

Article

Fish Biomonitoring and Ecological Assessment in the Dianchi Lake Basin Based on Environmental DNA

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Abstract: Fish are among the main target groups when conducting biomonitoring and ecological assessment of aquatic environments. Environmental DNA (eDNA) metabarcoding provides a unique opportunity to enhance biomonitoring throughput, precision, and standardization. However, few fish monitoring studies based on eDNA metabarcoding have been conducted at the basin scale. Taking Dianchi Lake and the three inflowing rivers (Panlong River, Baoxiang River, and Chai River) as research objects, this study monitored fish diversity via eDNA metabarcoding, and the Fish Index of Biological Integrity (F-IBI) was constructed and applied for the ecological assessment of the aquatic environment. Here, we detected 41 species belonging to 9 orders, 15 families, and 35 genera in the sampling areas, including 17 native fish species such as *Hemiculter leucisculus*, *Cyprinus carpio*, and *Silurus soldatovi*. Distinct diversity patterns were observed among Dianchi Lake and the three inflowing rivers. Particularly, the fish diversity of Panlong River was richer than that of Dianchi Lake, Baoxiang River, and Chai River. Water temperature (WT), chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) significantly influenced the distribution of fish communities in most sampling sites of the Dianchi Lake basin ($p < 0.05$). Moreover, according to the F-IBI assessment system, 25% of the sampling sites were defined as ‘fine’ or above, and 75% were ‘impaired’ or below. This study provided insights into the ecological restoration and precise management of the aquatic environment of the Dianchi Lake basin.

Keywords: fish community; biodiversity; eDNA metabarcoding; environmental factors; biotic integrity



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1. Introduction

Accurate biomonitoring is crucial for ecological assessment and the protection of aquatic environments [1]. Fish communities are at the top of the aquatic food chain, and therefore their composition and distribution are often used as indicators of the ecological condition of rivers and lakes [2–4]. Dianchi Lake is the largest freshwater lake in Yunnan Plateau, and therefore the governance and restoration of the Dianchi Lake basin have garnered increasing attention in recent years [5]. The discharge of wastewater, industrial waste, municipal sewage, pesticides, and fertilizers has led to the deterioration of the aquatic environment and loss of biodiversity, both of which have a bi-directional worsening effect [6]. Biodiversity is an important indicator of the ecological state of aquatic environments such as the Dianchi Lake basin [7]. The status of aquatic environmental stress and ecological restoration can be effectively analyzed through the biomonitoring of fish diversity in the Dianchi Lake basin.

Traditional fish surveys are generally based on capture (e.g., net fishing, cage fishing, electric fishing), which is highly invasive and defeats the original purpose of protecting biodiversity [8,9]. Moreover, some fish species are difficult to catch, and the identification of fish species requires time, resources, and expertise [10,11]. Therefore, traditional methods are often not well suited for the biomonitoring of rivers and lakes on a large scale. Environmental DNA (eDNA) metabarcoding provides an effective means to overcome these challenges. This novel biomonitoring method consists of directly extracting DNA from environmental samples, after which aquatic organisms can be identified down to the species level based on the eDNA sequences. This approach is generally more efficient than traditional methods [12], and therefore an increasing number of studies have adopted this method. The purpose of eDNA analysis is to obtain taxonomic information and gene function information of the species to which these environmental samples belong [13]. This is consistent with the purpose of traditional taxonomic identification methods based on morphological features but it is faster, more accurate, and more cost-effective [14–16]. Currently, eDNA is widely used in the field of fish biomonitoring to assess species richness [17,18], species abundance, as well as to conduct biomass assessments [18,19] and detect invasive species [20,21] or endangered species [22,23]. However, few studies have employed eDNA-based approaches to monitor the biodiversity of the Dianchi Lake basin and conduct ecological assessments.

In 1981, the Fish Index of Biological Integrity (F-IBI) was first proposed by Karr to assess aquatic environments in rivers in Indiana, USA [24]. Since then, the F-IBI has been widely used in the ecological assessment of aquatic environments worldwide [25–28]. However, given the unique environmental features of different regions, the F-IBI assessment system in one region is usually only suitable for that specific region. In China, the F-IBI has been used to conduct ecological assessments of the Yangtze River [29,30], the Yellow River [31], and Taihu Lake [32], among others. The creation of an F-IBI based on eDNA metabarcoding is of great significance in the assessment and protection of aquatic environments. However, previous studies have not established an F-IBI for the Dianchi Lake basin.

Our study employed eDNA metabarcoding to explore (1) fish diversity, (2) the relationship between fish communities and environmental factors, and (3) F-IBI and ecological assessments in Dianchi Lake and its three inflowing rivers (Panlong River, Baoxiang River, and Chai River). Therefore, this study provides basic data on fish biodiversity and a scientific basis for promoting the application of eDNA at the watershed scale, as well as to enable the ecological restoration and precise management of the Dianchi Lake basin.

2. Methods

2.1. Study Area

Our study focused on Dianchi Lake and its three inflowing rivers (Baoxiang River, Chai River, and Panlong River). The Baoxiang River is classified as a suburban river, which is mainly influenced by urban pollution and agricultural pollution. The Chai River is classified as an agricultural river, which is mainly influenced by agricultural irrigation and aquaculture pollution. The Panlong River is classified as an urban river, which runs through Kunming from north to south and is mainly influenced by domestic wastewater. A total of 24 sampling sites including 8 sampling sites in Dianchi Lake (D1–D8), 9 sampling sites in Panlong River (P1–P9), 4 sampling sites in Baoxiang River (B1–B4), and 3 sampling sites in Chai River (C1–C3) were set up (Figure 1). Sampling was conducted on 18 October 2020.

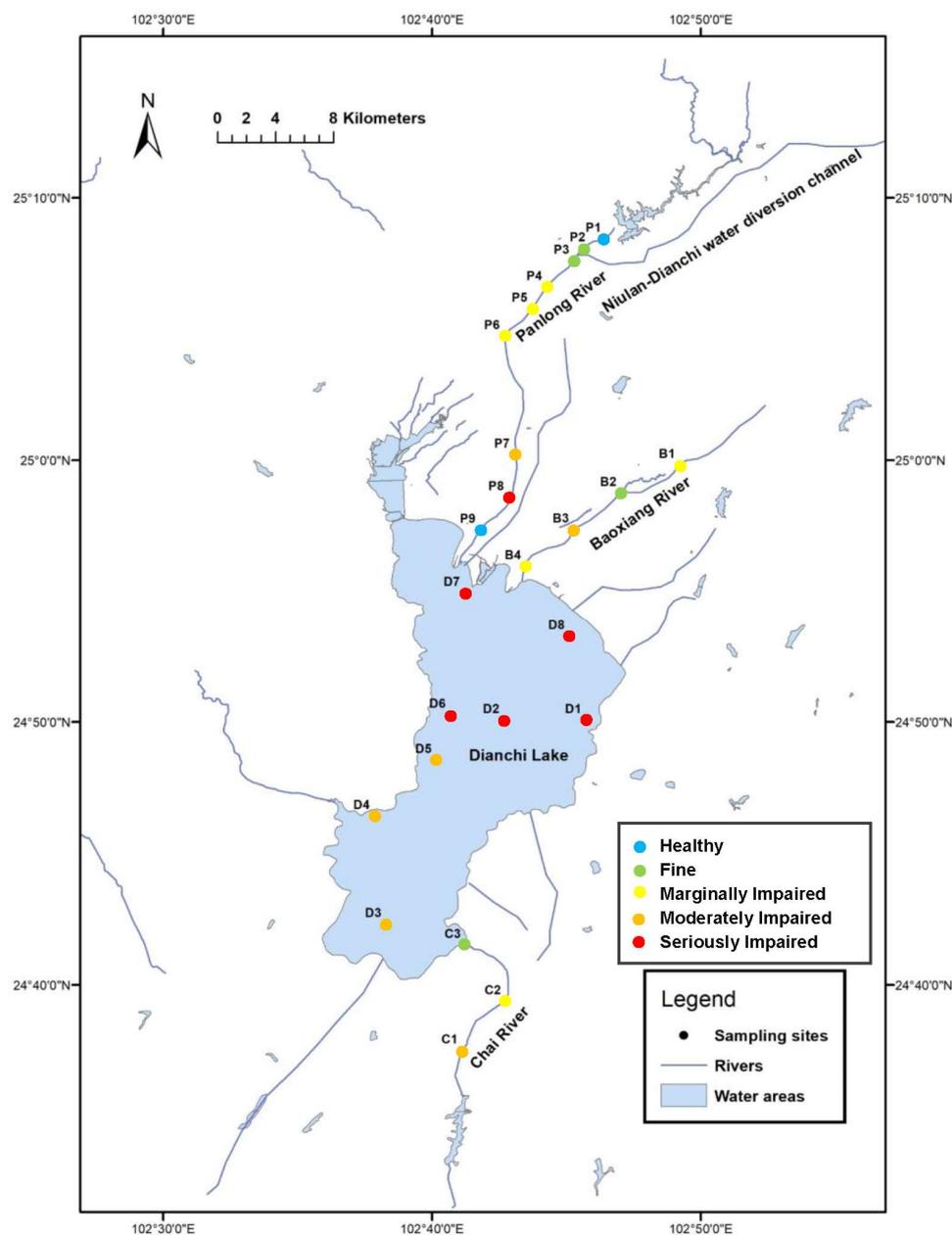


Figure 1. Location of sampling sites in Dianchi Lake and the three inflowing rivers. The colors represent different grades of aquatic ecological condition based on the F-IBI assessment.

2.2. Collection and Treatment of Water Samples

Surface water (3 L) was collected at each sampling site to monitor fish diversity (gloves and masks were worn throughout the sampling period to prevent cross-contamination). These samples were maintained in cold storage, transported back to the laboratory, and filtered using a Multi-channel Water eDNA Enrichment (WD-6, E-genomic Technology, Hong Kong, China) within 24 h. The pore diameter of the filter membrane was 0.45 μm , and the filtration volume of a single filter membrane was 500 mL. There were three biological replicates per site, and a total of 75 eDNA samples were filtered, which included 72 environmental freshwater samples, and 3 blank filter membranes processed with ddH₂O. Additionally, 1 L water samples were collected at each site to detect physicochemical indices including water temperature (WT), pH and dissolved oxygen (DO) (tested on site immediately), total nitrogen (TN), total phosphorus (TP), ammonia nitrogen (NH₃-N), chemical oxygen demand (COD), and electrical conductivity (Cond) (tested in a laboratory) (Table S1).

2.3. DNA Extraction, PCR Amplification, and High-Throughput Sequencing

DNA was extracted from filtered water samples using the DNeasy Blood & Tissue Kit (Qiagen, Dusseldorf, Germany). Three blank filter membranes were set as negative controls. The purity, quality, and concentration of the extracted DNA were detected using a NanoDrop spectrophotometer, Qubit 2.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and 1% agarose gel electrophoresis. The extracted DNA samples were stored in a $-20\text{ }^{\circ}\text{C}$ refrigerator until required for PCR amplification [33]. In this study, the Teleo 12S-rDNA primer pair (Teleo-F:5'-ACA CCG CCC GTC ACT CT-3', Teleo-R:5'-CTT CCG GTA CAC TTA CCA TG-3') was used for amplification [34]. The 30 μL PCR system included 15 μL of $2 \times$ Rapid Taq Master MIX (Vazyme, Nanjing, China), 1 μL of forward and reverse primers, 2 μL of DNA template, and 11 μL of DEPC water. The polymerase chain reaction (PCR) procedure included an initial denaturing step of $95\text{ }^{\circ}\text{C}$ for 3 min, 32 cycles of $95\text{ }^{\circ}\text{C}$ for 15 s of denaturation, $60\text{ }^{\circ}\text{C}$ for 15 s of annealing, $72\text{ }^{\circ}\text{C}$ for 3 s of extension, and a final extension step of $72\text{ }^{\circ}\text{C}$ for 5 min. Three negative PCR controls for every PCR process were simultaneously performed using negative controls as DNA templates. The PCR products were checked by 2% agarose gel electrophoresis [35], and the target PCR products were purified using the E-Z 96 Cycle Pure Kit (Omega, New York, NY, USA). All purified products were quantified and the same amount of DNA was combined for subsequent sequencing. Libraries were constructed using the Ion Xpress Plus Fragment Library kit (Thermo Fisher Scientific, Waltham, MA, USA) and were diluted to a final concentration of 100 pM, then sequenced in an Ion Torrent sequencer (Life Technologies, Carlsbad, CA, USA). This sequencing pool also contained 10 μL of the extraction negative controls; there was no sequence contamination in the negative controls.

2.4. Bioinformatics and Fish Biodiversity Analysis

Raw data were analyzed following the QIIME2 pipeline [36]. The primary information processing was as follows: low-quality sequences ($Q < 20$) and mismatched sequences (the maximum number of mismatches was set to 2) were eliminated, and 1,151,270 high-quality sequences were obtained. The UPARSE pipeline [37] was used to cluster the sequences into operational classification units (OTUs) at a similarity threshold of 97%. The taxonomy of representative OTUs were analyzed using the GenBank database (identity $\geq 95\%$, coverage = 100%) using QIIME. A total of 826 OTUs were annotated as fish, among which 658 OTUs could be annotated to the genus level, accounting for 79.66% of the total annotated fish OTUs, and 580 OTUs could be annotated to the species level, accounting for 70.21% of the total annotated OTUs. The rarefaction curves of all sampling sites are shown in Figure S1. The OTU reads present simultaneously in at least two replicates were averaged as the actually detected reads of each OTU at a given site.

A bubble map was generated with the OriginPro 2018 software to visualize the composition and relative abundance of fish at each sampling site. The Shannon–Wiener's diversity index was used to calculate the alpha diversity of fish in each sampling site. The dimensionality of the data was reduced to facilitate the comparison between the fish community composition in Dianchi Lake and the three inflowing rivers. Principal coordinates analysis (PCoA) and the PERMANOVA test were used to compare the differences in fish communities in the lake and rivers. Redundancy analysis (RDA) was used to reflect the relationship between the fish community data and environmental factors in each sampling site. Pearson correlation analysis was used to reveal the correlation between environmental factors and the relative abundance of the top 22 species of fish. All of the aforementioned statistical analyses were conducted using the “vegan” package in R version 3.6.0 [38].

2.5. Construction of F-IBI

The F-IBI was constructed based on the OTU cluster results. Due to a lack of relevant historical data, sites with little human interference (B1, B2, P1, and P2) were selected as reference sites, whereas the others were considered impacted sites [39–41]. Based on current studies [42,43], and considering the applicability and comprehensiveness of the candidate indices and the actual species composition, 21 primary indices in 4 categories (Table 1),

including 9 species composition and abundance indices (M1–M9), 4 nutritional structure indices (M10–M13), 3 tolerance indices (M14–M16), and 5 reproductive co-population indices (M17–M21) were established.

The F-IBI of Dianchi Lake and the three inflowing rivers were determined by screening the distribution range, discriminant ability screening, and correlation analysis and screening. The indices whose number of species was less than 5 or those in which the difference between sampling sites was less than 10% were removed. The overlaps of the box diagram (25–75% quantile range) between the reference sites and the impaired sites were compared to analyze the scores of each index after screening. Only the indices whose IQ (interquartile ranges) were greater than or equal to 2 were retained for further analysis. Pearson correlation analysis was conducted for the remaining indices to test the redundancy of paired candidate indexes. If there was a strong correlation between two indices ($|r| > 0.8$) [32,44], only the more important index was retained. Then, the ratio method was used to calculate the scores of each retained index. For the indices with negative interference response, the 95% quantile of the index score at all sampling sites was considered the best expected score:

$$\text{score} = (\text{actual score}/\text{the best expected score})$$

For the indices with positive interference response, the 5% quantile of the index score at all sampling sites was considered the best expected score:

$$\text{score} = (\text{maximum score}-\text{actual score})/(\text{maximum score}-\text{the best expected score}).$$

The final F-IBI score was obtained by adding the scores of all of the indices. The 95% quantile of the F-IBI scores of all sampling sites was used as the grade line of the “healthy” classification, after which the ‘fine,’ ‘marginally impaired,’ ‘moderately impaired,’ and ‘seriously impaired’ classifications were divided via the quartile method [45–47].

Table 1. Candidate indices for F-IBI and their expected responses to environmental stresses in Dianchi Lake and the three inflowing rivers.

Number	Type of the Candidate Parameter	Name of the Candidate Parameter	Expected Response to Disturbance	References
M1	Species composition and abundance	Number of fish species	Negative	[45]
M2		Sequence number of fish	Negative	[45]
M3		Relative abundance of exotic fish	Positive	[45]
M4		Percentage of indigenous fish species in Yunnan, China *	Negative	[45]
M5		Percentage of Perciformes	Negative	[48]
M6		Percentage of Siluriformes	Positive	[48]
M7		Percentage of Cyprinidae	Negative	[48]
M8		Percentage of Cobitidae	Positive	[48]
M9		Shannon–Wiener diversity index	Negative	[48]
M10	Nutrition structure	Relative abundance of omnivorous fish	Positive	[45]
M11		Relative abundance of herbivorous fish	Negative	[45]
M12		Relative abundance of carnivorous fish	Negative	[45]
M13		Relative abundance of plankton-eating fish	Negative	[32]

Table 1. Cont.

Number	Type of the Candidate Parameter	Name of the Candidate Parameter	Expected Response to Disturbance	References
M14	Tolerance	Relative abundance of sensitive fish	Negative	[45]
M15		Relative abundance of Pollution-resistant fish	Positive	[45]
M16		Relative abundance of moderately pollution-resistant fish	Positive	[32]
M17	Breeding co-located group	Relative abundance of slimy egg fish	Positive	[32]
M18		Relative abundance of demersal egg fish	Negative	[32]
M19		Relative abundance of floating egg fish	Negative	[48]
M20		Relative abundance of drifting egg fish	Negative	[32]
M21		Relative abundance of fish by other spawning methods	Negative	[45]

* Note: Because there were only five species of native fish in Dianchi Lake, Yunnan native fish were chosen.

In our study, ecological assessments were conducted in all sampling sites based on the constructed F-IBI and classification standard, where higher F-IBI scores indicate a healthier aquatic environment.

3. Results

3.1. Overall Fish Diversity

A total of 41 fish species were detected by eDNA metabarcoding in the Dianchi Lake basin, which belonged to 9 orders, 15 families, and 35 genera of Osteichthyes, including 17 native fishes, e.g., *Hemiculter leucisculus*, *Cyprinus carpio*, and *Silurus soldatovi*. A total of 38 species were found in Panlong River, 31 species in Baoxiang River and Dianchi Lake, and 30 species in Chai River (Table S2). A total of 26 species of fish were detected in both Dianchi Lake and the three inflowing rivers including five unique fish species in Panlong River, two unique fish species in Dianchi Lake, and one unique fish species in Chai River (Figure 2).

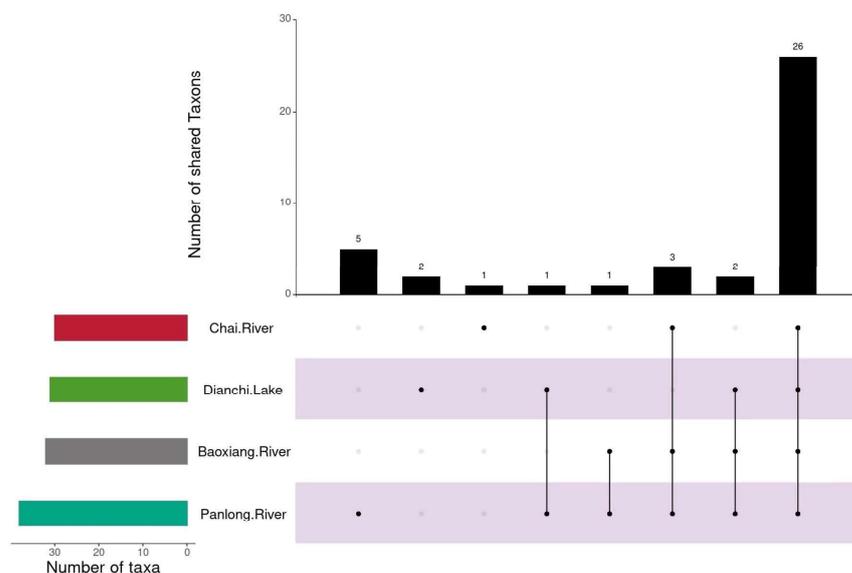


Figure 2. Upset plot with the number of shared taxa among habitats and the total number of taxa detected.

Obvious differences in the distribution of fish species were detected among all sampling sites. *Paramisgurnus dabryanus*, *Neosalanx taihuensis*, *Ctenopharyngodon Idella*, *Hypophthalmichthys nobilis*, *Gambusia affinis*, *Acipenser baerii*, *Cyprinus carpio*, *Acanthorhodeus chankaensis*, *Coilia brachygnathus*, *Carassius auratus*, *Hemibarbus* sp. and *Toxabramis swinhonis* were detected in all sampling sites, but there were significant differences in their relative abundance at each sampling site. The most dominant taxon in Dianchi was *Neosalanx taihuensis*, the most dominant taxon in Baoxiang River and Panlong River was *Paramisgurnus dabryanus*, and the most dominant taxon in Chai River was *Gambusia affinis* (Figure 3).

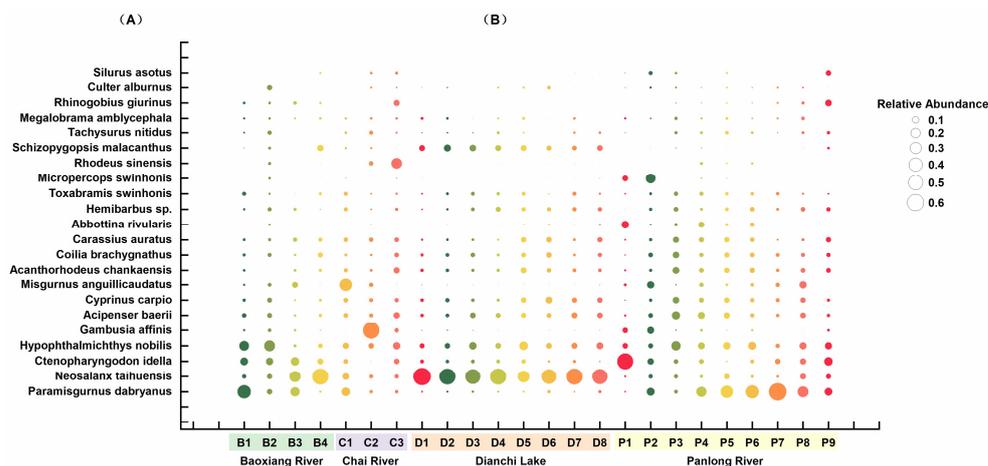


Figure 3. Community composition and relative abundance distribution of fish species (top 22) were revealed by eDNA metabarcoding at each sampling site. (A) Top 22 fish species in order of abundance. (B) Relative abundance distribution of each species at the sampling sites.

3.2. Differences in the Composition of the Fish Community in Dianchi Lake and the Three Inflowing Rivers

Distinct differences in the composition of fish communities were observed between Dianchi Lake and the three inflowing rivers. PCo1 and PCo2 explained 45.5% of the differences, and Dianchi Lake was significantly different from the three inflowing rivers. However, the fish community structures among the three rivers were not significantly separated (Figure 4).

There were significant differences in the composition of fish communities among the three inflowing rivers (PERMANOVA test, $p < 0.01$) (Table 2). The explicable part (R^2) of the PCoA diagram that overlapped with more groups was small. Although there were significant differences in the composition of fish communities among the three rivers, R^2 was only 13.42%, indicating that the location of the three rivers contributed significantly but accounted for a small proportion.

Table 2. PERMANOVA test results of the three inflowing rivers in Dianchi Lake basin.

Group	Fish	
Baoxiang River, Chai River, and Panlong River	R^2	0.1342
	F	3.4872
	p	0.001 ***
Baoxiang River and Chai River	R^2	0.1518
	F	3.4004
	p	0.002 **
Baoxiang River and Panlong River	R^2	0.0870
	F	3.5246
	p	0.001 ***
Chai River and Panlong River	R^2	0.0932
	F	3.4956
	p	0.001 ***

Note: ** means significant difference at 0.01 level, *** means significant difference at 0.001 level.

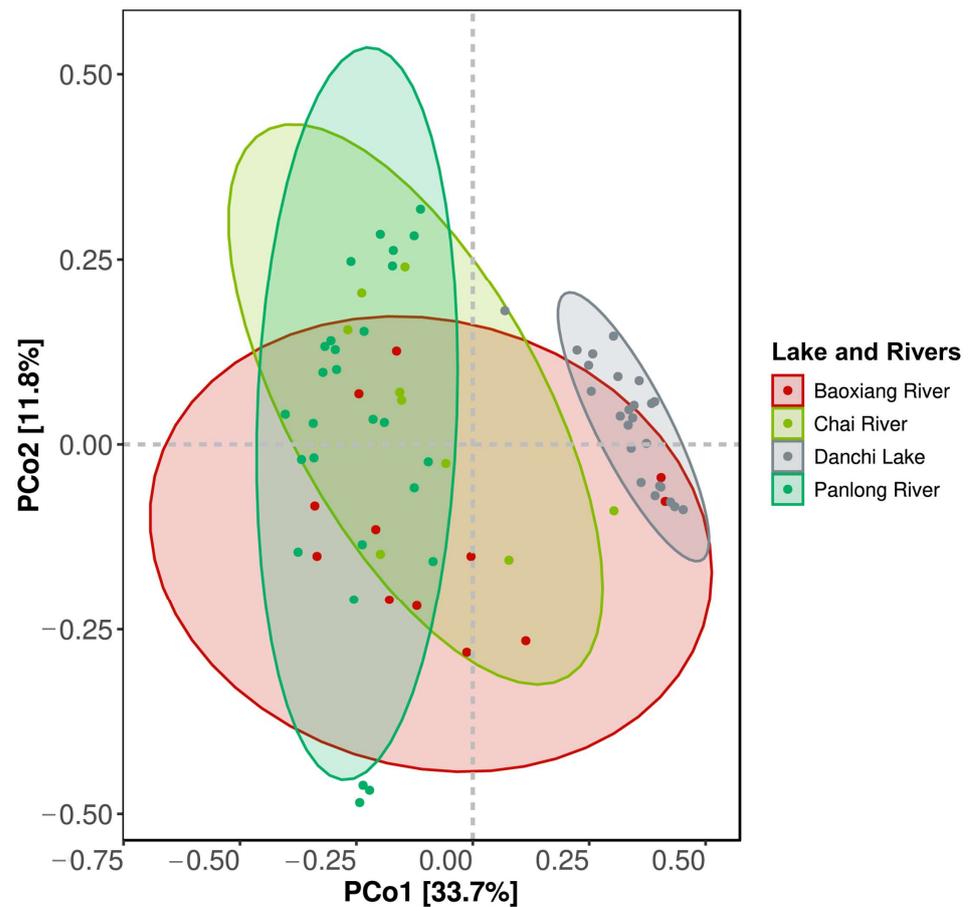


Figure 4. Principal coordinates analysis (PCoA) of the composition of the fish community of Dianchi Lake and the three inflowing rivers.

3.3. Relationship between the Fish Community and Environmental Factors in Dianchi Lake and the Three Inflowing Rivers

RDA analysis demonstrated that the composition and distribution of the fish community in most sampling sites were significantly affected by WT, COD, TN, and TP ($p < 0.05$). COD and TP were the primary environmental factors affecting the composition and distribution of the fish community at Dianchi Lake sites. The primary environmental factors affecting the composition and distribution of the fish community at most sampling sites in Panlong River, Chai River, and Baoxiang River were WT, COD, TN, TP, and DO (Figure 5).

WT, pH, DO, COD, TN, TP, and Cond were significantly correlated with the relative abundance of fish (Pearson, $R > 0.4$, $p < 0.05$), whereas $\text{NH}_3\text{-N}$ had no significant correlation (Figure 6). The relative abundance of *Neosalanx taihuensis* and *Schizopygopsis malacanthus* were significantly positively correlated with pH, WT, COD, and TP. The relative abundance of *Hypophthalmichthys nobilis*, *Acipenser baerii*, and *Coilia brachygnathus* was significantly positively correlated with DO, but negatively correlated with COD and TP. The relative abundance of *Micropercops swinhonis* was negatively correlated with TN and TP. The relative abundance of five fish including *Ctenopharyngodon idella*, *Gambusia affinis*, and *Micropercops swinhonis* showed a significant negative correlation with WT.

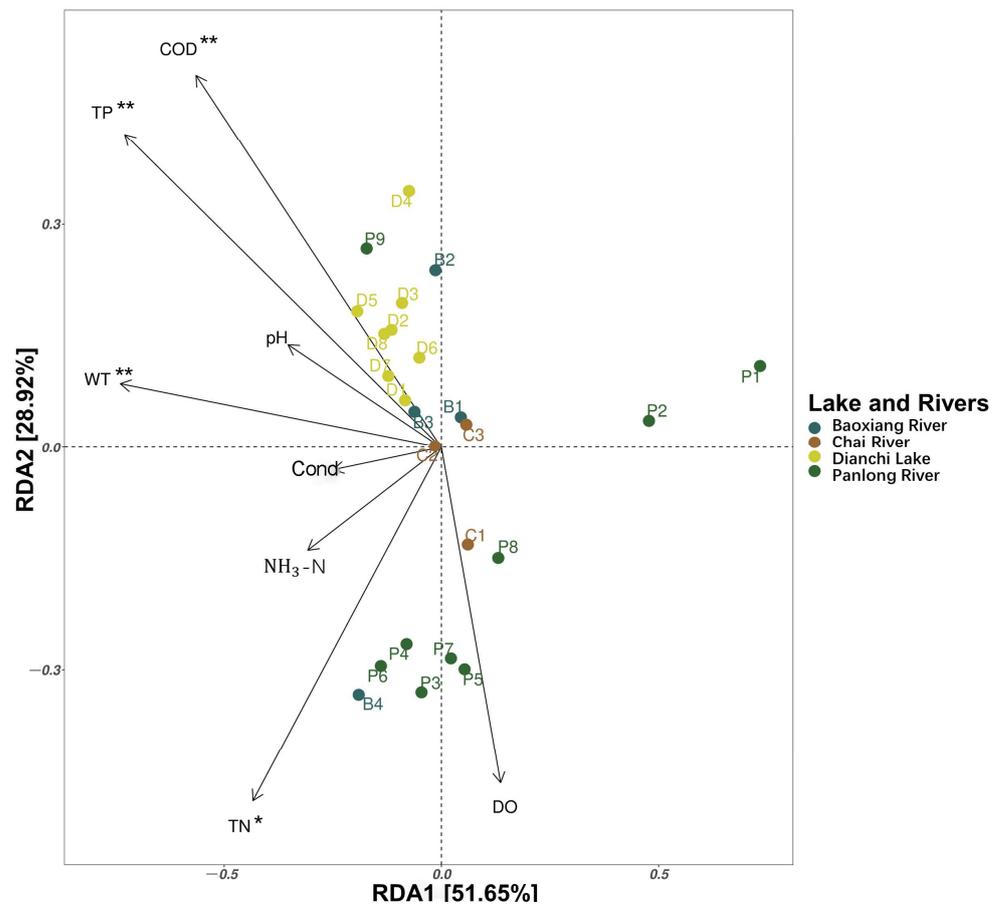


Figure 5. Redundancy analysis of the sampling point and environmental factors; * means significant affection at 0.05 level, ** means significant affection at 0.01 level.

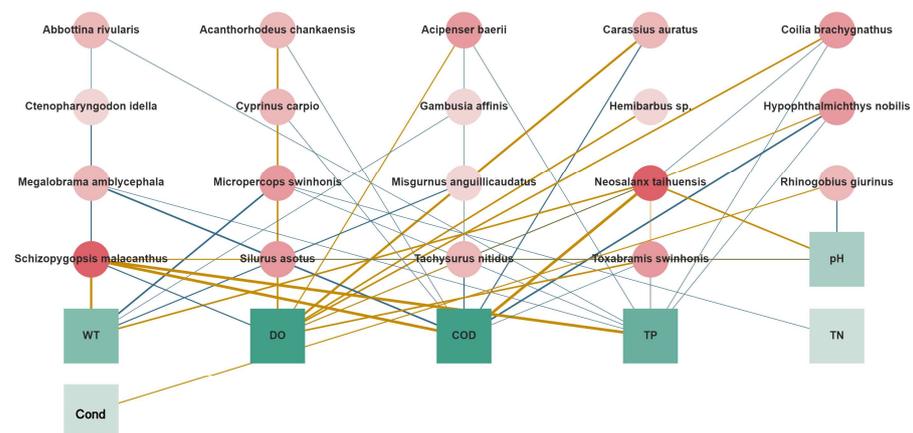


Figure 6. Network diagram of the correlation between environmental factors and fish communities. Pearson correlation analysis was conducted to characterize the correlations between environmental factors and the relative abundances of the top 22 species of fish. Among them, 19 species of fish were significantly correlated with environmental factors ($p < 0.05$). The yellow lines represent significantly positive correlations, the blue lines represent significantly negative correlations, and the thickness of the lines represents the correlation, with thicker lines indicating a stronger correlation. The thickest line represents a strong correlation ($R > 0.7$) and the thinnest line represents a moderate correlation ($R > 0.4$).

3.4. Ecological Assessment of Aquatic Environments Based on F-IBI

F-IBI was constructed based on the fish diversity data, and 21 candidate indices were preliminarily screened out (Table 1). After screening the distribution range, the numerical distribution of all indices of each sampling site was deemed acceptable and therefore all of them were included in box discriminant analyses. There was a significant difference between the scores of impaired sites and reference sites of M1, M4, M5, M8, M11, M12, M14, M19, and M21 ($IQ \geq 2$, Figure S2). A total of 9 indices were retained and Pearson correlation analysis was performed (Table S3). The results of the Pearson correlation analysis demonstrated that M11 and M19, and M12 and M21 were strongly correlated. M12 and M19 were retained as they possessed more abundant and comprehensive data. Therefore, the F-IBI was composed of M1, M4, M5, M8, M12, M14, and M19.

The detailed classification standard for the F-IBI scores was previously described in the Materials and Methods section. Based on this classification standard (Table S4), the aquatic ecological condition of Dianchi Lake and the three inflowing rivers was evaluated and exhibited in Figure 1. The colors represent different grades of aquatic ecological condition. The results showed that among the 24 sampling sites, 2 sampling sites were 'healthy,' 4 were 'fine,' and the other sampling sites were classified as 'impaired.' The aquatic ecological condition of P1, P9, B2, C3, P2, and P3 were relatively healthier; B1, B4, C2, P4, P5, and P6 were marginally impaired, and other sampling sites were seriously impaired.

4. Discussion

4.1. Patterns of Fish Diversity in Dianchi Lake and the Three Inflowing Rivers

In our study, 41 fish species, including 17 native fishes from Yunnan, were detected in Dianchi Lake and the three inflowing rivers via eDNA metabarcoding. The number of fish species in Panlong River was higher than in Dianchi Lake, Chai River, and Baoxiang River. This could be related to the fact that Panlong River was the most intensively sampled, and therefore the total number of fish species increased accordingly. This could also be related to the fact that Panlong River is the catchment channel of the Niulan River–Dianchi Lake water diversion project, which might result in the influx of more biological groups. Therefore, the similarity in water quality, habitat, and aquatic community among the connected water areas was accentuated by the water diversion project [49]. Our study demonstrated that there were similarities in fish species between waters connected by water diversion projects, which was similar to previous reports from other diversion projects. For example, similar fish communities were found in water areas along China's South-to-North Water Diversion Project [50]. Similar phytoplankton communities were found in water areas along the water diversion projects between the Yangtze River and Tai Lake [51]. Moreover, The Nicaragua Canal caused the genetic homogenization of differentiated gene pools by breaking down the geographic isolation between the Punta Gorda and San Juan basins [52].

A total of 12 fish species accounted for 79% of the total sequence abundance and were detected in all sampling sites, and were therefore considered the dominant taxa. Additionally, our findings were consistent with those of previous studies that characterized the common fish species in the Dianchi Lake basin using traditional identification methods [53]. Among the five existing indigenous fish species in Dianchi Lake, *Carassius auratus*, *Monopterus albus*, and *Cyprinus carpio* were detected, whereas *Sinocyclocheilus grahami* and *Anabarilius alburnops* were not, and these findings were consistent with those of Luo [54]. These results may be due to the scarcity of *Sinocyclocheilus grahami* and *Anabarilius Alburnops* in Dianchi Lake [55], but may also be related to the limitations of eDNA metabarcoding monitoring technology, including its low amplification efficiency, its short, amplified fragments, and the incomplete Yunnan native species information found in the database [56]. Therefore, in addition to enriching fish databases, the species monitoring coverage can also be improved in future studies by combining fish universal primers [57]. Our study also detected a variety of exotic fish, which not only included *Paramisgurnus dabryanus*, *Acanthorhodeus chankaensis*, *Coilia brachygnathus*, *Hypomesus olidus*, *Sarcocheilichthys nigripinnis*, *Acheilognathus macropterus*, *Toxabramis swinhonis*, and *Mugilogobius myxodermus* above alien

invasion level III detected by traditional methods, but also *Neosalanx taihuensis*, *Megalobrama amblycephala*, *Culter oxycephaloides*, *Rhinogobius formosanus*, and *Bangana tungting*. These findings demonstrate that eDNA metabarcoding, an emerging biodiversity survey method, is a highly sensitive approach for the monitoring and protection of aquatic organisms, as well as for the detection of alien species invasion [58–60].

The Beta diversity analysis based on the Bray–Curtis distance matrix showed that the fish composition of Dianchi Lake was significantly different from that of the three rivers, and so was the fish composition of each of the three inflowing rivers. This result was consistent with the previous conclusion that there were significant regional differences in the biological composition of lakes and rivers, as well as between rivers [61]. These differences might be attributed to the water quality [62], habitat [63], natural shoreline [64], and pollution sources [65] of the sampling sites.

4.2. Effects of Environmental Factors on the Fish Communities in Dianchi Lake and the Three Inflowing Rivers

The composition and distribution of the fish communities were affected by environmental factors. COD and TP were the primary environmental factors affecting the composition and distribution of the fish community at Dianchi Lake sites. The primary environmental factors affecting the composition and distribution of the fish community at most sampling sites in Panlong River, Chai River, and Baoxiang River were WT, COD, TN, TP, and DO. This was consistent with the environmental pollution characteristics around the basin. Due to the nearby phosphate mining areas and the richness of agricultural greenhouses and pesticide- and fertilizer-oriented enterprises surrounding the basin, the nutrient pollution of Chai River is prominent. The Panlong River is a typical urban river that flows through the residential area of Kunming. Tailwater from sewage treatment plants are discharged into the middle part of the river. However, the Niulan River has been diverted into Dianchi lake through the Panlong River since 2014, and the water quality indices of Panlong river have been significantly improved [66]. The Baoxiang River is a suburban river and therefore exhibits the characteristic signs of agricultural non-point source pollution and urban non-point source pollution [67,68]. The pollution from urban, industrial, and agricultural non-point sources has increased the concentration of COD, TP, and TN in water and eutrophication, which ultimately impacts the structure of the fish community.

The results of Pearson correlation analyses indicated that the relative abundance of fish was mainly correlated with pH, WT, DO, COD, TN, TP, and Cond, but was not correlated with NH₃-N, which was consistent with the results of Wang [69]. *Neosalanx taihuensis* and *Schizopygopsis malacanthus* were significantly positively correlated with pH, T, COD, and TP. The relative abundance of *Neosalanx taihuensis* and *Schizopygopsis malacanthus* in Dianchi Lake was higher than that of the three rivers. Previous studies have reported that TN and TP concentrations of lakes are positively correlated with the TP and TN input of rivers, with nutrient levels being typically higher in lakes compared to the inflowing rivers [70,71]. Higher levels of organic matter and phosphorus in water increase the algae biomass [72]. In these cases, herbivorous fish quickly become dominant due to an increase in the available food sources [73]. Therefore, fish with moderate pollution resistance that feed primarily on algae were shown to adapt to living in water rich in organic matter and nutrient salts, which is reflected by higher relative abundances. *Acipenser baerii*, *Hypophthalmichthys nobilis*, and *Coilia brachygnathus* were significantly positively correlated with DO but negatively correlated with COD and TP. The main pollutants in the tailwater of the surrounding farmland runoff, rural domestic sewage, and urban sewage treatment plant that did not reach the standard were nitrogen, phosphorus, and organic matter, which was reflected in an increase in NH₃-N, TN, TP, and COD levels when discharged into the river [68,74]. Water quality deteriorates as the concentration of nutrients in water increases, resulting in the death of young fish and shrimp, and decreases in the diversity of cladocerans and copepods [75]. In turn, this limits the survival of fish that primarily feed on cladocerans and

copepods. Additionally, DO is a major limiting factor for fish growth, and excessively low $\rho(\text{DO})$ will affect fish feeding and breeding [76]. The sensitive species *Micropercops swinhonis* was negatively correlated with TN and TP, and the abundance of the sequence of this species was higher in sites with better water quality. The survival of sensitive fish is affected by the increase in nitrogen and phosphorus concentration in water. *Ctenopharyngodon idella* and *Gambusia affinis* were sensitive to water temperature. Climate warming may directly or indirectly change plankton algae, zooplankton biomass, community structure, and habitat by affecting the physicochemical properties of water, the composition of aquatic plants, and the structure of the food chain [77,78], thus affecting the fish community.

4.3. Aquatic Ecological Condition of Dianchi Lake and the Three Inflowing Rivers

Due to their position at the top of the aquatic food chain, fish represent the highest trophic level, and the aquatic ecological condition can be reflected by its species diversity and community distribution. In our study, the F-IBI was used to assess the aquatic environment in Dianchi Lake and the three inflowing rivers. According to Figure 1, the aquatic ecological condition of the south of Dianchi Lake was better than that of the north, which was consistent with the findings of Yu [79]. This difference between the south and north may be due to the high urbanization, large population, and large sewage discharge in the northern part and the perennial prevailing southwest wind in the Dianchi Lake area. Therefore, organic pollutants and nitrogen/phosphorus in the southern water area were accumulated in the north, which coincided with disturbances in the water flow and wind currents [80].

There were obvious differences in the aquatic ecological conditions at each site of the three inflowing rivers. Importantly, our findings indicated that the ecological status of the studied waterbodies was not simply determined by the relative position of the upstream, midstream, and downstream, but was closely related to the environment surrounding the basin. P1 was close to Kunming Songhuaba Reservoir, an important drinking water source, whereas P9 was located in the Xinghai Peninsula Wetland Park near the site where the Panlong river enters Dianchi Lake. The water quality was good, the F-IBI scores were high, and the aquatic ecological conditions were classified as 'healthy.' P3 was located at the site where the Niulan River diverts water into the Panlong River, and the aquatic ecological condition was classified as 'fine.' P7 was located near a site with dense urban residential areas and enterprises' activity. A large area of open-air and greenhouse farmland around C1 was planted with vegetables and flowers. A large number of residual pesticides and fertilizers entered the river channels along with farm runoff. B3 was located near the Yunnan Provincial Museum, Guandu Ancient Town, and other scenic spots where there is abundant sewage discharge from dining and entertainment establishments. The aquatic ecological conditions of these sites were classified as 'moderately impaired.' All of these findings were consistent with the characteristics of the environment surrounding the sampling sites, suggesting that the F-IBI based on eDNA biomonitoring data can be used to assess the ecological conditions of the Dianchi Lake basin. The changes in some indices are likely not only caused by environmental quality but also by human factors such as fishing and the freeing of captive fishes, which should be taken into account in actual ecological evaluations.

5. Conclusions

In this study, 41 species of fish, including 17 native fishes, were detected by eDNA metabarcoding in Dianchi Lake and the three inflowing rivers. The species richness of fish communities was higher in Panlong River than in Dianchi Lake, Baoxiang River, and Chai River. There were significant differences in the composition and distribution of fish communities between Dianchi Lake and the three inflowing rivers, as well as among each river. The composition and distribution of the fish community in most sampling sites were significantly affected by WT, COD, TN, and TP, which was related to the complex environment surrounding the basin, as well as many sources of exogenous pollution. The

pattern of fish diversity could partly reflect the local environmental stressors. Additionally, a site-specific F-IBI composed of 7 crucial indices was constructed and applied to assess the aquatic environment of 24 sampling sites in the Dianchi Lake basin. According to our findings, 25% of the sampling sites were classified as ‘fine’ or above and 75% were ‘impaired’ or below. More importantly, our results highlighted the high efficiency, high sensitivity, and large-scale capabilities of eDNA metabarcoding, demonstrating that this approach provides a promising and powerful means on which to base future river and lake biomonitoring and assessment efforts.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w15030399/s1>, Figure S1: Rarefaction curves of fish species at sampling sites. (each line represents a sampling site). Figure S2: Box-plots of nine accepted candidate metrics between reference and impacted sites. Table S1: Detection methods and instruments used for physicochemical indices analyses. Table S2: List of fish species in Dianchi Lake and the three inflowing rivers. Table S3: Correlation coefficients of Pearson tests between nine candidate metrics. Table S4: Classification of F-IBI in Dianchi lake and the three inflowing rivers.

Author Contributions: Y.L., H.M., S.X. and W.Z. designed the experiments. Y.L., H.M., Z.Z., L.S., Z.L. and K.Z. performed the experiments and analyzed the data. H.M. and Y.L. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are openly available in the Sequence Read Archive (SRA) with the accession number PRJNA896695.

Conflicts of Interest: The authors declare that they have no competing interests.

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