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Research Article

Using Environmental DNA to Assess Fish Communities Post-Waterway Regulation in the Yangtze River

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Aquatic ecosystems, essential for global biodiversity and human health, are increasingly threatened by hydraulic engineering projects. This study examines the impact of waterway regulation on fish communities in the Jiujiang section of the Yangtze River using environmental DNA (eDNA) technology. Water samples were collected from multiple locations within and outside the construction areas during the low and high water periods (HWPs) of 2022 and 2023. The results reveal differences in fish community composition and diversity between construction and nonconstruction areas, as well as between low and HWPs. Notably, fish diversity was higher during HWPs, with Cyprinidae being the predominant family in the study area. Canonical correspondence analysis (CCA) identified pH and water temperature as key environmental factors affecting fish communities. This study demonstrates the effectiveness of eDNA technology in monitoring aquatic ecosystems and provides valuable insights for the sustainable management and conservation of regulated waterways. The findings highlight the necessity of long-term ecological monitoring to address the negative impacts of hydraulic engineering projects.

Keywords: beta diversity; ecological monitoring; eDNA; fish diversity; waterway regulation

1. Introduction

Aquatic ecosystems, including rivers, lakes, wetlands, and oceans, are among the most diverse and vulnerable on earth, supporting a significant portion of global biodiversity [1, 2]. They provide critical habitats for aquatic organisms and offer numerous ecological services to human societies, such as water purification, climate regulation, nutrient cycling, and food supply [3]. These ecosystems play a crucial role in maintaining ecological balance and ensuring human health [1, 2, 4].

However, with the expansion of human activities and increased utilization of water resources, aquatic ecosystems face unprecedented threats [3, 5, 6]. Hydraulic engineering projects significantly impact these ecosystems. The construction and management of hydraulic projects often alter the natural forms and flow dynamics of rivers, lakes, and oceans to meet human needs, such as flood control, irrigation, and water resource management. While these projects provide substantial economic and social benefits, they also result in negative impacts, including changes in flow patterns, habitat destruction, water quality degradation, ecosystem deterioration, biodiversity loss, and ecological imbalance [3, 6–8]. Therefore, assessing and mitigating the impacts of hydraulic engineering on aquatic ecosystems has become an urgent research priority.

Among various hydraulic projects, waterway regulation has a significant impact on aquatic ecosystems due to its specific purposes and designs [9, 10]. Activities such as dredging, bank reinforcement, and dike construction, primarily aimed at improving navigation conditions, ensuring shipping safety, and enhancing transportation efficiency, have promoted economic development and trade. However, these activities also alter flow patterns, destroy original habitat structures, affect water quality and sediment transport, and disrupt the living and breeding conditions of fish and other aquatic organisms, potentially leading to ecosystem degradation [11, 12]. For fish, which are an essential part of aquatic communities, waterway regulation can have particularly direct and significant impacts [13–15], such as destroying spawning grounds along riverbeds and banks, affecting fish reproduction and growth [15]. Similarly, dike repairs and bank reinforcements can lead to the disappearance of wetlands and changes in river ecosystems, impacting fish habitats and survival conditions [14, 16, 17]. Therefore, in-depth research on the impacts of waterway regulation on aquatic ecosystems, particularly in major water bodies like the Yangtze River, holds significant ecological and economic importance.

To date, research on river health assessment is extensive. For example, Camp et al. [18] explored how low-carbon emission strategies could improve waterway management, while Caris et al. [19] studied the environmental impact and sustainability strategies of green waterways. Goulding et al. [20] analyzed the effectiveness of green waterways in reducing pollution. Panov et al. [21] assessed the risks of invasive aquatic species in European inland waterways. Zhang et al. [22] estimated the navigation risks of the Yangtze River using the Formal Safety Assessment (FSA) concept and Bayesian network (BN) techniques. These studies provide valuable insights into waterway pollution control and risk management but do not thoroughly explore the ecological impacts of waterway regulation. Li et al. [23] and Liu et al. [9] used systematic assessment frameworks and models to analyze the impacts of waterway projects on the Yangtze River's ecosystems, highlighting the importance of implementing ecological restoration and compensation measures during and after construction. However, they did not adequately cover the specific impacts of waterway regulation on aquatic organisms, particularly the evaluation of these impacts postregulation.

Science and technology play vital roles in aquatic ecosystem protection and management. Recently, the development and application of biomonitoring technologies, particularly environmental DNA (eDNA) technology, have provided more comprehensive methods to assess the structure and changes of aquatic communities [24]. eDNA technology, which detects DNA fragments left by organisms in water, enables noninvasive monitoring of the presence and abundance of aquatic organisms. Compared to traditional biomonitoring methods, eDNA technology offers high sensitivity, high resolution, and high efficiency [25], allowing for a more accurate reflection of the structure and dynamic changes of aquatic communities [25, 26]. eDNA technology can provide detailed temporal and spatial variation data, helping to identify the specific impacts of engineering activities on species or overall communities. Therefore, this technology shows great potential in assessing the impacts of hydraulic engineering on aquatic organisms, providing new technical means and scientific bases for water resource management and ecological protection [27]. Currently, reports on using eDNA technology to investigate the impacts of hydraulic engineering on aquatic organisms are abundant [28–30]. For example, by collecting water samples upstream and downstream of dams and analyzing the eDNA, it is possible to monitor changes in the diversity and abundance of aquatic communities to evaluate the impacts of dams on aquatic ecosystems [28]. In water resource management, eDNA analysis of regular water samples can track seasonal and management strategy-based distribution changes of aquatic organisms, optimizing water resource management plans to ensure ecosystem health and sustainability, and providing new perspectives and methods for evaluating river restoration projects [29, 30]. However, studies on using eDNA to investigate the impacts of waterway regulation on aquatic organisms are still lacking.

Fish play a crucial role in freshwater ecosystems, contributing to ecosystem services, habitat formation, nutrient cycling, and biodiversity maintenance [3]. They are also highly sensitive to environmental pressures and human activities. Therefore, understanding the species composition and distribution of fish communities can provide valuable information for ecological protection [30–32]. This study focuses on fish, using eDNA technology to analyze water samples from the Jiujiang section of the Yangtze River 2 years after the completion of waterway regulation projects, to explore the spatiotemporal dynamics of fish communities postregulation. The objectives of this study are: (1) to reveal the composition and community differences of fish in different areas based on the location of sampling points relative to the waterway regulation zone; (2) to reveal the composition and community differences of fish in different water periods based on the water levels and flow rates of each year; and (3) to determine the dominant environmental factors affecting fish communities postregulation. The results of this study will provide deeper and more comprehensive insights into the impacts of waterway regulation projects and offer a scientific basis and decision support for waterway regulation and ecological protection.

2. Materials and Methods

2.1. Study Area. The Jiujiang section of the midstream Yangtze River, located between Wuhan and Anqing (Figure 1(a)). This segment serves as a critical junction, connecting the upper, middle, and lower reaches of the river, and is a crucial waterway for material transportation. It is also one of the key shallow sections in the middle reaches. To improve navigation conditions, channel regulation projects were conducted by waterway authorities in 2011 and 2018. While these projects have maintained channel stability, they have also altered local hydrological regimes and aquatic habitats, impacting the aquatic biota in this section of the river [9].

2.2. Sampling Locations and Procedure. Water samples were collected in March, April, May, and September of 2022 and in April, June, July, and December of 2023. Eight sampling points were established. Based on their spatial relationship to

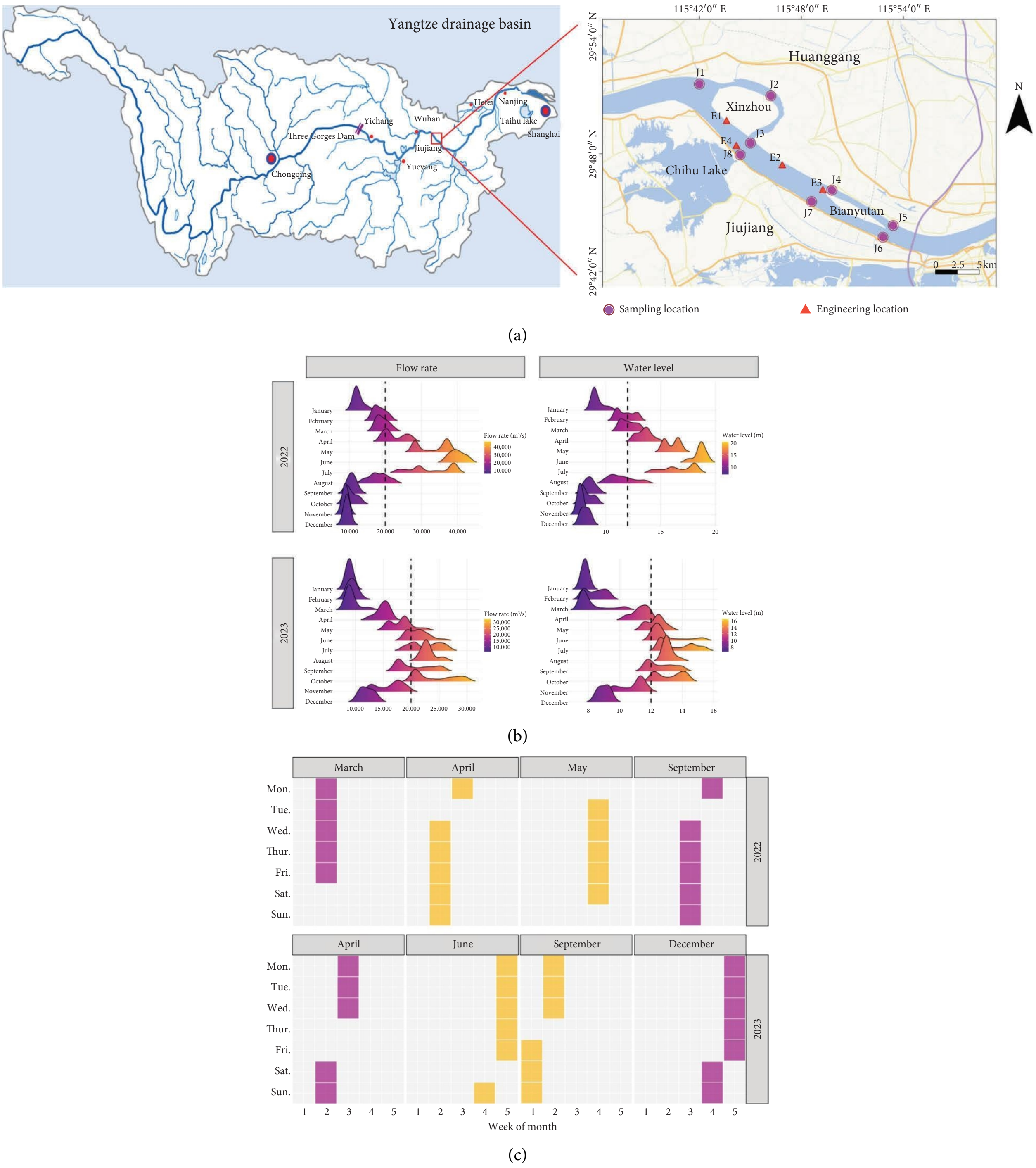


Figure 1: Study area and sampling sites in the Jiujiang section of the Yangtze River (a), changes in flow rate and water levels (b), and specific sampling times (c) for the Jiujiang section in 2022 and 2023.

the engineering sites (E1–E4) (Figure 1(a)), sampling points J3, J4, J7, and J8 were classified as within the construction area (WCA), while J1, J2, J5, and J6 were classified as outside the construction area (OCA). Hydrological data, including flow rate and water level, were obtained from nearby hydrological stations. According to the annual hydrological data of the basin (Figure 1(b)), sampling periods were classified into high water period (HWP) and low water period (LWP) using a flow rate threshold of 20,000 m^3/s and a water level threshold of 12 m. HWP included April and May of 2022 and June and July of 2023, while LWP included March and September of 2022 and April and December of 2023 (Figure 1(c)). Quantitative collection of water samples was conducted using an ESBL-BACKPACK eDNA multifunctional sampler (Smith-Root, USA), with a sample volume of 6 L per sampling point. Water samples were filtered using 0.45 μm PVDF membranes (Merck Millipore, Germany), and the membranes were stored in 5 mL cryogenic vials. A final filtration of 6 L of sterile distilled water was performed as a blank control. All samples were temporarily stored in dry ice containers and subsequently transferred to a −80°C ultra-low temperature freezer. To prevent cross-contamination between sampling locations, new disposable gloves were used at each point, and all equipment in contact with the filter membranes was disinfected with 10% sodium hypochlorite. Following these procedures, a total of 72 independent samples were obtained.

2.3. Water Quality Parameter Measurement. Physicochemical parameters measured at each sampling site included pH, dissolved oxygen (DO), water temperature (WT), total dissolved solids (TDSs), salinity (S), electrical conductivity (EC), total nitrogen (TN), and total phosphorus (TP). TN was measured following the Chinese National Standard HJ 636-2012 using the alkaline potassium persulfate digestion UV spectrophotometric method. TP was measured according to the Chinese National Standard GB 11893-89 using the ammonium molybdate spectrophotometric method. The remaining water quality parameters were measured in situ using a YSI EXO2 multiparameter sonde (Xylem, USA) and its associated probes.

2.4. DNA Extraction and High-Throughput Sequencing. The cryogenic tubes containing the filters were removed from the freezer and thawed on ice for eDNA extraction. Fish eDNA was extracted from the water samples using the E.Z.N.A. Soil DNA Kit (Omega Bio-tek, USA) according to the manufacturer's protocols, with strict contamination control measures in place throughout the extraction process. Fish 16S ribosomal RNA genes were amplified using the Ac16S primer pair (Ac16S-F: CCTTTTGCATCATGATTT AGC and Ac16S-R: CAGGTGGCTGCTTTTAGGC) [33]. The PCR amplification program consisted of an initial denaturation at 95°C for 2 min, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. PCRs were performed in triplicate in 20 μL mixtures containing 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA. The PCR products from triplicate reactions per sample were pooled and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA) according to the manufacturer's instructions. The purified DNA was used to construct an Illumina Pair-End library following Illumina's genomic DNA library preparation procedure. The amplicon library was sequenced using the Illumina MiSeq platform (2 × 250) according to standard protocols.

2.5. Bioinformatic Analyses on Sequencing Data. Raw fastq files were initially demultiplexed using Perl scripts based on the barcode sequence information for each sample. The processing criteria were as follows: (i) 250 bp reads were truncated at any site with an average quality score below 20 over a 10-bp sliding window, and truncated reads shorter than 50 bp were discarded; (ii) exact barcode matching was performed, allowing a maximum of 2 nucleotide mismatches in primer matching, and reads containing ambiguous characters were removed; and (iii) only sequences with an overlap longer than 10 bp were assembled based on their overlap sequence, and reads that could not be assembled were discarded.

The high-quality retained data were processed as follows. Paired-end reads were merged using VSEARCH, primers were trimmed, and quality control was applied, requiring an error rate of less than 1% [34]. Redundant sequences were removed, retaining only unique sequences that appeared at least 10 times in the dataset. The UNOISE algorithm was used to denoise and generate Amplicon Sequence Variants (ASVs), which were then clustered at 98% similarity to create a feature table [35]. Samples were rarefied to the minimum read count using the vegan package in R for subsequent analysis. Representative sequences were annotated using the MitoFish fish mitochondrial genome database with BLAST searches [36]. Only results with the highest scores and coverage ≥ 97% were retained. The annotation results were manually verified using fish survey records from Jiujiang and nearby waters [37, 38], as well as unpublished environmental assessment reports. This verification established the assignment of each ASV, following the species assignment criteria outlined by Zhang et al. [39]. Specifically, when a query sequence matched a single local species, that species was assigned. If multiple local species matched, the lowest common taxonomic level was assigned. The resulting species list was cross-checked with FishBase (https://fishbase.mnhn.fr/search.php) and GBIF (https://www.gbif.org/) to confirm species distribution and exclude species not present in the study area. The final fish species list was used for downstream analysis.

2.6. Statistical Analysis and Visualization. All data analyses were conducted using R software (Version 4.4.2). Alpha diversity indices were calculated using the *alphaDiversity* function, and beta diversity indices, based on Bray–Curtis distances, were computed using the *vegdist* function from the vegan package. The alpha diversity indices included observed richness (Richness), Shannon diversity (Shannon), Simpson diversity (Simpson), Chao1 richness (Chao1), and Pielou's evenness (Pielou). Linear fitting and Spearman's correlation were used to assess variations between groups. Principal Coordinate Analysis (PCoA) was employed to evaluate differences in fish communities across various groups, such as water periods, years, and spatial locations. Significant differences were tested using Permutational Multivariate Analysis of Variance (PERMANOVA) with the adonis2 function. The stability of fish community composition was assessed by calculating the Average Variation Degree (AVD), which measures the variation in species composition by evaluating the deviation of species relative abundance from the mean [40]. The formula used for AVD is

$$\mathrm{AVD} = \frac{\Sigma_{i=1}^{n}\left(\left(\left|x_i - \overline{x_i}\right|\right)/\left(\delta_i\right)\right)}{k \times n}, \tag{1}$$

where k is the number of samples, x_i is the abundance of OTU in each sample, and $\overline{x_i}$ and δ_i are the mean and standard deviation of OTU abundance across all samples, respectively. n is the number of OTUs in each sample. A lower AVD value indicates higher community stability and greater resistance to disturbance.

Beta diversity was partitioned into replacement and nestedness components to assess temporal and spatial changes in fish assemblages. Replacement reflects species substitution between different samples, while nestedness indicates species loss or gain without replacement. Qualitative beta diversity estimation using binary Jaccard dissimilarity was performed with the betapart package. One-way analysis of variance (ANOVA) and independent samples nonparametric tests (Kruskal–Wallis test) were used for all group difference tests. ANOVA was applied only to data meeting homogeneity of variance and normal distribution criteria.

Detrended correspondence analysis (DCA) was conducted to determine the length of environmental gradients. DCA helps decide between linear and unimodal methods for subsequent analysis by assessing gradient length in species distribution data. With maximum axis values of 5.83 and 4.02, Canonical correspondence analysis (CCA) was selected to relate fish communities to environmental factors. Environmental data were standardized using z-scores. The variance inflation factor (VIF) was used to identify collinearity among environmental factors. Parameters with the highest multicollinearity were removed using the vifstep function from the usdm package. Hellinger-transformed species abundance was used for CCA, with 999 permutation tests to assess the significance of the relationships between environmental variables and ordination axes.

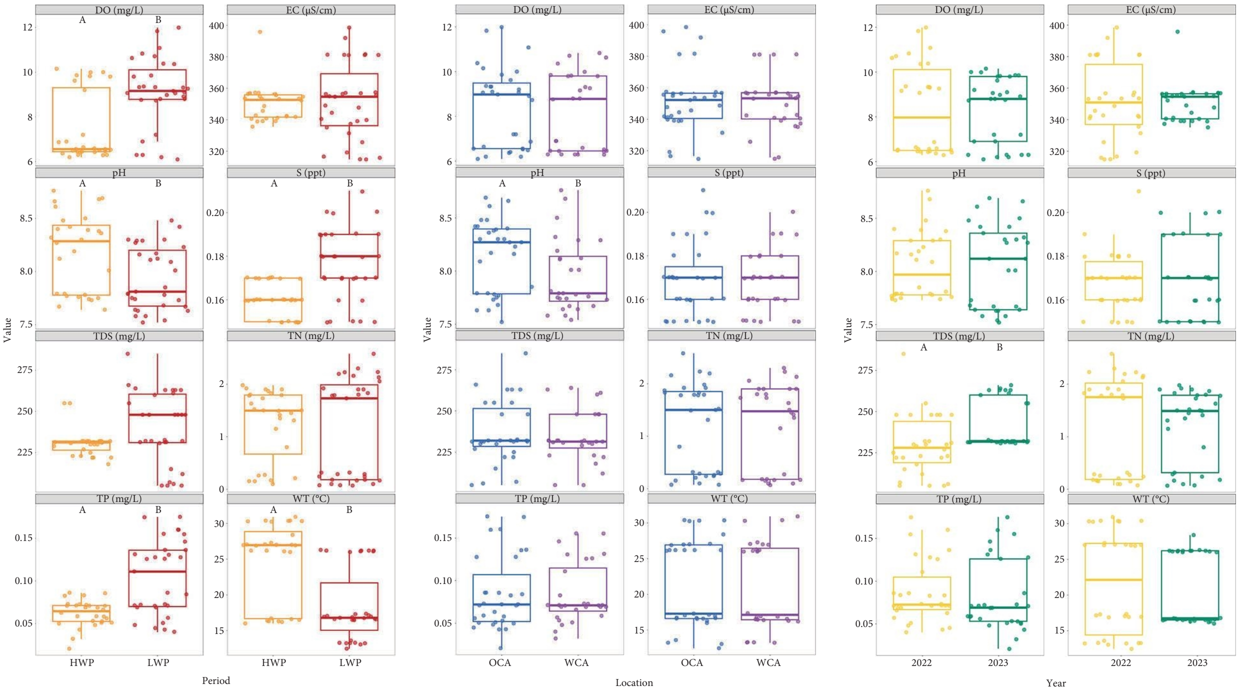
Sampling areas and points were visualized using ArcMap 10.8 and QGIS 3.36.2. Graphical visualizations were created using various R packages: ggridges, UpSetR, ComplexHeatmap, ggplot2, ggpubr, and ggtern. The ggridges package was used to visualize hydrological data. The UpSetR package created UpSet plots to show fish species co-occurrence across different sampling points, including unique and shared species. The ComplexHeatmap package plotted species abundance variations across different sampling points. Box plots were created using ggplot2 and ggpubr, while ternary plots were generated using ggtern.

3. Results

3.1. Changes in Water Environmental Variables. Compared to the HWP, the LWP showed significant increases in DO, TP, and S ($p < 0.001$). Conversely, pH and WT significantly decreased ($p < 0.01$). The remaining environmental variables (EC, TDS, and TN) showed no significant changes (Figure 2(a)). For the OCA and WCA, only pH significantly increased in OCA ($p < 0.05$) (Figure 2(c)). The other environmental variables showed no significant changes. Annual comparisons of water quality indicated a significant increase in TDS ($p < 0.05$), with no significant changes in the other environmental variables.

3.2. Fish Community Composition. Merging the raw data yielded 4,542,358 sequences. After quality control, 35,828 low-quality sequences were discarded. Following filtering, deduplication, and denoising, 4,447,447 clean reads were retained, representing 922 ASV sequences. BLASTN annotation and manual correction identified 37 fish species across 3 orders, 9 families, and 28 genera (Figure 3(b)). The predominant species were *Cyprinus* sp. (20.27%), *Hypophthalmichthys nobilis* (18.63%), and *Hypophthalmichthys molitrix* (15.08%), indicating high prevalence and abundance in the study area. Other notable species included *Megalobrama* sp. (8.66%), *Ctenopharyngodon idella* (4.47%), *Carassius gibelio* (4.93%), and *Silurus* sp. (4.06%). *H. nobilis* and *H. molitrix*, the most abundant taxa, were detected at all sites (Figures 3(a) and 3(b)). *Pomatoschistus* sp., *Carassius auratus*, and *Xenocypris argentea* were more frequently found in the WCA, specifically at sites J3, J4, and J7, with only occasional detection at J1 in the OCA. Conversely, *C. idella*, *Rhinogobius similis*, and *C. auratus auratus* were more frequently detected in the OCA. Other species, such as *Megalobrama* sp., *C. idella*, and *C. gibelio*, showed high abundance at specific sampling points and times. Sites J6 and J8 had the highest species richness, each detecting 18 species. In 2022, 27 species were identified, compared to 23 species in 2023 (Figure 4). The number of species detected in OCA and WCA was similar, with 28 and 29 species, respectively. The HWP had more species (29) compared to the LWP (25).

3.3. Spatial and Temporal Variations in Fish Diversity and Community Stability. Fish diversity exhibited spatial and temporal differences across various groups. Comparative analysis of different water periods showed consistent trends between 2022 and 2023. The median Shannon index was higher during the HWP compared to the LWP. The Shannon index also displayed greater variability during the HWP, indicating higher overall fish diversity, whereas the LWP showed lower fish diversity and less variability in the Shannon index. Similar trends were observed in the richness and Chao1 indices (Figure 5(a)). In 2022, the Pielou index during the HWP was lower than during the LWP, but in 2023, it was higher during the HWP (Figure 5(a)). Overall, the analysis from both years indicated that alpha diversity was consistently higher during the HWP compared to the LWP, with the Shannon index being significantly higher during the HWP ($p < 0.05$). Comparative analysis of different locations revealed that the Shannon index for the WCA was slightly higher than those for the OCA in both 2022 and 2023, though the differences were not statistically significant (Figure 3(a)). Other indices showed the opposite trend, with OCA slightly higher than WCA (Figure 5(b)). Linear fitting results indicate that the slopes for different water periods are −0.2 (OCA) and −0.34 (WCA) (Figure 6(a)), while for different locations, they are −0.15 (LWP) and 0.0095 (HWP) (Figure 6(b)). This suggests that the rate of change in alpha diversity is more pronounced



(a)

(b)

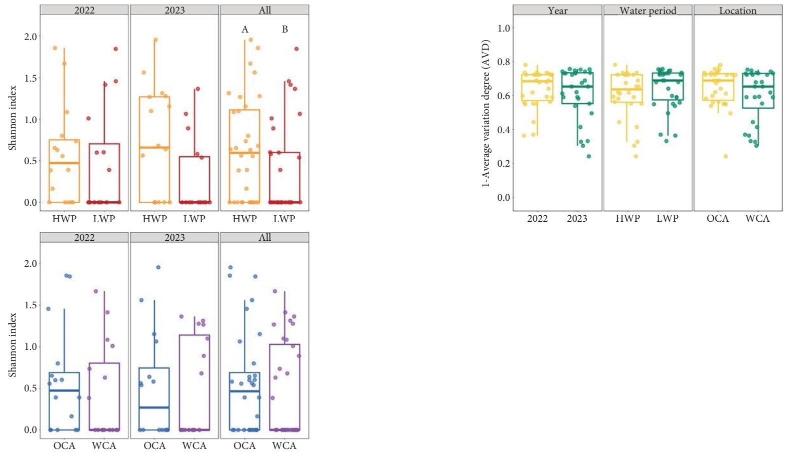
(c)

Figure 2: Variations in environmental factors between different water periods, locations, and years. Letters A and B above box plots indicate significant differences.

(a)

(b)

Figure 3: Continued.



(c)

(d)

Figure 3: Fish community composition at each site (a) and relative abundance (b). Alpha diversity (c) and AVD (d) across different water periods and locations. Letters A and B above box plots indicate significant differences.

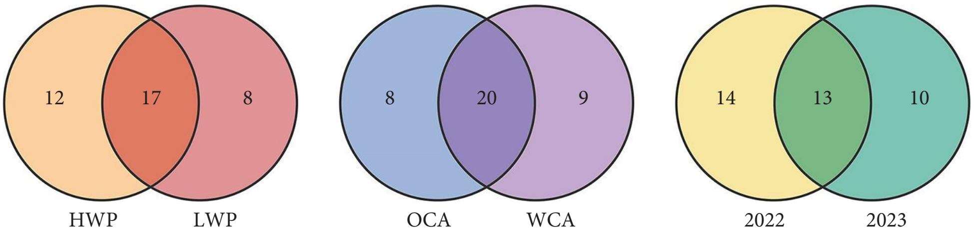
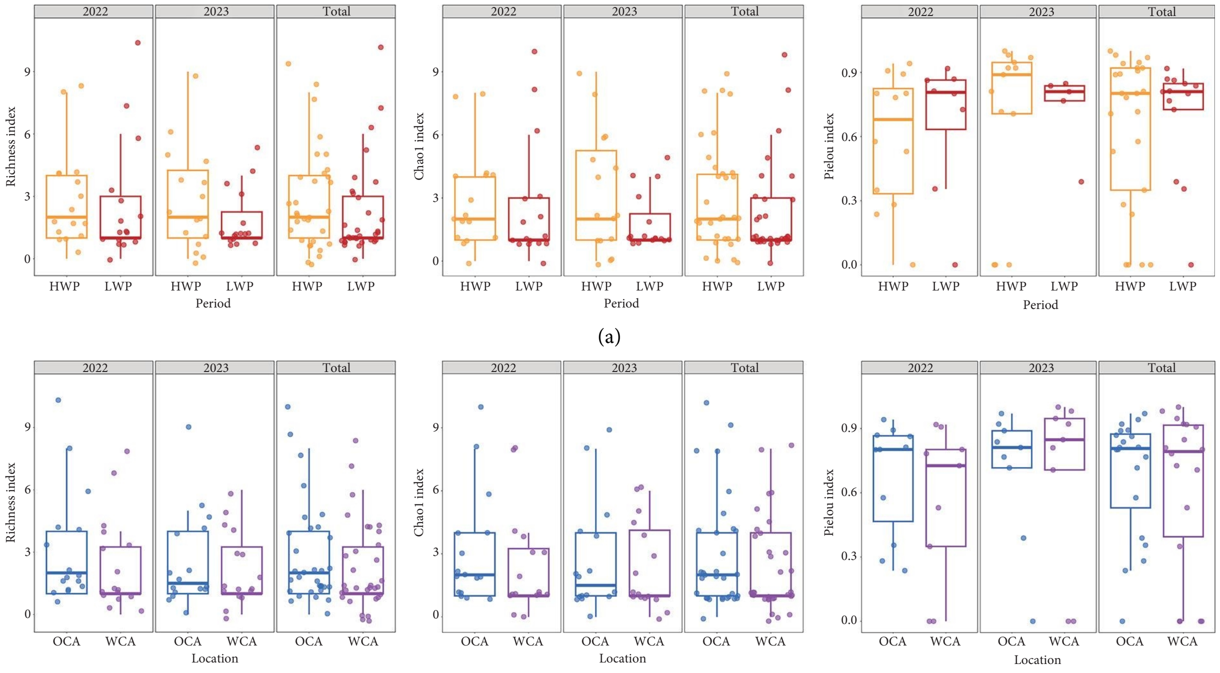


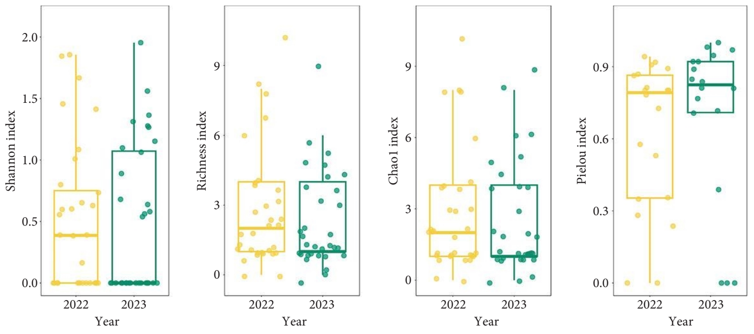
Figure 4: Venn diagram comparison of fish community composition under different water periods, locations, and years.



(a)

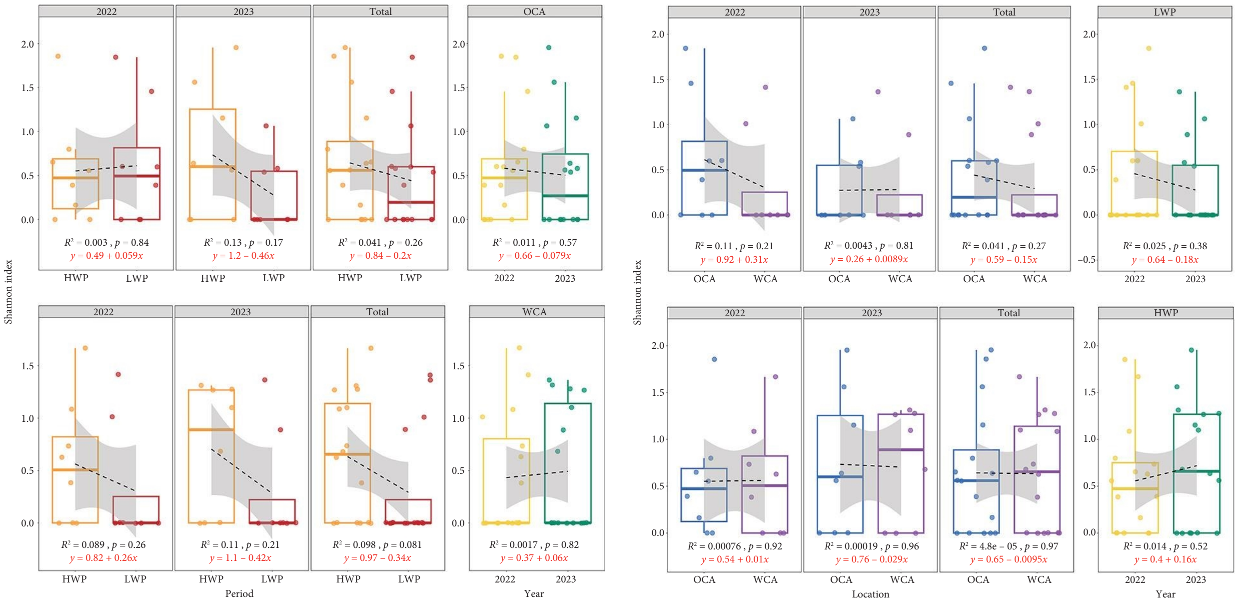
(b)

Figure 5: Continued.



(c)

Figure 5: Other alpha diversity indices besides the Shannon index across different water periods (a), locations (b), and years (c).



(a) (b)

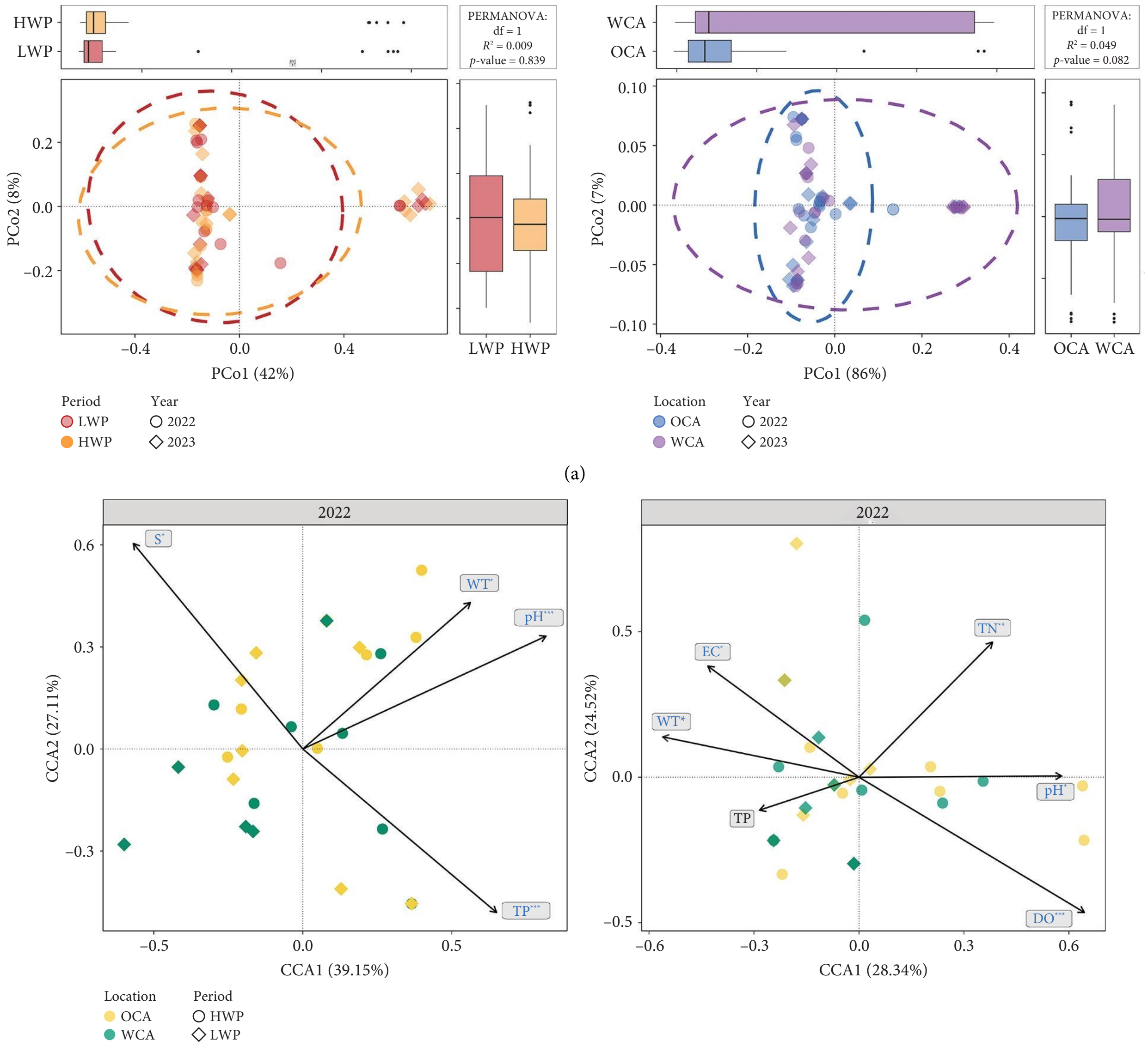
Figure 6: Changes in the Shannon index across different water periods (a) and locations (b) in 2022 and 2023, along with the fitted curves.

between different water periods than between different locations. Comparisons between different years showed that the Shannon index, richness index, and Chao1 index were all higher in 2022 than in 2023. Conversely, the Pielou index was higher in 2023, indicating a more even species distribution in that year (Figure 5(c)). The rates of change (slopes) for different water periods and locations between the 2 years were LWP: −0.18, HWP: 0.16, OCA: −0.079, and WCA: 0.06 (Figure 6). This demonstrates that the rate of change in water periods between the 2 years was greater than the change between locations.

The AVD method was used to further evaluate changes in fish community composition. The average AVD values were 0.36 ± 0.11 in 2022 and 0.39 ± 0.15 in 2023 (Figure 3(d)). The average AVD for HWP was 0.39 ± 0.14, and for LWP, it was 0.36 ± 0.12. Similarly, the average AVD for WCA was 0.40 ± 0.15, and for OCA, it was 0.35 ± 0.11. These results indicate that community stability was lower in 2023 compared to 2022. Additionally, community stability was lower during HWP compared to LWP and lower in WCA compared to OCA.

To illustrate community changes, we analyzed fish communities across different groups. PCoA based on Bray–Curtis dissimilarity showed that the first two axes explained 42% and 8% of the variance for different water periods (PCoA1 and PCoA2, respectively). For different locations, the first two axes explained 86% and 7% of the variance, respectively. Despite the high variance explained by these axes, the samples from different water periods and locations exhibited mixed distribution patterns (Figure 7(a)). Furthermore, PERMANOVA results indicated no significant differences in fish communities across different water periods, locations, and years.

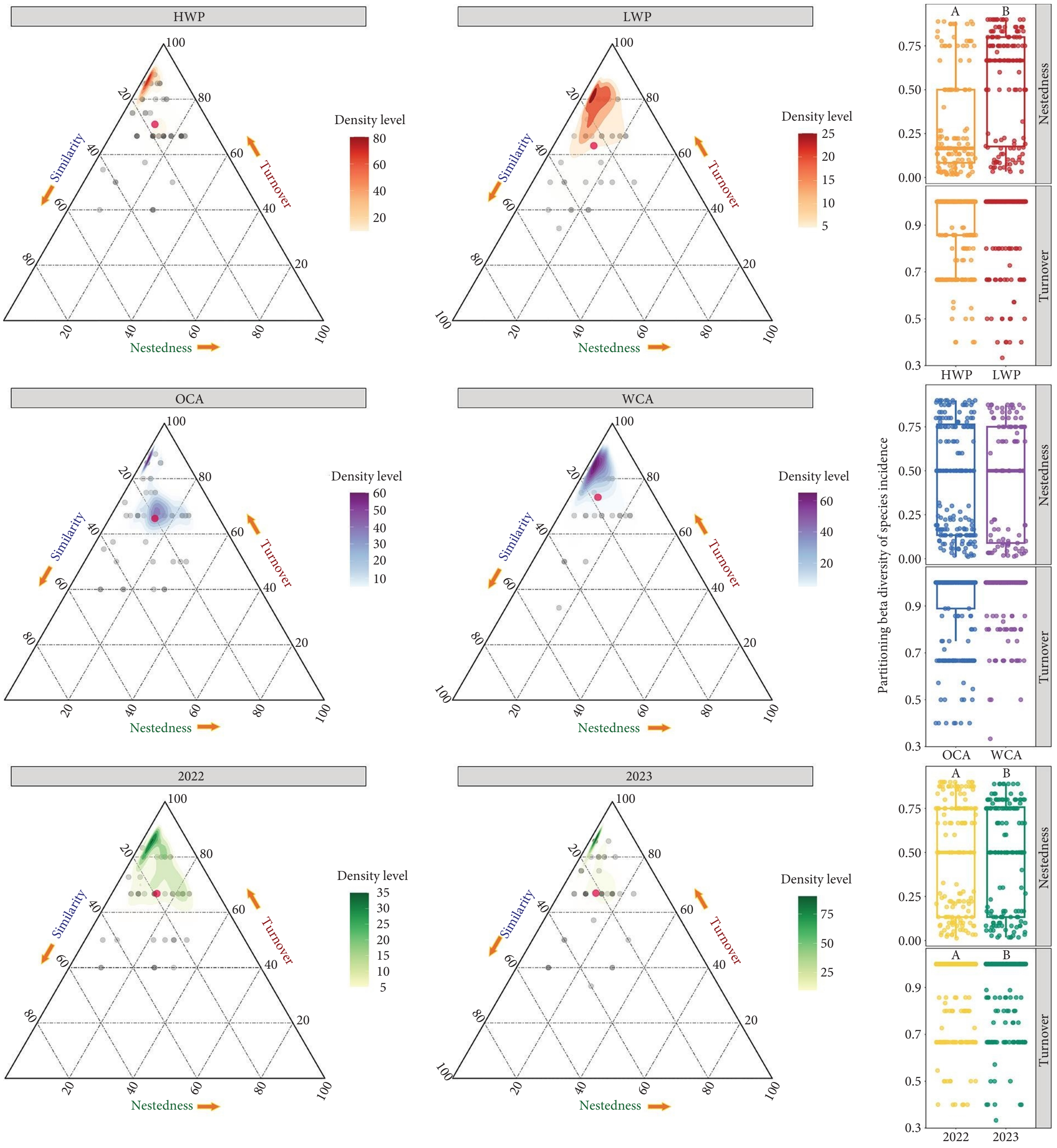
Beta diversity, partitioned into turnover and nestedness components using incidence-based Jaccard dissimilarity, revealed consistent total beta diversity across different times



(a)

(b)

FIGURE 7: Continued.



(c)

Figure 7: The results of PCoA, ANOVA of PCoA1 and PCoA2, and PERMANOVA significance based on the Bray–Curtis dissimilarity (a) of fish communities (a). The CCA showing the correlation between environmental factors and fish communities ordination plot (b). *Environmental factors that have a significant impact on the fish community ($p < 0.05$), $^{**}p < 0.01$, $^{***}p < 0.001$. β-diversity (total, turnover, and nested diversity) of fish assemblages across groups based on incidence-based Jaccard dissimilarity (c). Letters A and B above box plots indicate significant differences.

and spaces, with values of 0.97 for both the HWP and LWP (Figure 7(c)). The mean values were 0.83 ± 0.08 and 0.76 ± 0.12, respectively. For the OCA and WCA, the values were 0.97 and 0.98, with means of 0.80 ± 0.09 and 0.82 ± 0.09, respectively. Both 2022 and 2023 had beta diversity values of 0.97, with mean values of 0.81 ± 0.10 and 0.78 ± 0.10, respectively. In all groups, the turnover component contributed more to spatial differences than nestedness, indicating significant species turnover. Notably, the turnover and nestedness components in 2022 showed significant differences compared to 2023. Specifically, nestedness was higher in 2022 than in 2023 (means of 0.14 ± 0.08 and 0.11 ± 0.07, respectively), while species turnover was lower in 2022 than in 2023 (means of 0.668 ± 0.13 and 0.669 ± 0.13, respectively). This suggests that annual environmental changes differently impact species diversity. Compared to HWP, LWP had higher nestedness (HWP: 0.12, LWP: 0.13). Species turnover was higher during the HWP (HWP: 0.71, LWP: 0.63), while nestedness was higher during the LWP, indicating that water level changes significantly influence species composition. Although not significantly different, nestedness was higher in OCA than in WCA, while species turnover was higher in WCA, suggesting that engineering activities have a low spatial impact on the fish community.

3.4. Influencing Factors of Fish Communities. Using VIF testing, we selected independent environmental factors and identified four factors in 2022 and six in 2023. Then we conducted CCA to explore the impacts of these factors on fish community structure (Figure 7(b)). In 2022, all selected factors (S, WT, pH, and TP) were significantly correlated with fish communities ($p < 0.05$). The first canonical axis (CCA1) explained 39.15% of the variance, primarily influenced by pH and TP. The second axis (CCA2) explained 27.11% of the variance, dominated by S and WT. In 2023, all factors except TP were significantly correlated with fish community structure ($p < 0.05$). CCA1 explained 28.34% of the variance, primarily driven by DO, pH, and WT, while CCA2 explained 24.52% of the variance, dominated by TN and DO.

4. Discussion

This study utilizes eDNA technology to investigate the impact of waterway regulation on fish communities in the Jiujiang section of the Yangtze River, 2 years after the project's completion. The research reveals changes in fish diversity and community composition temporally (across water periods and annual variations) and spatially (relative to the regulated waterway). Compared to traditional biomonitoring methods, eDNA offers high sensitivity and efficiency in detecting species presence and abundance, providing detailed temporal and spatial variation data [41]. Moreover, the noninvasive nature of eDNA is crucial for ecosystems undergoing ecological restoration, especially under the current Yangtze River fishing ban, making eDNA an ideal choice [42]. Compared to a traditional sampling survey conducted in the same location in 2015, which included 19 random samples, they identified a total of 44 fish species from 12 families and 35 genera. The dominant family was Cyprinidae, representing 56.82% of the total species, followed by Serranidae, Bagridae, and Cobitidae, which collectively accounted for 15.91% [38]. The eDNA results from this study similarly indicated that Cyprinidae dominated, constituting 55.56% of the fish population, with Serranidae, Bagridae, and Cobitidae making up 22.22%. This consistency with previous research confirms the effectiveness of eDNA in assessing the impact of waterway regulation on fish communities. In fact, eDNA technology has been widely used to evaluate the impact of engineering projects or human activities on aquatic ecosystems. Examples include the South-to-North Water Diversion Project [30], ecological water replenishment [29], agricultural run-off [43], abandoned mining ponds [44], and reservoir cascade [31]. eDNA has demonstrated significant advantages in assessing the ecological impacts of these projects. Additionally, an increasing number of countries are incorporating eDNA methods into their river ecological restoration assessment frameworks [45, 46]. eDNA technology enables a comprehensive understanding and monitoring of aquatic ecosystem health, providing a scientific basis for water resource management and ecological protection.

Fish communities can adapt to different habitat conditions and exhibit significant mobility, allowing them to migrate upstream or downstream to avoid disturbed areas [47]. The lack of significant differences in fish diversity and community structure across different locations suggests that the spatial impact of waterway regulation on fish communities might be limited. During river maintenance work, certain fish species that are less sensitive to environmental changes tend to migrate short distances and return to their original habitats shortly after [48]. This might explain why there are no significant differences in fish diversity and community structure between the OCA and WCA. Previous studies have indicated that the impact of river dredging on fish communities is mainly confined to reduced species richness and diversity downstream of the project area. This is reflected in our study, in that the upstream sites (J1–J3 and J8) show higher diversity and richness compared to the downstream sites (J4–J7) (Figure 3(a)). Notably, the J8 sampling point near the E4 engineering site exhibited the highest fish community richness and diversity. This is likely due to the implementation of spur dikes at E4, a common riverbank protection structure that can increase water depth and flow velocity, significantly improving fish habitats [49–51]. Existing research indicates that the use of ecological structures and emerging materials in waterway regulation projects can produce positive ecological effects [52–54]. For instance, in projects aiming to protect mid-channel bars, the use of implantable ecological stabilization methods has gradually transformed bare riverbanks into vegetated areas. The growth of vegetation effectively stabilizes the riverbanks, and evidence of bird habitation has also been observed [52]. Another waterway regulation project that employed ecological restoration measures, including artificial reefs and ecological floating beds, showed significant improvements, with an increase in benthic organisms within the reefs [53]. Therefore, in the long run, ecologically integrated and appropriately designed waterway regulation projects may have positive impacts on aquatic ecosystems.

Fish communities exhibit more significant differences across different water periods. In both 2022 and 2023, fish diversity was consistently higher during the HWP compared to the LWP, with overall diversity significantly greater during HWP. This can be attributed to the favorable hydrological conditions during HWP, which support higher species richness and abundance. Waterway regulation activities, including dredging, bank reinforcement, and dike construction, often alter river flow and depth to enhance navigational connectivity [55, 56]. However, the dynamic flow states and seasonal flow variations, which regulate the spatial complexity of physical habitats, are crucial for supporting aquatic biodiversity and ecosystem functions. These factors are key to maintaining and supporting river community diversity [11, 57]. Fish are particularly sensitive to flow changes, especially when flow is reduced, leading to significant negative impacts [58, 59]. As water areas decrease, habitats become fragmented and isolated. Since certain species can only survive in specific habitats, the species composition across different habitats becomes more similar, a phenomenon known as nestedness, which is particularly pronounced during LWP. Additionally, more water quality indicators show significant differences across water periods. Linear fitting results indicate that the rate of change in alpha diversity across different water periods is greater than the rate of change across different locations. Conversely, the rate of change in alpha diversity across different locations is smaller during different water periods. This evidence suggests that temporal factors (water periods) may have a greater impact on fish communities than spatial factors. Therefore, the impact of water period changes on fish communities should be prioritized in future assessments of ecological restoration following waterway regulation.

The impacts of hydraulic engineering projects, such as waterway regulation and dam construction, on aquatic ecosystems are prolonged and often subtle. Many components of these ecosystems, including substrates and species, may take decades or even centuries to fully recover [60]. In this study, the environmental factors influencing fish community composition changed over the years. However, pH and WT consistently showed significant correlations with fish communities in both years, highlighting their crucial roles in influencing fish distribution and abundance. These two factors have been widely reported to affect fish diversity [31, 61, 62]. Specifically, pH is important for stress responses, osmoregulation, and growth hormone regulation in fish [63]. WT affects fish diversity primarily by influencing their survival and reproduction [64]. Besides the environmental factors measured in this study, suspended sediments are frequently reported as a major factor impacted by waterway regulation projects. Suspended sediments can inhibit fish behaviors such as foraging [65] and evasion and stress responses [65–67]. And they also affect fish physiology and function, with impacts that can be both immediate and long-lasting [68]. Therefore, future research should focus on long-term monitoring and integrating more environmental variables to explore the effects of hydraulic engineering on aquatic ecosystems. Different taxonomic groups respond to environmental factors at varying rates and degrees [69, 70]. Evaluating the ecological impact and recovery of hydraulic engineering based on a single taxonomic group may not provide an accurate assessment. Other species, such as fungi, bacteria, aquatic vascular plants, and invertebrates, should also be considered. For clearer results, future assessments should include a broad range of taxonomic groups.

5. Conclusion

In summary, this study utilized eDNA data to examine the composition of fish communities and the influence of environmental factors on their structure in the Jiujiang section of the Yangtze River, 2 years after waterway regulation projects were completed. The findings revealed that Cyprinidae were the dominant fish family in the study area. On a spatial scale, comparing the OCA and WCA, fish community diversity and environmental factors showed minimal variation. In contrast, on a temporal scale, comparing HWP and LWP, there were significant differences in fish communities. The Shannon index was significantly higher during HWP, while nestedness was significantly lower during LWP. Many environmental factors also differed significantly between HWP and LWP. In 2022 and 2023, pH and WT were consistently and significantly correlated with fish communities, while other environmental factors exhibited dynamic changes. We emphasize that prioritizing water periods and long-term ecological monitoring is crucial for the sustainable development of waterways. The findings of this study provide new insights into assessing the ecological status of waterways postregulation and offer a scientific basis for future conservation and management efforts.

Data Availability Statement

The data used in this study are available upon reasonable request from the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Yichen Zheng: investigation, data curation, writing–review and editing. Haobo Zhang: writing–original draft, writing–review and editing, visualization, software, methodology, investigation, formal analysis, data curation. Shuran Yin: writing–review and editing, data curation. Xiaoyan Wang: writing–review and editing. Jianbo Chang: writing–review and editing, funding acquisition, supervision, conceptualization.

Yichen Zheng and Haobo Zhang contributed equally to this work.

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