AS. Composition of reef fish as a success indicator of the bio-transplants method in Tunda Island, Serang Regency, Banten Province. E3S Web Conf. 2021; 322: 01027.

Pasquaud S, Courrat A, Fonseca VF, Gamito R, Goncalves CI, Lobry J, et al. Strength and time lag of relationships between human pressures and fish-based metrics used to assess ecological quality of estuarine systems. Estuarine, Coastal and Shelf Science 2013; 134: 119-127.

Perez-Dominguez R, Maci S, Courrat A, Lepage M, Borja A, Uriarte A, et al. Current developments on fish-based indices to assess ecological-quality status of estuaries and lagoons. Ecological Indicators 2012; 23: 34-45.

Petry A, Schulz U. Longitudinal changes and indicator species of the fish fauna in the subtropical Sinos River, Brazil. Journal of Fish Biology 2006; 69: 272-290.

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biology 2011; 12: 1-18.

Sosa-Lopez A, Mouillot D, Do Chi T, Ramos-Miranda J. Ecological indicators based on fish biomass distribution along trophic levels: an application to the Terminos coastal lagoon, Mexico. ICES Journal of Marine Science 2005; 62: 453-458.

Southerland MT, Rogers GM, Kline MJ, Morgan RP, Boward DM, Kazyak PF, et al. Improving biological indicators to better assess the condition of streams. Ecological Indicators 2007; 7: 751-767.

Souza GBG, Vianna M. Fish-based indices for assessing ecological quality and biotic integrity in transitional waters: A systematic review. Ecological Indicators 2020; 109: 105665.

Vorosmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, et al. Global threats to human water security and river biodiversity. Nature 2010; 467: 555-561.

Wang S, Luo B-K, Qin Y-J, Zhao J-G, Wang T-T, Stewart SD, et al. Fish isotopic niches associated with environmental indicators and human disturbance along a disturbed large subtropical river in China. Science of The Total Environment 2020a; 750: 141667.

Wang S, Su L-H, Luo B-K, Qin Y-J, Stewart SD, Tang J-P, et al. Stable isotopes reveal effects of natural drivers and anthropogenic pressures on isotopic niches of invertebrate communities in a large subtropical river of China. Environmental Science and Pollution Research 2020b; 27: 36132-36146.

Wang S, Tang J-P, Su L-H, Fan J-J, Chang H-Y, Wang T-T, et al. Fish feeding groups, food selectivity, and diet shifts associated with environmental factors and prey availability along a large subtropical river, China. Aquatic Sciences 2019; 81: 31.

Wang S, Wang L, Chang H-Y, Li F, Tang J-P, Zhou X-A, et al. Longitudinal variation in energy flow networks along a large subtropical river, China. Ecological Modelling 2018a; 387: 83-95.

Wang S, Wang T-T, Lin H-J, Stewart SD, Cheng G, Li W, et al. Impacts of environmental factors on the food web structure, energy flows, and system attributes along a subtropical urban river in southern China. Science of The Total Environment 2021; 794: 148673.

Wang S, Wang T-T, Tang J-P, Wang L, Yang Y, Lin H-J, et al. Longitudinal variation in fish prey utilization, trophic guilds, and indicator species along a large subtropical river, China. Ecology and Evolution 2018b; 8: 11467-11483.

Wang T-T, Wang X-D, Wang D-Y, Fan S-D, Wang S, Chen Z-B, et al. Aquatic invertebrate diversity profiling in heterogeneous wetland habitats by environmental DNA metabarcoding. Ecological Indicators 2023; 150: 110126.

Welcomme RL, Winemiller KO, Cowx IG. Fish environmental guilds as a tool for assessment of ecological condition of rivers. River Research and Applications 2006; 22: 377-396.

Wiens JA. Riverine landscapes: taking landscape ecology into the water. Freshwater biology 2002; 47: 501-515.

Winemiller KO. Spatial and temporal variation in tropical fish trophic networks. Ecological monographs 1990; 60: 331-367.

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